TSETSE AND TRYPANOSOMOSIS INFORMATION
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Edited by
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Bisamberg
Austria

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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) and the Institut de recherche pour le développement (IRD).

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

Since the value of this information service depends to a great extent on the receipt of relevant material from research workers, campaign planners and organizers and field workers themselves, readers are requested to submit news items and copies of scientific papers and reports to the Editor: Dr James Dargie, Brunstubengasse 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.

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<tr>
<td>a.i.</td>
<td>active ingredient</td>
</tr>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
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<tr>
<td>ALAT</td>
<td>alanine aminotransaminase</td>
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<tr>
<td>ASAT</td>
<td>aspartic acid aminotransaminase</td>
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<tr>
<td>b.w.</td>
<td>body weight</td>
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<tr>
<td>BIIT</td>
<td>blood incubation infectivity test</td>
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<td>CATT</td>
<td>card agglutination test for trypanosomiasis</td>
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<tr>
<td>CD50</td>
<td>median curative dose</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
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<td>HAT</td>
<td>human African trypanosomiasis</td>
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<tr>
<td>HCT</td>
<td>haematocrit centrifugation technique</td>
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<tr>
<td>GIS</td>
<td>geographic information system(s)</td>
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<tr>
<td>GPS</td>
<td>global positioning system(s)</td>
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<tr>
<td>i.m.</td>
<td>intramuscular(ly)</td>
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<tr>
<td>i.p.</td>
<td>intraperitoneal(ly)</td>
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<tr>
<td>i.v.</td>
<td>intravenous(ly)</td>
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<tr>
<td>IFAT</td>
<td>indirect fluorescent antibody test</td>
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<tr>
<td>KIVI</td>
<td>kit for in vitro isolation of trypanosomes</td>
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<tr>
<td>LC50</td>
<td>median lethal concentration</td>
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<tr>
<td>LD50</td>
<td>median lethal dose</td>
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<tr>
<td>M</td>
<td>molar</td>
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<tr>
<td>mAEC</td>
<td>miniature anion-exchange centrifugation technique</td>
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<tr>
<td>McAb</td>
<td>monoclonal antibody</td>
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<tr>
<td>MW</td>
<td>molecular weight</td>
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<td>NARS</td>
<td>National Agricultural Research Services/Systems</td>
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<td>p.i.</td>
<td>post-infection</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PCV</td>
<td>packed cell volume</td>
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<tr>
<td>ppb</td>
<td>parts per billion (10^9)</td>
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<td>ppm</td>
<td>parts per million</td>
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<tr>
<td>r.h.</td>
<td>relative humidity</td>
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<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<td>SIT</td>
<td>sterile insect technique</td>
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<tr>
<td>sp(p).</td>
<td>species (plural)</td>
</tr>
<tr>
<td>ssp(p).</td>
<td>subspecies (plural)</td>
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<tr>
<td>UV</td>
<td>ultra-violet</td>
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<td>VAT</td>
<td>variable antigen type</td>
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<td>VSG</td>
<td>variant surface glycoprotein</td>
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<tr>
<td>WBC</td>
<td>white blood cell</td>
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### 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
- **CIRIDES**: Centre International de Recherche-Développement sur l’Elevage en Zone Subhumide
- **CNERV**: Centre National d’Elevage et de Recherches Vétérinaires
- **CNR**: 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
- **EDF**: European Development Fund
- **FAO**: Food and Agriculture Organization of the United Nations
- **FITCA**: Farming in Tsetse Control Areas of Eastern Africa
Tsetse and Trypanosomosis Information

GTZ Deutsche Gesellschaft für Technische Zusammenarbeit
IAEA International Atomic Energy Agency
IBAR Interafrikan Bureau for Animal Resources
ICIVE International Centre of Insect Physiology and Ecology
ICPTV Integrated Control of Pathogenic Trypanosomes and their Vectors
IFAD International Fund for Agricultural Development
ILRI International Livestock Research Institute
INRA Institut National de Recherche Agronomique
IPR Institut Pierre Richet
IRD Institut de Recherche et de Développement (formerly ORSTOM)
ISCTRC International Scientific Council for Trypanosomiasis Research and Control
ISRA Institut Sénégalais de Recherches Agricoles
ITC International Trypanotolerance Centre
KARI Kenya Agricultural Research Institute
KETRI Kenya Trypanosomiasis Research Institute
LCV Laboratoire Central Vétérinaire
LNERV Laboratoire National de l’Elevage et de Recherches Vétérinaires
LSHTM London School of Hygiene and Tropical Medicine
MRC Medical Research Council
MRU Mano River Union
NITR Nigerian Institute for Trypanosomiasis Research
NRI Natural Resources Institute
OCCGE Organisation de Coopération et de Coordination pour la Lutte contre les Grande Endémies
OCEAC Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale
OGAPROV Office Gabonais pour l’Amélioration de la Production de la Viande
OIE Office International des Epizooties
OMVG Organisation pour la Mise en Valeur du Fleuve Gambie
PAAT Programme against African Trypanosomosis
PATTEC Pan-African Tsetse and Trypanosomiasis Eradication Campaign
PRCT Projet de Recherches Cliniques sur la Trypanosomiase
RDI Rural Development International
RUCA Rijksuniversitair Centrum Antwerpen
SADC Southern African Development Community
SIDA Swedish International Development Authority
SODEPRA Société pour le Développement des Productions Animales
TDR UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases
TDRC Tropical Diseases Research Centre
TPRI Tropical Pesticides Research Institute
TTRI Tsetse and Trypanosomiasis Research Institute
UNDP United Nations Development Programme
USAID United States Agency for International Development
USDA United States Department of Agriculture
UTRO Uganda Trypanosomiasis Research Organisation
WHO World Health Organization
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This regional project was implemented successfully by FAO and its partners between 2013 and 2015. The Government of Italy renewed its commitment to this initiative and its trust in FAO by funding a second phase of the project for implementation between 2016 and 2017 (budget US$ 1 million). As was the case for Phase 1, Phase 2 also targets six countries as a priority (Burkina Faso, Ethiopia, Ghana, Kenya, Mali and Uganda), while also providing training, assistance and tools to all trypanosomosis-affected countries in Africa.

During Phase 1, over 300 technical staff from 20 African countries were trained, with a focus on geographic information systems (GIS) for decision making, as well as tsetse and trypanosomosis risk assessment and interventions. Technical assistance was provided to a range of affected countries and other stakeholders by means of expert missions, workshops and technical and scientific publications. All training and technical assistance activities were carried out in close collaboration with the African Union – Pan African Tsetse and Trypanosomosis Eradication Campaign (AU-PATTEC), International Atomic Energy Agency (IAEA) and World Health Organization (WHO).

A continental Atlas of tsetse and African animal trypanosomosis (AAT) was initiated and piloted in Ethiopia, Kenya and Uganda. The development of national Atlases of tsetse and AAT was supported. Assistance was provided to countries affected by sleeping sickness and to WHO, in particular in the framework of the Atlas of human African trypanosomosis (HAT). Leadership and/or contributions were provided to 13 peer-reviewed scientific publications, thus ensuring that the innovative methodologies piloted by the project reached all potential beneficiaries and maximized their impact. Peer-review also testified to the high technical and scientific standards of all project activities. Pilot field interventions were also included in the project: in particular, the innovative livestock protective fences (LPFs) were introduced in southern Ethiopia to enhance food security and alleviate rural poverty. To this end, 90 farming households benefitted from materials (LPFs), sensitization and training.

During Phase 2 the achievements of Phase 1 will be consolidated and upscaled. Advanced capacity development will be addressed, and strengthened capacities will be used to develop evidence-based, cost-effective plans for field interventions against trypanosomosis. Piloting of LPFs will be extended to a larger number of countries (i.e. Burkina Faso and Kenya) and to different regions of Ethiopia. The continental Atlas of tsetse and animal trypanosomosis will be completed and the development of national Atlases by affected countries will be supported. FAO assistance to WHO and to countries affected by HAT will be further strengthened, thus contributing to the goals of sleeping sickness control and elimination, as set by WHO and its Member States.

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

ACTIVITIES IN EASTERN AFRICA: PROMOTING A PROGRESSIVE CONTROL PATHWAY (PCP) FOR AFRICAN ANIMAL TRYPANOSOMOSIS

The FAO sub-regional office for Eastern Africa convened a tsetse and trypanosomosis (T & T) planning workshop in Addis Ababa, Ethiopia from 2–4 December 2015. The meeting was organized in the framework of PAAT in collaboration with AU-PATTEC and CIRAD to
support Eastern African countries affected by African animal trypanosomosis (AAT). The workshop was attended by representatives from eight countries (Democratic Republic of the Congo, Ethiopia, Kenya, Rwanda, South Sudan, Sudan, Tanzania and Uganda), as well as from a number of organizations and stakeholders (i.e. AU-IBAR, AU-PATTEC, CIRAD, the Desert Locust Control Programme for East Africa (DLCO-EA), the East African Community (EAC), FAO, GALVmed, IAEA, IGAD, WHO).

The workshop aimed to form a sub-regional network on AAT and to introduce programme managers in affected countries to the concept of Progressive Control Pathway (PCP) as applied to AAT. PCP is a stepwise, internationally recognized approach already applied to the control and elimination of a number of diseases such as Foot-and-Mouth Disease (FMD), rabies, peste des petits ruminants (PPR), etc.

The workshop gave participants the opportunity to familiarize themselves with PCP, and to discuss how the PCP framework can be utilized to develop national and sub-regional roadmaps for the progressive control and elimination of AAT. A number of issues were raised and discussed within working groups, including (i) coordination at the sub-regional level, (ii) funding mechanisms, and (iii) the required support to affected countries from international and regional organizations. Working groups also enabled national and sub-regional/multinational roadmaps to be drafted, and a sub-regional workplan for the Eastern African T&T network to be proposed for the period 2016–17. The latter includes the following: (i) two training workshops for programme managers and technical officers, (ii) two sub-regional meetings addressing the planning and implementation of T & T control programmes, (iii) refinement/validation of two multinational projects, focusing on (a) EAC and (b) Ethiopia, Sudan, South Sudan, (c) joint missions for monitoring national and regional T & T control activities, (d) joint resource mobilization missions/events, and (e) development of an FAO sub-regional Technical Cooperation Project (TCP) to support the activities of the network for two years (2016–2017).

For more information, please contact Oumar Diall (oumar.diall@fao.org)

**FROM THE JOINT FAO/IAEA PROGRAMME**

**STANDARD OPERATIONAL PROCEDURES TO DETECT AND MANAGE GLOSSINA PALLIDIPES SALIVARY GLAND HYPERTROPHY VIRUS (GPSGHV) IN TSETSE FLY “FACTORIES”**

Many species of tsetse flies are infected with a virus that causes salivary gland hypertrophy (SGH) symptoms associated with a reduced fecundity and fertility. A high prevalence of SGH has been correlated with the collapse of two laboratory colonies of *Glossina pallidipes* and colony maintenance problems in a mass rearing facility in Ethiopia. Mass-production of *Glossina* species is crucial for tsetse control programmes incorporating the sterile insect technique (SIT), and therefore requires a management strategy for this virus.

During the last decade the Joint FAO/IAEA Programme investigated different strategies to manage the salivary gland hypertrophy virus (SGHV) in *G. pallidipes* mass production colonies, and developed an efficient virus management strategy, which succeeded in controlling and eliminating the virus from the tsetse mass production facility in Kality, Ethiopia. The virus management strategy that was developed is based on a combination of using antiviral drug (Valacyclovir at a dose of 300 μg/ml of blood) and a clean feeding system. In addition to these methods, regular monitoring for the prevalence of SGH by dissection and the confirmation of SGHV by PCR is strongly recommended. In certain cases when attempting to start a new colony from field collected flies a new procedure to avoid using virus infected material is recommended.
To facilitate the monitoring for SGH or the SGHV infections and to properly implement the virus management strategy using a combination of the antiviral drug and the clean feeding system, a step-by-step standard operation procedure (SOP) has been developed, based on current knowledge and experiences from large-scale production of *G. pallidipes*. This document provides useful information for both large and small scale rearing facilities to maintain tsetse flies free from SGHV infection.

This SOP is addressed to staff involved in tsetse rearing with sufficient education to recognize SGH symptoms, and with the ability to regularly monitor variations from normal “healthy” flies. Such variations include reproductive disturbances (reduced matings and egg production) and reduced longevity (premature mortalities and prolonged larviposition cycles). For some parts of this SOP, more sophisticated experience in molecular biology techniques is required to conduct virus diagnosis using the polymerase chain reaction (PCR) method.

**BLOOD PROCESSING MANUAL AND DATABASE FOR TSETSE FLIES**

Although the blood processing procedures manual and the database were developed for the tsetse rearing facility in Kality, Ethiopia, the procedures and database are standard and can be used in relation to any tsetse production facility.

Tsetse flies are fed on quality-tested fresh defibrinated blood, which has been stored in a frozen condition (Wetzel and Luger 1978).

The procedures described concern the collection of animal blood in the abattoir, radiation with gamma-rays (decontamination), preservation and storage in a deep freeze, quality control assurance and processing of the blood into diets for feeding tsetse flies. The procedures aim to provide a constant quality of nutrition to mass-reared fly colonies maintained over a long period for field programmes. In brief, the standard procedure specifically created for the insectary at Kaliti is as follows:

Before collecting the blood, all equipment that will be in contact with blood must be thoroughly cleaned and sterilized. During blood collection in the abattoir, fibrin is removed by mechanically stirring the blood or by adding anticoagulants to stop the blood from clotting. If slaughtering takes place on the floor, the blood collection equipment used is selected accordingly.

After collection, a sample of blood from each batch is checked for microbial contamination, and bioassayed for its nutritive value using female adult flies. All blood fed to flies must first be decontaminated, for example by radiation treatment.

Blood will then be stored in a frozen condition (-20 °C) until the results of quality control tests are available. If results show no bacterial contamination and good nutritional value, the blood will be thawed and prepared for use as required.

**ZANZIBAR REMAINS FREE OF TSETSE, 18 YEARS AFTER ERADICATION WAS DECLARED**

Up until the late 1990s, the island of Unguja, Zanzibar (United Republic of Tanzania) was infested with tsetse—*Glossina austeni* Newstead. Trypanosomosis prevalence among the mostly indigenous cattle averaged around 19 percent and posed a constraint to livestock production in mixed farming systems by the rural farming communities.

A successful eradication project that included the systematic aerial release of sterile male tsetse flies was implemented from 1994 to 1997 by the Government of Zanzibar with support from the FAO/IAEA and IAEA Technical Cooperation Programmes. Following suppression of the tsetse population with insecticides and the subsequent phase of sterile male releases, intensive monitoring for tsetse flies and trypanosome infections in livestock demonstrated that the sterile insect technique (SIT) campaign had been successful in completely eradicating the *Glossina austeni* population from the island.
The removal of the tsetse fly vector resulted in the disappearance of animal trypanosomosis, thus enabling farmers to rear improved breeds of cattle and integrate livestock keeping with cropping contributed significantly to improvements in areas where this had previously been impossible. The increased livestock and crop productivity contributed significantly to an increase in the quality of people’s lives.

A recent entomological and parasitological survey (2015), carried out jointly by independent experts and the Department of Veterinary Services of Zanzibar, has confirmed the continued tsetse-free status of the island, 18 years after eradication was declared.

This survey was carried out by first conducting a GIS analysis of land use (based on the most recent GlobCover information from 2009) and vegetation index (based on eMODIS, January 2015) that was combined with entomological data obtained from baseline surveys carried out prior to the eradication campaign. From this analysis, 23 one-km² square grids covering the most suitable habitat for Glossina austeni were selected. The Jozani National Park, with its dense gallery forest, high humidity suitable for tsetse breeding sites and with wild pigs and primates to provide host blood meals for tsetse, was the most suitable habitat for tsetse reinvasion and was the main focus of the survey during the dry season baseline data collection. It was, indeed, in these areas where the tsetse population density had reached its highest levels before and during the eradication project.

Two-hundred and eleven sticky panels of two types (described by Vreysen et al., 2001) consisting of blue and white cloth coated with a sticky glue, were deployed and left on site for at least 10 days before being checked for tsetse catches and removed. The tsetse panels captured many non-target species but not a single tsetse fly was caught during the survey. The island has therefore remained tsetse free since 1996.

In addition, a total of 356 cattle aged between 6 and 12 months from 13 villages in Unguja were sampled. The blood was checked for trypanosome prevalence by microscopic examination of the buffy coat layer after micro-haematocrit centrifugation. In addition, serum samples were collected and sent to the mainland Tanzania Veterinary Laboratory Agency for examination using the more sensitive Pan Tryp LAMP molecular diagnosis technique. Microscopic examination detected no trypanosome infections. The molecular tests identified no T. congolense infections, which can only be transmitted by tsetse, but did identify nine cases of T. vivax infection that need to be followed up to confirm if they are real cases or false positives and whether the possible infection could reach symptomatic levels as the animals sampled had no clinical symptoms of trypanosomosis. Livestock are occasionally imported from the mainland of Tanzania into Zanzibar so these animals may have been imported with pre-existing infections. A follow-up of the history of the nine cases of infected animals would therefore be useful.
distributions of tsetse populations from existing baseline entomological data and to be able to analyse the degree of isolation from adjacent tsetse populations. The training course was hosted by the National Institute for Control and Eradication of Tsetse and Trypanosomosis (NICETT), the Director of which is Dr Thomas Cherenet.

**DRONES FOR GOOD: ENTRY OF A DRONE FOR THE RELEASE OF STERILE MALE TSETSE FOR A COMPETITION HELD IN DUBAI, UNITED ARAB EMIRATES, 4–6 FEBRUARY 2016**

The UAE Drones for Good Award was dedicated to transforming the innovative technologies behind civilian drones into practical, realisable solutions for improving people's lives today. Two entries from the Joint FAO/IAEA Programme were submitted and selected among the ten semi-finalists of the competition. They consisted of one unmanned aerial system (UAS) for release sterile tsetse and one for release of sterile mosquitoes. A total of 1 017 proposals were submitted from 165 countries for the competition.

The first entry was developed in support of the ongoing Southern Tsetse Eradication Project under the Ethiopian Ministry of Livestock and with the invaluable participation of Embention, a private company which developed the prototype. The drone for release of sterile male tsetse is a fixed wing aircraft that conducts full autonomous missions, including take-off and landing. It has a flight endurance of 200 km or three hours’ flying time and can carry a payload of 2.5 kg that would allow 2,500 sterile males to be carried whilst kept chilled in each of the two release pods fixed under the wings. The UAS would therefore be capable of accurately releasing 5,000 sterile male tsetse at pre-defined coordinates on each flight. A single flight would cover an area of approximately 100 km² of suitable tsetse habitat. Although the entry did not reach the finals of the competition, the drone will be tested in Ethiopia and ultimately used as a more cost-effective means of implementing SIT in the Deme area of the southern Rift Valley.

**FROM WHO**

**SLEEPING SICKNESS OUTBREAKS PREVENTED BY CLIMATE CHANGE RESEARCH**

Climate change has serious implications for public health. According to WHO estimates, climate change is already causing tens of thousands of deaths every year - from shifting patterns of disease, from extreme weather events, such as heat-waves and floods, and from the degradation of air quality, food and water supplies, and sanitation.

This photo story highlights how climate change is impacting the Maasai in northern United Republic of Tanzania and shows how WHO’s tropical disease research programme (TDR), is working to protect their cattle, livelihoods and health from the impacts of climate change.

In the United Republic of Tanzania poor communities reliant on crops and livestock are seeing their livelihoods and food security wrecked by unexpected weather patterns such as increasing temperatures, late rainfall onset and droughts. The Maasai pastoralists in the northern part of the United Republic of Tanzania are particularly vulnerable to the combined effects of climate change and zoonotic diseases (diseases that can be passed from animals to humans) because they live close to large wildlife populations that can act as reservoirs of infection, and compete for access to water and food for their cattle.

Cattle are the lifeblood of the Maasai tribe. Their milk is a critical source of income and nutrition. However, persistent droughts have reduced pasture growth in recent years leaving the fields dry and dusty. As a result men are taking their herds further and further away to get good pasture.
The Maasai are migrating to northern United Republic of Tanzania’s woodlands where large wildlife populations live and are exposing their cattle to human African Trypanosomiasis or sleeping sickness, which has been dormant for years. Sleeping sickness can dry up the cattle’s milk permanently, and impact the community’s health. Human African Trypanosomiasis or sleeping sickness threatens millions of people in 36 sub-Saharan African countries where there are tsetse flies that transmit the disease. The Maasai, the people most exposed to these flies and therefore the disease, live in remote rural areas and depend on agriculture, fishing, animal husbandry or hunting.

Beyond sleeping sickness, rabies, bacterial infections and diseases carried by ticks are also impacting the Maasai. Yet, these zoonotic diseases do not get much attention. Merging the sciences of the flies, parasites, the environment, water and climate, WHO’s Special Programme on Research and Training in Tropical Diseases, called TDR, is working with the community to research and develop solutions.

A TDR study, launched in 2013, through a grant agreement with the International Development Research Centre of Canada is working to identify the safest areas for Maasai to take their cattle. Data collected shows that up to 29 percent of the flies carry the parasite causing sleeping sickness, but this varies by season and location. However, blood samples from cattle that graze in these areas will show how this translates into infection rates.

To understand vector abundance and parasite prevalence rates, tsetse flies are collected in a wide range of sampling areas during both the dry and wet months, and then identified in the laboratory. Molecular tools are used for analysis to identify the numbers of the flies and the infection rates.
The Maasai have always known they need to move their cattle away from grazing areas for a period of time so that the grasses can recover. However, more land is being taken over by farms, and expanding populations and wildlife are increasing the competition for grazing areas. As a result, pastoralists have seen a large number of their cattle die, and their incomes reduced.

The community is a key link in both the research and the solutions. All data are presented to community groups, and potential adaptations are discussed and developed together. The goal is targeted and sustainable solutions that can be integrated into daily life long after the research is completed.

Results from the United Republic of Tanzania study and four others that look at additional diseases impacted by climate change, like malaria, will be finalized at the end of 2016. By working with communities directly impacted by climate change, the studies will offer a new model of approaching the complicated issue that touches on land use, water, animal and human health.

**FROM GALVmed**

**NEW DRUGS TO FIGHT NAGANA**

Animal African Trypanosomosis (AAT), widely known in Africa as nagana, is a deadly scourge of livestock, killing an estimated 3 million cattle each year, as well as many sheep and goats. Spread in the bite of tsetse flies, and caused – like malaria – by a blood-dwelling protozoan parasite, the disease causes fever, severe anaemia and ultimately death if not treated. In infested areas, which cover around 10 million km² across 40 African countries, from Senegal and Ethiopia in the north to South Africa in the south, the disease is responsible for up to 50 percent loss in milk and meat production in affected cattle.

Drugs to treat AAT are available, but their efficacy is limited, particularly as there is increasing parasite resistance to existing drugs, which were first introduced over 40 years ago. Another key concern is drug safety, in terms of residues in food-producing animals. To effectively protect and treat cattle against nagana requires the development of new drugs, which is at the heart of GALVmed’s approach. This work is funded by the Bill & Melinda Gates Foundation and the UK’s Department for International Development.

**Giving drug developers a head-start**

Following consultation with a wide range of stakeholders, including livestock farmers, local vets and staff in departments of agriculture, as well as commercial companies, GALVmed developed a series of Target Product Profiles for new drugs and novel control tools. Various features of the candidate product that were considered ideal (the ‘wants’) or minimal (the ‘musts’), such as efficacy against drug-resistant strains, were included. Developing new drugs for neglected tropical diseases is an expensive and high-risk process, especially when there is a
necessary focus on consumer safety. Therefore, with a number of project partners, GALVmed has tested a range of novel drugs to identify those that are effective against *T. congolense* and *T. vivax*, the most important parasites species that cause AAT.

The search for new trypanocides has unearthed several potential candidates that fit the bill; their development has been de-risked to the point where they are near to handover to a commercial partner to develop further and market. The results on candidate molecules were shared with a number of potential commercial partners in Franschhoek, South Africa during a meeting in November 2015. Those who are interested in taking the potential candidates through full development have been asked to submit their Expressions of Interest to GALVmed by January 2016.

Any companies with experience in pharmaceutical development and an interest in products for Africa who have an interest in developing and marketing these products but who did not attend the meeting, can contact GALVmed’s project lead, Dr Rose Peter for a copy of the non-confidential information package and further discussions.

**FROM FIND**

**EXPERTS CONCLUDE INTERIM RESULTS LOOK GOOD FOR SECOND-GENERATION HAT RDT**

Interim results look promising in a clinical trial evaluating the prototype second-generation rapid diagnostic test (RDT) for sleeping sickness, according to a group of experts that met at FIND. In 2014 and 2015, FIND and partners (Standard Diagnostics/Alere, University of Cambridge, University of Dundee and National Sleeping Sickness Control Programme of DRC) intensified efforts to develop a RDT for sleeping sickness using recombinant antigens. The new test will be easier and cheaper to manufacture than the first-generation RDT, which is made of native antigens generated from pathogenic trypanosomes. A clinical trial to evaluate the new test is currently underway in 15 sites in the Democratic Republic of the Congo. As of January 2016, more than 250 human African trypanosomiasis (HAT) cases and 43,000 controls had been enrolled. Final results of this study will be available by the second quarter of 2016. The January meeting concluded that the performance of the new test was comparable to the performance of the first-generation RDT and therefore satisfactory, although the implementation strategies for the test have yet to be defined. One option that was considered was to combine this new test with other screening tools to further improve test sensitivity. The second-generation RDT is planned to be made commercially available during the first half of 2016.

**FROM DNDi**

**DRUGS FOR NEGLECTED DISEASES INITIATIVE UNVEILS NEW PLAN FOR NEGLECTED PATIENTS**

*Aims to Deliver 16–18 Treatments for Up to 10 Diseases with EUR 650 Million*

After having built the world’s largest drug development pipeline for the most neglected diseases, the Drugs for Neglected Diseases initiative (DNDi) has unveiled plans for a more flexible, dynamic portfolio approach, integrating various operating models to better respond to the needs of patients, notably in low- and middle-income countries. The plan also paves the way for new diseases to be taken up in DNDi’s portfolio. As part of its updated Business Plan for the period 2015–2023, DNDi remains committed to developing treatments for African sleeping sickness, leishmaniasis, and Chagas disease as well as filarial diseases and paediatric HIV. Having recently transferred its malaria activities to the Medicines for Malaria Venture
(MMV), DNDi will soon be launching new research and development (R&D) projects for hepatitis C and mycetoma, two very different diseases that share, with other important global health issues such as anti-microbial resistance, one key challenge: the existing system of biomedical innovation has failed to deliver safe, effective, quality products that are affordable to poor populations.

“New threats are emerging at breakneck speed in today’s rapidly changing global health R&D landscape”, said Dr Bernard Pécoul, Executive Director of DNDi. “DNDi will remain focused on reaching our treatment targets for the most neglected diseases, but we are now in a position to apply new R&D models, where patient needs drive drug development over profits, and where prices of drugs are delinked from the cost of their development,” he added.

The Business Plan 2015–2023 was elaborated through a 24-month process and in-depth consultation with DNDi’s founding partners, governments, key stakeholders, and experts in global health research. It was approved by the Board of Directors in June 2015. The new plan emphasizes DNDi’s commitment to addressing the needs of neglected patients, while allowing for more flexibility to extend the scope of diseases to address current and future unmet and/or urgent patient needs as they arise. A range of operating and support models has been designed to ensure DNDi’s engagement is tailored and appropriate to the need.

For example, the high-cost of a new generation of drugs for hepatitis C has become one of the world’s most pressing and high-profile public health challenges, leaving millions of patients behind. To develop an affordable public health tool for hepatitis C, DNDi will conduct clinical trials for combinations consisting of recently approved drugs and clinical-stage compounds in middle-income countries. For mycetoma, DNDi will test a promising drug candidate for this devastating illness for which there has been virtually no R&D – leaving patients to suffer with toxic and ineffective drugs. To help address the global threat posed by anti-microbial resistance, DNDi will create an internal task force, in collaboration with WHO, to assess the potential of an incubator to house a new initiative focused on developing antibiotics.

DNDi will continue developing its pipeline of over 30 projects for the most neglected diseases. By its 20th anniversary in 2023, DNDi aims to deliver 16 to 18 new treatments with an estimated total budget of EUR 650 million. Importantly, DNDi will use its own experience to forcefully advocate for a global R&D framework that guarantees both innovation and equitable patient access to health technologies.

“The Ebola crisis showed the world the need for highly effective collaborations and timely response,” said Professor Marcel Tanner, Chair of the Board of Directors, DNDi. “As the organization matures, DNDi will continue to learn, innovate, and adapt its operational models to guarantee that R&D capacity is built where needed while continuously identifying and addressing neglected patients needs through the best science.”

FROM CIRAD

OPTIMIZING TSETSE FLY ERADICATION USING SATELLITE IMAGERY AND GENETICS

Isolated populations of tsetse flies constitute the best targets for eradication campaigns, but they are difficult to detect. By combining analysis of satellite images and genetics, researchers at CIRAD and their partners have developed a methodology for identifying these populations at the continental level. This innovative approach, the result of eight years of work, could be applied to the targeting of other vectors of disease, as well as to the protection of endangered species.
The reinfestation problem
In sub-Saharan Africa, tsetse flies spread parasitic diseases, trypanosomoses, which not only present a threat to human health but also ravage agriculture. For example, nagana, or animal African trypanosomiasis, kills more than three million head of cattle every year, resulting in losses exceeding 4 billion dollars. Eradication campaigns are essential tools to control these diseases. Unfortunately, they are very costly and relatively ineffective: so far, they have been successful in less than 2 percent of infested areas. “Often, eradication campaigns target fly populations that are not truly isolated from one another. Cleared zones are therefore gradually reinfested by tsetse flies from neighbouring areas”, explains Jérémy Bouyer, a researcher at CIRAD who led this study.

Geographers come to the rescue
Targeting isolated populations therefore helps to ensure their eradication will be definitive. But how can they be identified? Generally, to do so, researchers analyse genetic exchange between fly populations. But this approach requires costly tsetse capture campaigns in zones that are often very difficult to reach, which prevents its systemisation. To avoid this problem, researchers at CIRAD and their French, African and European partners have developed another method based on the concept of “landscape friction”. Borrowed from geographers, who use it in particular to model traffic flows, in biology this term defines the way in which the landscape elements modulate the movement of animal species.

A map to predict isolation
First, the scientists determined the genetic distance separating 37 populations of the species Glossina palpalis gambiensis, the main vector of trypanosomoses. To do so, they analysed the genome of 1 158 flies caught in four West African countries (Burkina Faso, Mali, Guinea and Senegal, or 80 to 90 percent of the distribution area of this fly). Using satellite images of these regions, they also identified the natural barriers likely to limit the dissemination of tsetse flies and determined the most likely dispersal patterns for these flies. They thus established a “friction map” revealing the connections between the different populations of tsetse flies. Eight populations presenting different degrees of isolation were consequently revealed in the “tsetse belt” (the area in which tsetse flies live, which crosses 38 sub-Saharan African countries and covers more than 10 million km²). These are all potential targets for eradication campaigns. One population in Senegal is already the object of an elimination programme selected for the Milano 2015 World Expo because of its exemplary nature for the sustainable development of small communities of livestock farmers.

A wide variety of practical applications
“This original methodology could not have been developed without close cooperation between ecologists, geographers, population geneticists and modellers. In particular, it has the advantage of moving away from expert opinions, which are subjective and may in some cases be a source of error”, says Jérémy Bouyer. This approach is currently being transferred to other vectors, such as the midge Culicoides imicola, in the Mediterranean basin. It can also be used to study the genetic structure of virus populations on the scale of a whole continent in order to develop the most appropriate vaccination strategies based on this. Finally, it will facilitate the work of conservation biologists, by helping them for example to identify exchange corridors between certain endangered animal populations living in increasingly fragmented ecosystems.

The originality of this research, published in the prestigious journal Proceedings of the National Academy of Sciences, has attracted the attention of managers at Earth Engine, the remote sensing data analysis platform belonging to Google. See also Abstract No. 17673 in this part of TTI.

Partners: IAEA, FAO, IRD
SECTION B – ABSTRACTS

1. GENERAL (INCLUDING LAND USE)


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Efforts to control neglected tropical diseases have increasingly focused on questions of implementation. But how should we conceptualize the implementation process? Drawing on ethnographic fieldwork between 2010 and 2012, this article explores efforts by a small-scale public-private partnership to use private veterinarians to sustainably control zoonotic sleeping sickness in Uganda. With a fundamental tension between business incentives and vector control, I show how divergences in knowledge, power, values, and social norms shaped project implementation and community responses. Reflecting more widely on the relationships between project plans and local realities, I argue that these encounters reveal the heuristic value in approaching global health interventions as evolving “social experiments”. This metaphor reveals the uncertainty inherent to dominant narratives and models, the role of available expertise in defining the limits of action, and the need for continuous adaption to synchronize with emergent social and institutional topographies.


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So far, research on trypanosomatid infections has been driven by “disease by disease” approaches, leading to different concepts and control strategies. It is, however, increasingly clear that they share common features such as the ability to generate long-lasting asymptomatic infections in their mammalian hosts. Trypanotolerance, long integrated in animal African trypanosomosis control, historically refers to the ability of cattle breeds to limit Trypanosoma infection and pathology, but has only recently been recognized in humans. Whilst trypanotolerance is absent from the vocabulary on leishmaniasis and Chagas disease, asymptomatic infections also occur. We review the concept of trypanotolerance across the trypanosomatids and discuss the importance of asymptomatic carriage in the current context of elimination.

Neglected tropical diseases (NTDs) are a group of tropical infectious diseases affecting the poorest people. Of 17 NTDs managed by WHO, two, guinea worm disease (by 2015) and yaws (by 2020) are targeted for eradication, and four (blinding trachoma, human African trypanosomiasis, leprosy, and lymphatic filariasis) for elimination by 2020. The goals look promising but 11 others are still highly prevalent. Soil-transmitted helminths (STHs) are one NTD which prevail over the world including temperate zones. They had been highly prevalent in Korea but are mostly disappearing at present through systematic and sustainable control activity. The successful experience of STH control enables Korean experts to develop many programmes of NTD control in developing countries. Several programmes involving both official development aid and non-governmental organizations are now targeting NTDs. Most NTDs are low in health priority compared with their health threats because they are chronic, insidious, and of low mortality. No-one, including the victims, raised the priority of NTD control with a loud voice. After the millennium development goals declared disease control over the world, NTDs are becoming less neglected globally. Even with limited resources, beginning a sustainable national programme is the key for the control and elimination of NTDs. Neglect, especially self-neglect, cannot eliminate diseases and upgrade the quality of life of the neglected people.


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 host. 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 the recent work on the different escape mechanisms employed by African trypanosomes to ensure infection chronicity and transmission potential.
Developing countries face numerous barriers to conducting effective and efficient ethics reviews of international collaborative research. In addition to potentially overlooking important scientific and ethical considerations, inadequate or insufficiently trained ethics committees may insist on unwarranted changes to protocols that can impair a study's scientific or ethical validity. Moreover, poorly functioning review systems can impose substantial delays on the commencement of research, which needlessly undermine the development of new interventions for urgent medical needs. In response to these concerns, the Drugs for Neglected Diseases Initiative (DNDi), an independent non-profit organization founded by a coalition of public sector and international organizations, developed a mechanism to facilitate more effective and efficient host country ethics review for a study of the use of fexinidazole for the treatment of late stage African trypanosomiasis (HAT). The project involved the implementation of a novel “pre-review” process of ethical oversight, conducted by an ad hoc committee of ethics committee representatives from African and European countries, in collaboration with internationally recognized scientific experts. This article examines the process and outcomes of this collaborative process.


Control of insect vector populations is an integral part of disease management but has many challenges. Area-wide campaigns, mainly based on insecticide administration, are most effective for control of insect populations, whereas disease prevention is more localised and protects a smaller number of animals against insect vector contact. Various control and prevention techniques are available for use against most insect vectors and are illustrated here by focusing on two important insect groups: biting midges and tsetse flies. Biting midges (*Culicoides*) present a major threat and challenge to disease and vector control because of limited large-scale control options and the huge population sizes and wide distribution of these insects. Localised disease prevention forms the basis for control, and there is a need for better understanding of the ecology and biology of these insects in order to develop large-scale control techniques. The necessary techniques to effectively control tsetse flies (*Glossina*) and trypanosomosis exist for both localised and area-wide control. The development of a new, cost-efficient device has had a significant impact in the control of both human and animal trypanosomosis. This is especially relevant in Uganda, where the movement of livestock for trading purposes is implicated in disease distribution and poses an immediate health threat where the two forms of the disease overlap. Although many successes have been achieved,
continued research and development are needed to keep abreast of the multitude of challenges in insect vector control.


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The singular opportunity to eliminate human African trypanosomiasis (HAT) has come to be widely acknowledged, both by the World Health Organization (WHO) and within the broad network it coordinates. The Atlas of HAT is the cornerstone of a system required to provide evidence and monitor the elimination process. We present here how the Atlas is rising to the challenge and we discuss the yet-to-be-resolved issues. Information on all HAT cases reported from endemic and non-endemic countries is collated, standardized, georeferenced, and included in a database (start year: 2000). Results of all active screening campaigns are also included. Maps of HAT distribution and risk are subsequently generated at a variety of scales. For risk estimation, a kernel smoothed function is calculated, based on HAT reported cases and Landscan databases of human population. The Atlas also maps health facilities providing HAT diagnosis and treatment, and the coverage of active and passive surveillance. Ninety-three percent of reported cases are mapped at the village level (181 872/196 459, period 2000–2012), the others being referenced at the focus level. The average accuracy is estimated at 900 m. Completeness and accuracy of mapping are steadily improving, as reporting is standardized and the use of global positioning system (GPS) expands. Trends in *gambiense* HAT risk between the periods 2003–2007 and 2008–2012 show a sharp drop, with a 57% reduction for the population at moderate, high and very high risk. A similar decrease is observed for *rhodesiense* HAT (i.e. 62.3% reduction in the same risk categories). In conclusion, the Atlas of HAT increasingly underpins a wide range of control, research and advocacy activities. Capacity building and transfer of national database and the technology for its exploitation in endemic countries are ongoing. Under-detection and under-reporting pose the main challenges to the Atlas. To address them, statistical models will be used to estimate how many cases go undetected or unreported, and where. Ecological modelling could also shed some light on the risk of HAT away from the known focal areas of endemicity. To enhance timeliness, efforts will also be made to reduce the turnaround time between data reporting and mapping. As we look ahead at HAT elimination, the importance of putting and keeping the disease on the map cannot but increase.


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Despite the fact that we are immersed in an era of important technological advances, neglected tropical diseases represent a significant health burden in large parts of the world. Human African trypanosomiasis (HAT), also known as sleeping sickness, is a vector-borne disease responsible for epidemics that devastated several African countries last century and still threaten scores of others today in sub-Saharan Africa. It is caused by the flagellated protozoa *Trypanosoma brucei gambiense* and *T. b. rhodesiense* and transmitted by tsetse flies. The disease presents two clinical stages. The early stage is characterized by the presence of the parasite in the blood and the haemolymphatic system. The late stage, or central nervous system stage, occurs when the parasites cross the blood–brain barrier leading to severe sleep cycle disruptions, paralysis, progressive mental weakening and, ultimately, results in death if not treated.


This paper explores the framings of trypanosomiasis, a widespread and potentially fatal zoonotic disease transmitted by tsetse flies (*Glossina* species) affecting both humans and livestock. This is a country case study focusing on the political economy of knowledge in Zambia. It is a pertinent time to examine this issue as human population growth and other factors have led to migration into tsetse-inhabited areas with little historical influence from livestock. Disease transmission in new human-wildlife interfaces such as these is a greater risk, and opinions on the best way to manage this are deeply divided. A qualitative case study method was used to examine the narratives on trypanosomiasis in the Zambian policy context through a series of key informant interviews. Interviewees included key actors from international organisations, research organisations and local activists from a variety of perspectives acknowledging the need to explore the relationships between the human, animal and environmental sectors. Diverse framings are held by key actors looking from, variously, the perspectives of wildlife and environmental protection, agricultural development, poverty alleviation, and veterinary and public health. From these viewpoints, four narratives about trypanosomiasis policy were identified, focused around four different beliefs: that trypanosomiasis is protecting the environment, is causing poverty, is not a major problem, and finally, that it is a Zambian rather than international issue to contend with. Within these narratives there are also conflicting views on the best control methods to use and different reasoning behind the pathways of response. These are based on apparently incompatible priorities of people, land, animals, the economy and the environment. The extent to which a One Health approach has been embraced and the potential usefulness of this as a way of reconciling the aims of these framings and narratives are considered throughout the paper. It is concluded that while there has historically been a lack of One Health working in this context, the complex, interacting factors that impact the disease show the need for cross-sector, interdisciplinary decision making to stop rival narratives leading to competing actions. Additional recommendations include implementing: surveillance to assess under-reporting of disease and consequential under-estimation of disease risk; evidence-based decision making;
increased and structurally managed funding across countries; and focus on interactions between disease drivers, disease incidence at the community level, and poverty and equity impacts.


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Infectious diseases account for a significant global burden of disease and substantial investment in research and development. This paper presents a systematic assessment of research investments awarded to UK institutions and global health metrics assessing disease burden. We systematically sourced research funding data awarded from public and philanthropic organisations between 1997 and 2013. We screened awards for relevance to infection and categorised data by type of science, disease area and specific pathogen. Investments were compared with mortality, disability-adjusted life years (DALYs) and years lived with disability (YLDs) across three time points. Between 1997 and 2013, there were 7 398 awards with a total investment of Pounds Sterling (GBP) 3.7 billion. An increase in research funding across 2011-2013 was observed for most disease areas, with notable exceptions being sexually transmitted infections and sepsis research where funding decreased. Most funding remains for pre-clinical research (GBP 2.2 billion, 59.4%). Relative to global mortality, DALYs and YLDs, acute hepatitis C, leishmaniasis and African trypanosomiasis received comparatively high levels of funding. Pneumonia, shigellosis, pertussis, cholera and syphilis were poorly funded across all health metrics. Tuberculosis (TB) consistently attracts relatively less funding than HIV and malaria. In conclusion, most infections have received increases in research investment, alongside decreases in global burden of disease in 2013. The UK demonstrates research strengths in some neglected tropical diseases such as African trypanosomiasis and leishmaniasis, but syphilis, cholera, shigellosis and pneumonia remain poorly funded relative to their global burden. Acute hepatitis C appears well funded but the figures do not adequately take into account projected future chronic burdens for this condition. These findings can help to inform global policymakers on resource allocation for research investment.

Quantitative analysis and mathematical models are useful tools in informing strategies to control or eliminate disease. Currently, there is an urgent need to develop these tools to inform policy to achieve the 2020 goals for neglected tropical diseases (NTDs). In this paper we give an overview of a collection of novel model-based analyses which aim to address key questions on the dynamics of transmission and control of nine NTDs: Chagas disease, visceral leishmaniasis, human African trypanosomiasis, leprosy, soil-transmitted helminths, schistosomiasis, lymphatic filariasis, onchocerciasis and trachoma. Several common themes resonate throughout these analyses, including: the importance of epidemiological setting on the success of interventions; targeting groups who are at highest risk of infection or re-infection; and reaching populations who do not have access to interventions and may act as a reservoir for infection. The results also highlight the challenge of maintaining elimination “as a public health problem” when true elimination is not reached. The models elucidate the factors that may be contributing most to persistence of disease and discuss the requirements for eventually achieving true elimination, if that is possible. Overall, this collection presents new analyses to inform current control initiatives. These papers form a base from which further development of the models and more rigorous validation against a variety of datasets can help to give more detailed advice. At the moment, the models' predictions are being considered as the world prepares for a final push towards control or elimination of neglected tropical diseases by 2020.

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Protozoan parasitic diseases are endemic in many countries worldwide, especially in developing countries, where infertility is a major burden. It has been reported that such infections may cause infertility through impairment in male and female reproductive systems. We searched Medline, PubMed, and Scopus databases and Google Scholar to identify the potentially relevant studies on protozoan parasitic infections and their implications in human and animal model infertility. Literature described that some of the protozoan parasites such as *Trichomonas vaginalis* may cause deformities of the genital tract, cervical neoplasia, and tubal and atypical pelvic inflammations in women and also non-gonococcal urethritis, asthenozoospermia, and teratozoospermia in men. *Toxoplasma gondii* could cause endometritis, impaired folliculogenesis, ovarian and uterine atrophy, adrenal hypertrophy, vasculitis, and cessation of oestrus cycling in female and also decrease in semen quality, concentration, and motility in male. *Trypanosoma cruzi* inhibits cell division in embryos and impairs normal implantation and development of placenta. Decrease in gestation rate, infection of hormone-producing glands, parasite invasion of the placenta, and overproduction of inflammatory cytokines in the oviducts and uterine horns are other possible mechanisms induced by *Trypanosoma cruzi* to infertility. *Plasmodium* spp. and *Trypanosoma brucei* spp. cause damage to the pituitary gland, hormonal disorders, and decreased semen quality. *Entamoeba histolytica* infection leads to pelvic pain, salpingitis, tubo-ovarian abscess, and genital ulcers. Cutaneous and visceral leishmaniasis can induce genital lesions, testicular amyloidosis, inflammation of epididymis, prostatitis, and sperm abnormality in human and animals. In addition, some epidemiological studies have reported that rates of protozoan infections in infertile patients are higher than healthy controls. The current review indicates that protozoan parasitic infections may be an important cause of infertility. Given the widespread prevalence of parasitic protozoa diseases worldwide, we suggest further studies to better understand the relationship between such infections and infertility.


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Among the many complex relationships between insects and microorganisms such as viruses, bacteria and parasites, some have resulted in the establishment of biological systems within which the insects act as a biological vector for infectious agents. It is therefore advisable to understand the identity and biology of these vectors in depth, in order to define procedures for epidemiological surveillance and anti-vector control. The following are successively reviewed in this article: Anoplura (lice), Siphonaptera (fleas), Heteroptera (bugs: Cimicidae, Triatoma, Belostomatidae), Psychodidae (sandflies), Simuliidae (black flies), Ceratopogonidae
Tsetse and Trypanosomosis Information

(bitings midges), Culicidae (mosquitoes), Tabanidae (horseflies) and Muscidae (tsetse flies, stable flies and pupipara). The author provides a rapid overview of the morphology, systematics, development cycle and bio-ecology of each of these groups of vectors. Finally, their medical and veterinary importance is briefly reviewed.


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African trypanosomiases are infectious diseases caused by trypanosomes. African animal trypanosomiasis (AAT) remains an important threat to livestock production in some affected areas whereas human African trypanosomiasis (HAT) is targeted for elimination in 2020. In West and Central Africa, it has been shown that the parasites causing these diseases can coexist in the same tsetse fly or the same animal. In such complex settings, the control of these diseases must be put in the general context of trypanosomiasis control or the "one health" concept where the coordination of control operations will be beneficial for both diseases. In this context, implementing control activities on AAT will help to sustain HAT control. It will also have a positive impact on animal health and economic development of the regions. The training of inhabitants on how to implement and sustain vector control tools will enable a long-term sustainability of control operations that will lead to the elimination of HAT and AAT.


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Epidemics of both forms of human African trypanosomiasis (HAT) are confined to spatially stable foci in sub-Saharan Africa while tsetse distribution is widespread. Infection rates of Trypanosoma brucei gambiense in tsetse are extremely low and cannot account for the catastrophic epidemics of Gambian HAT (gHAT) seen over the past century. Here we examine the origins of gHAT epidemics and evidence implicating human genetics in HAT epidemiology. We discuss the role of stress causing breakdown of heritable tolerance in silent disease carriers generating gHAT outbreaks and see how peculiarities in the epidemiologies of gHAT and Rhodesian HAT (rHAT) impact on strategies.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

For the control of *Glossina brevipalpis* and *Glossina austeni* that occur in South Africa an area-wide integrated pest management (AW-IPM) programme with a sterile insect technique (SIT) component has been proposed. The quality of the released sterile male tsetse flies will greatly determine the success of the SIT component of the programme. Sterile males need to be able to compete with wild males immediately after their release in the affected area. The mating competitiveness can be affected by many factors including the optimal mating age of the fly which can have an impact on the timing of the release. To assess the optimal mating age for *G. brevipalpis* and *G. austeni*, mating competitiveness studies were carried out in a walk-in field cage. First, the time of peak fly activity was determined by performing the experiment in the morning and then again in the afternoon. Thereafter, 3, 6 and 9-day-old male flies competed for 3-day-old virgin females. There were no significant differences in mating performance when the field cage experiments were done in the morning or in the afternoon. However, the mating latency was shorter in the afternoon than in the morning. For both species 9-day-old males mated significantly more often than 6 or 3-day-old males. Age did not affect the males' ability to transfer sperm, mating duration or the mating latency. All females that mated were inseminated. In conclusion, age did influence the mating competitiveness of *G. brevipalpis* and *G. austeni* and it is recommended that sterile males are not released before the age of 9 days. Keeping the male flies in the rearing facility for 8 days will have economic and logistic consequences for AW-IPM programmes that have a SIT component.


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*Glossina pallidipes* salivary gland hyperplasia (GpSGH) syndrome caused by the salivary gland hyperplasia virus reduces the reproductive potential of tsetse flies, posing a serious threat for rearing of sufficient colonies for use in tsetse and trypanosomiasis control using the sterile insect technique. This research was conducted in the Kaliti Tsetse Mass Rearing and Irradiation Centre in Ethiopia with the objective of studying the prevalence of GpSGH syndrome in laboratory colonies of *G. pallidipes* (Tororo and Arbaminch) reared for release in the implementation of the sterile insect technique and a field strain of *G. pallidipes* Arbaminch. Presence or absence of GpSGH was determined when pathological features of the salivary gland were revealed after dissection. The overall prevalence of GpSGH syndrome in laboratory colonies was 48.3% (747/1 548) with a statistically significant (p = 0.001) prevalence of 70.2% (544/775) in Arbaminch colonies and 26.26% (203/773) in Tororo colonies. The prevalence of GpSGH in laboratory flies fed according to the clean blood feeding protocol was 68.9% and 22.4% in Arbaminch and Tororo strains, respectively. It was 70.5% and 27.2% respectively in laboratory colonies of Arbaminch and Tororo strains fed according to the standard membrane
feeding protocol. The difference in prevalence of the disease between the two feeding protocols was not statistically significant in either Arba Minch (p = 0.359) or Tororo (p = 0.111) strains. The prevalence of SGH in wild *G. pallidipes* Arba Minch strain was 3% (15/500) and was significantly (p < 0.001) lower than in the laboratory strain. The effect of age and density-related stress on the development of GpSGH was not statistically significant. The prevalence of GpSGH in the newly emerging (teneral) flies in the laboratory colonies was 66.7% and 20% in the Arba Minch and Tororo strains, respectively. For all considered risk factors, the prevalence was much higher in *G. pallidipes* Arba Minch laboratory colonies.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY


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The tsetse fly *Glossina morsitans morsitans* is an important insect vector of African trypanosomes which cause human African trypanosomiasis (HAT). As with other haematophagous arthropods, tsetse flies rely heavily on the pharmacological properties of their saliva to suppress their hosts’ immune reactions and get a blood meal. However, little information is available on the immune regulators of tsetse testes flies. An immunoregulatory peptide named Gloss 2 containing the amino acid sequence QKNDTAFSCHFFEIYL SNCFNKKEYIKNYLQIM has been identified from the salivary glands of the tsetse fly *G. morsitans morsitans* (Diptera: Glossinidae). Gloss 2 has the ability to inhibit the secretion of tumour necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), interleukin-6 (IL-6) and interleukin-10 (IL-10) induced by lipopolysaccharide (LPS) in mouse splenocytes. Besides, Gloss 2 significantly suppressed the LPS-induced activation of the MAPK (mitogen activated protein kinases) signalling pathway through blocking phosphorylation of JNK (Jun amino-terminal kinases), Erk (extracellular-signal-regulated kinases) and P38. Gloss 2 probably inhibits host inflammatory responses by inhibiting secretion of TNF-alpha, IFN-gamma and IL-6. Considering IL-10’s ability to promote humoral immune responses by enhancing class II expression B cells and inducing immunoglobulin (Ig) production, Gloss 2 may inhibit host humoral immune response by inhibiting IL-10 secretion. The immune-suppression may facilitate the blood feeding of tsetse fly and transmission of African trypanosomes to hosts.


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Adipokinetic hormones (AKH) are well known regulators of energy metabolism in insects. These neuropeptides are produced in the corpora cardiaca and perform their hormonal function by interacting with specific G protein-coupled receptors (GPCRs) at the cell membranes of target tissues, mainly the fat body. Here, we investigated the sequences, spatial and temporal distributions, and pharmacology of AKH neuropeptides and receptors in the tsetse fly, Glossina morsitans morsitans. The open reading frames of two splice variants of the Glomo-akh receptor (Glomo-akhr) gene and of the AKH neuropeptide encoding genes, gmmhrth and gmmakh, were cloned. Both tsetse AKHR isoforms showed strong sequence conservation when compared with other insect AKHRs. Glomo-AKH prepropeptides also had the typical architecture of AKH precursors. In an in vitro Ca\(^{2+}\) mobilization assay, Glomo-AKH neuropeptides activated each receptor isoform up to nanomolar concentrations. We identified structural features of tsetse AKH neuropeptides essential for receptor activation in vitro. Gene expression profiles suggest a function for AKH signalling in regulating Glossina energy metabolism, where AKH peptides are released from the corpora cardiaca and activate receptors mainly expressed in the fat body. This analysis of the ligand-receptor coupling, expression, and pharmacology of the two Glomo-AKHR variants facilitates further elucidation of the function of AKH in G. m. morsitans.


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Microarray is a powerful and cheap method to identify and quantify gene expression, in particular in a mix of total RNA extracted from biological samples such as the tsetse fly gut, including several organisms (here, the fly tissue and the intestinal microorganisms). Besides, biostatistics and bioinformatics allow comparing the transcriptomes from samples collected from differently treated flies, and thus to identify and quantify differentially expressed genes. Here, we describe in detail a whole microarray transcriptome dataset produced from tsetse fly symbionts, Sodalis glossinidius and Wigglesworthia glossinidia. The tsetse fly midguts were sampled at key steps of tsetse fly infection by trypanosomes, 3-day and 10-day sampling times, to target the differentially expressed genes involved, respectively, in early events associated with trypanosome entry into the midgut and with the establishment of infection; and 20 days to target the genes involved in events occurring later in the infection process. We describe in detail the methodology applied for analysing the microarray data including differential expression as well as functional annotation of the identified symbiont genes. Both the microarray data and design are available at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48360;http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48361;http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55931.
Transcription initiation regulation is mediated by sequence-specific interactions between DNA-binding proteins (transcription factors) and cis-elements, where BRE, TATA, INR, DPE and MTE motifs constitute canonical core motifs for basal transcription initiation of genes. Accurate identification of transcription start site (TSS) and their corresponding promoter regions are critical for delineation of these motifs. To this end, the genome scale analysis of core promoter architecture in insects has been confined to *Drosophila*. The recently sequenced tsetse fly genome provides a unique opportunity to analyse transcription initiation regulation machinery in blood-feeding insects. A computational method for identification of TSS in the newly sequenced tsetse fly genome was evaluated, using TSS seq tags sampled from two developmental stages namely; larvae and pupae. There were 3 134 tag clusters among which 45.4% (1 424) of the tag clusters were mapped to first coding exons or their proximal predicted 5'UTR regions and 1.0% (31) of the tag clusters mapping to transposons, within a threshold of 100 tags per cluster. These 1 393 non transposon-derived core promoters had propensity for AT nucleotides. The -1/+1 and 1/+1 positions in *D. melanogaster*, and *G. m. morsitans* had propensity for CA and AA dinucleotides respectively. The 1 393 tag clusters comprised narrow promoters (5%), broad with peak promoters (23%) and broad without peak promoters (72%). Two-way motif co-occurrence analysis showed that the MTE-DPE pair is over-represented in broad core promoters. The frequently occurring triplet motifs in all promoter classes are the INR-MTE-DPE, TATA-MTE-DPE and TATA-INR-DPE. Promoters without the TATA motif had higher frequency of the MTE and INR motifs than those observed in *Drosophila*, where the DPE motif occurs more frequently in promoters without the TATA motif. Gene ontology terms associated with developmental processes were over-represented in the narrow and broad with peak promoters. The study has identified different motif combinations associated with broad promoters in a blood-feeding insect. In the case of TATA-less core promoters, *G. m. morsitans* uses the MTE to compensate for the lack of a TATA motif. The increasing availability of TSS seq data allows for revision of existing gene annotation datasets with the potential of identifying new transcriptional units.


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New mode-of-action insecticides are sought to provide continued control of pesticide resistant arthropod vectors of neglected tropical diseases (NTDs). We previously identified
antagonists of the AaDOP2 D1-like dopamine receptor (DAR) from the yellow fever mosquito, *Aedes aegypti*, with toxicity to *Ae. aegypti* larvae as leads for novel insecticides. To extend DAR-based insecticide discovery, we evaluated the molecular and pharmacological characteristics of an orthologous DAR target, CqDOP2, from *Culex quinquefasciatus*, the vector of lymphatic filariasis and West Nile virus. CqDOP2 has 94.7% amino acid identity to AaDOP2 and 28.3% identity to the human D1-like DAR, hD1. CqDOP2 and AaDOP2 exhibited similar pharmacological responses to biogenic amines and DAR antagonists in cell-based assays. The antagonists amitriptyline, amperozide, asenapine, chlorpromazine and doxepin were between 35 to 227-fold more selective at inhibiting the response of CqDOP2 and AaDOP2 in comparison to hD1. Antagonists were toxic to both *C. quinquefasciatus* and *Ae. aegypti* larvae, with LC₅₀ values ranging from 41 to 208 μM 72 h post-exposure. Orthologous DOP2 receptors identified from the African malaria mosquito, *Anopheles gambiae*, the sand fly, *Phlebotomus papatasi* and the tsetse fly, *Glossina morsitans*, had high sequence similarity to CqDOP2 and AaDOP2. DAR antagonists represent a putative new insecticide class with activity against *C. quinquefasciatus* and *Ae. aegypti*, the two most important mosquito vectors of NTDs. There has been limited change in the sequence and pharmacological properties of the DOP2 DARs of these species since divergence of the tribes Culicini and Aedini. We identified antagonists selective for mosquito versus human DARs and observed a correlation between DAR pharmacology and the *in vivo* larval toxicity of antagonists. These data demonstrate that sequence similarity can be predictive of target potential. On this basis, we propose expanded insecticide discovery around orthologous DOP2 targets from additional dipteran vectors.


Tsetse flies (Diptera: *Glossinidae*) are the vectors of trypanosomes causing sleeping sickness in humans, and nagana (animal trypanosomosis) in domestic animals, in sub-Saharan Africa. They have been described as being strictly haematophagous, and transmission of trypanosomes occurs when they feed on a human or an animal. There have been indications however in old papers that tsetse may have the ability to digest sugar. Here we show that hungry tsetse (*Glossina palpalis gambiensis*) in the lab. do feed on water and on water with sugar when no blood is available, and we also show that wild tsetse have detectable sugar residues. We showed in laboratory conditions that at a low concentration (0.1%) or provided occasionally (0.1%, 0.5%, 1%), glucose had no significant impact on female longevity and fecundity. However, regular provision of water with 1% glucose increased the mortality and reduced the fecundity of female *G. p. gambiensis*. The proportion of wild tsetse caught by traps which have detectable sugar residue in their midgut varied between 5 and 10% according to species (*p* <10⁻³) and sex, with more females being found with sugar residues than males (*p* < 10⁻³). We also observed a higher frequency of sugar residues in the dry season than in the rainy season (*p* < 10⁻³). The infection status did not affect the frequency of sugar residues found (*p* = 0.65), neither did age (*p* = 0.23). These observations represent a fundamental change in our knowledge of this insect vector. They open the way for further research in the field to know more on tsetse feeding
behaviour regarding other sources of meal than blood, in particular with plants, and may constitute future new means of controlling this vector of neglected tropical disease.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES


In Senegal, a project has been undertaken to eradicate a population of tsetse flies (*Glossina palpalis gambiensis*) from a prime area for intensifying livestock production - the coastal region of Niayes. The project is intended to remove the constraint of trypanosomosis and allow the ecological intensification of cattle production. A cross-sectional analysis of ten case studies was the inductive phase of an assessment to gauge the impact of removing trypanosomosis on livestock production strategies. The methodology used was a comprehensive analysis, with participatory epidemiology tools to understand farmers' rationales. The authors analysed the strategies of three main types of livestock producer (agro-pastoralists, mixed crop/livestock farmers and intensive dairy farmers). The strategies were in line with the farmers' goals and their ability to mobilise the socio-technical network. The risk management of trypanosomosis has been incorporated into livestock management practices through the use of trypanotolerant breeds, medical prophylaxis or placing livestock in low-risk areas. Removing the risk of disease would therefore have a major impact on decisions about the composition and strategic direction of herds. This change in the animal health environment would steer livestock production along different routes of intensification in a highly competitive environment. The indicators of innovation capacity revealed by this study will be used to quantitatively monitor various change scenarios, taking livestock producers' reasoning into account, in order to assess the socio-economic impact of eradicating the tsetse fly population in this area. The methodology presented in the study can be used to understand the impact of controlling other vector-borne infections on the innovation dynamics of livestock producers.


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Tsetse flies are the cyclical vectors of deadly human and animal trypanosomoses in sub-Saharan Africa. Tsetse control is a key component for the integrated management of both plagues, but local eradication successes have been limited to less than 2% of the infested area. This is attributed to either resurgence of residual populations that were omitted from the eradication campaign or reinvasion from neighbouring infested areas. Here we focused on *Glossina palpalis gambiensis*, a riverine tsetse species representing the main vector of trypanosomoses in West Africa. We mapped landscape resistance to tsetse genetic flow, hereafter referred to as friction, to identify natural barriers that isolate tsetse populations. For this purpose, we fitted a statistical model of the genetic distance between 37 tsetse populations sampled in the region, using a set of remotely sensed environmental data as predictors. The least-cost path between these populations was then estimated using the predicted friction map. The method enabled us to avoid the subjectivity inherent in the expert-based weighting of environmental parameters. Finally, we identified potentially isolated clusters of *G. p. gambiensis* habitat based on a species distribution model and ranked them according to their predicted genetic distance to the main tsetse population. The methodology presented here will inform the choice on the most appropriate intervention strategies to be implemented against tsetse flies in different parts of Africa. It can also be used to control other pests and to support conservation of endangered species.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also 38: 17653, 17662, 17671, 17673]


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Control of *gambiense* sleeping sickness, a neglected tropical disease targeted for elimination by 2020, relies mainly on mass screening of populations at risk and treatment of
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cases. This strategy is however challenged by the existence of undetected reservoirs of parasites that contribute to the maintenance of transmission. In this study, performed in the Boffa disease focus of Guinea, we evaluated the value of adding vector control to medical surveys and measured its impact on disease burden. The focus was divided into two parts (screen and treat in the western part; screen and treat plus vector control in the eastern part) separated by the Rio Pongo river. Population census and baseline entomological data were collected from the entire focus at the beginning of the study and insecticide impregnated targets were deployed on the eastern bank only. Medical surveys were performed in both areas in 2012 and 2013. In the vector control area, there was an 80% decrease in tsetse density, resulting in a significant decrease of human tsetse contacts, and a decrease of disease prevalence (from 0.3% to 0.1%; \( p = 0.01 \)), and an almost nil incidence of new infections (< 0.1%). In contrast, incidence was 10 times higher in the area without vector control (> 1%, \( p < 0.0001 \)) with a disease prevalence increasing slightly (from 0.5 to 0.7%, \( p = 0.34 \)). Combining medical and vector control was decisive in reducing \( T. b. \) gambiense transmission and in speeding up progress towards elimination. Similar strategies could be applied in other foci.


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African trypanosomiasis, also known as "sleeping sickness" in humans and "nagana" in livestock is an important vector-borne disease in sub-Saharan Africa. Control of trypanosomiasis has focused on eliminating the vector, the tsetse fly (*Glossina* spp.). Effective tsetse fly control planning requires models to predict tsetse population and distribution changes over time and space. Traditional planning models have used statistical tools to predict tsetse distributions and have been hindered by limited field survey data. We developed an Agent-Based Model (ABM) to provide timing and location information for tsetse fly control without the presence/absence of training data. The model is driven by daily remotely-sensed environment data. The model provides a flexible tool linking environmental changes with individual biology to analyse tsetse control methods such as aerial insecticide spraying, wild animal control, releasing irradiated sterile tsetse males, and land use and cover modification. This is a bottom-up process-based model with freely available data as inputs that can be easily transferred to a new area. The tsetse population simulation more closely approximates real conditions than those using traditional statistical models making it a useful tool in tsetse fly control planning.


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Human African trypanosomiasis threatens human health across Africa. The subspecies *T. b. gambiense* is responsible for the vast majority of reported HAT cases. Over the past decade, expanded control efforts accomplished a substantial reduction in HAT transmission, spurring the WHO to include Gambian HAT on its roadmap for 2020 elimination. To inform the implementation of this elimination goal, we evaluated the likelihood that current control interventions will achieve the 2020 target in Boffa prefecture in Guinea, which has one of the highest prevalence for HAT in the country, and where vector control measures have been implemented in combination with the traditional screen and treat strategy. We developed a three-species mathematical model of HAT and used a Bayesian melding approach to calibrate the model to epidemiological and entomological data from Boffa. From the calibrated model, we generated the probabilistic predictions regarding the likelihood that the current HAT control programmes could achieve elimination by 2020 in Boffa. Our model projections indicate that if annual vector control is implemented in combination with annual or biennial active case detection and treatment, the probability of eliminating HAT as a public health problem in Boffa by 2020 is over 90%. Annual implementation of vector control alone has a significant impact but a decreased chance of reaching the objective (77%). However, if the ongoing control efforts are interrupted, HAT will continue to remain a public health problem. In the presence of a non-human transmission reservoir, intervention strategies must be maintained at high coverage, even after 2020 elimination, to prevent HAT re-emerging as a public health problem. In conclusion, complementing active screening and treatment with vector control has the potential to achieve the elimination target before 2020 in the Boffa focus. However, surveillance must continue after elimination to prevent re-emergence.


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Tsetse flies transmit trypanosomes that cause nagana in cattle, and sleeping sickness in humans. Therefore, optimising visual baits to control tsetse is an important priority. Tsetse are intercepted at visual baits due to their initial attraction to the bait, and their subsequent contact with it due to landing or accidental collision. Attraction is proposed to be driven in part by a chromatic mechanism to which a UV-blue photoreceptor contributes positively, and a UV and a green photoreceptor contribute negatively. Landing responses are elicited by stimuli with low luminance, but many studies also find apparently strong landing responses when stimuli have high UV reflectivity, which would imply that UV wavelengths contribute negatively to attraction at a distance, but positively to landing responses at close range. The strength of landing responses is often judged using the number of tsetse sampled at a cloth panel expressed as a proportion of the combined catch of the cloth panel and a flanking net that samples circling flies. I modelled these data from two previously published field studies, using calculated fly photoreceptor excitations as predictors. I found that the proportion of tsetse caught on the cloth panel increased with an index representing the chromatic mechanism driving attraction, as would be expected if the same mechanism underlay both long- and close-range attraction. However, the proportion of tsetse caught on the cloth panel also increased with excitation of the UV-sensitive R7p photoreceptor, in an apparently separate but interacting behavioural mechanism. This R7p-driven effect resembles the fly open-space response which is believed to underlie their dispersal towards areas of open sky. As such, the proportion of tsetse that contact
a cloth panel likely reflects a combination of deliberate landings by potentially host-seeking tsetse, and accidental collisions by those seeking to disperse, with a separate visual mechanism underlying each behaviour.


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Tsetse flies transmit trypanosomes that cause human and African animal trypanosomosis, a debilitating disease of humans (sleeping sickness) and livestock (nagana). An area-wide integrated pest management campaign against Glossina palpalis gambiensis has been implemented in Senegal since 2010 that includes a sterile insect technique (SIT) component. The SIT can only be successful when the sterile males that are destined for release have a flight ability, survival and competitiveness that are as close as possible to that of their wild male counterparts. Tests were developed to assess the quality of G. p. gambiensis males that emerged from pupae that were produced and irradiated in Burkina Faso and Slovakia (irradiation done in Seibersdorf, Austria) and transported weekly under chilled conditions to Dakar, Senegal. For each consignment a sample of 50 pupae was used for a quality control test (QC group). To assess flight ability, the pupae were put in a cylinder filtering emerged flies that were able to escape the cylinder. The survival of these flyers was thereafter monitored under stress conditions (without feeding). Remaining pupae were emerged and released in the target area of the eradication programme (RF group). The following parameter values were obtained for the QC flies: average emergence rate more than 69%, median survival of 6 days, and average flight ability of more than 35%. The quality protocol was a good proxy of fly quality, explaining a large part of the variances of the examined parameters. The quality protocol described here will allow the accurate monitoring of the quality of shipped sterile male tsetse used in operational eradication programmes in the framework of the Pan-African Tsetse and Trypanosomosis Eradication Campaign.

Gambian sleeping sickness (human African trypanosomiasis, HAT) outbreaks are brought under control by case detection and treatment although it is recognised that this typically only reaches about 75% of the population. Vector control is capable of completely interrupting HAT transmission but is not used because it is considered too expensive and difficult to organise in resource-poor settings. We conducted a full scale field trial of a refined vector control technology to determine its utility in control of Gambian HAT. The major vector of Gambian HAT is the tsetse fly *Glossina fuscipes* which lives in the humid zone immediately adjacent to water bodies. From a series of preliminary trials we determined the number of tiny targets required to reduce *G. fuscipes* populations by more than 90%. Using these data for model calibration we predicted we needed a target density of 20 per linear km of river in riverine savannah to achieve > 90% tsetse control. We then carried out a full scale, 500 km² field trial covering two HAT foci in Northern Uganda to determine the efficacy of tiny targets (overall target density 5.7/km²). In 12 months, tsetse populations declined by more than 90%. As a guide we used a published HAT transmission model and calculated that a 72% reduction in tsetse population is required to stop transmission in those settings. The Ugandan census suggests population density in the HAT foci is approximately 500 per km². The estimated cost for a single round of active case detection (excluding treatment), covering 80% of the population is US$ 433 333 (WHO figures). One year of vector control organised within the country, which can completely stop HAT transmission, would cost US$ 42,700. The case for adding this method of vector control to case detection and treatment is strong. We outline how such a component could be organised.

Zimbabwe, we developed catch methods for studying the efficiency of E-nets and E-cloth for tsetse, using improved transformers to supply the grids with electrical pulses of ~40kV. At energies per pulse of 35–215 mJ, the efficiency was enhanced by reducing the pulse interval from 3 200 to 1 ms. Efficiency was low at 35 mJ per pulse, but there seemed no benefit of increasing the energy beyond 70 mJ. Catches at E-nets declined when the fine netting normally used became either coarser or much finer, and increased when the grid frame was moved from 2.5 cm to 27.5 cm from the grid. Data for muscoids and tabanids were roughly comparable to those for tsetse. The catch method for studying efficiency is useful for supplementing and extending video methods. Specifications are suggested for E-nets and E-cloth that are ~95% efficient and suitable for estimating the absolute numbers of available flies. Grids that are less efficient, but more economical, are recommended for studies of relative numbers available to various baits.

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

[See also 38: 17662, 17672, 17673]


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The study investigated socio-cultural characteristics of pastoralists that influenced the tsetse-trypanosome-cattle reservoir interface, thereby predisposing them to HAT in Niger State, north-central Nigeria. It was a cross-sectional survey of adult pastoral herders aged 30 years and above, and conducted between October 2012 and February 2013. A face-to-face structured questionnaire was administered on the pastoralists associated with 96 cattle herds with questions focused on pastoralists' socio-cultural activities and behavioural practices related to HAT risk. Descriptive and analytic statistics were used to describe the obtained data. A total of 384 pastoralists participated, with mean age of 49.6 +/- 10.76 SD years. Male respondents constituted 86.7% of gender, while pastoralists of age group 40-49 years constituted 35.4% of respondents. About 59.4% of the pastoralists had knowledge about HAT and its symptoms and only 33.9% of them believed that cattle served as a reservoir of HAT. Knowledge/belief levels of the pastoralists about African trypanosomiasis occurrence in humans and animals were statistically significant. Males were four times more likely to be exposed to HAT (OR = 3.67; 95% CI: 1.42, 9.52); age group 60-69 was also four times more likely to be exposed (OR = 3.59; 95% CI: 1.56, 8.28); and nomadic pastoralists were two times more likely to be exposed to HAT (OR = 2.07; 95% CI: 1.37, 3.14). All cultural practices significantly influenced exposure to HAT with extensive husbandry system three times more likely to predispose pastoralists to HAT (OR = 3.21; 95% CI: 1.65, 6.24). Socio-cultural characteristics of pastoralists influenced exposure to HAT risk and, therefore, there is a need to sensitize them to bring changes to their socio-cultural practices and perceptions to achieve effective and long term sustainable HAT control. Elimination strategies of parasites in animals and vectors should be considered to avoid reintroduction from animal reservoirs.
Tsetse and Trypanosomosis Information


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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.


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Trypanosomiasis is a neglected tropical disease caused by the trypanosome parasite and transmitted by the tsetse fly vector. In sub-Saharan Africa, both the human and animal variants of the disease are a great obstacle towards agriculture, development, and health. In order to better understand and therefore combat trypanosomiasis, characterizing disease hotspots across species is critical. In this study, 193 samples from cattle, sheep, and goats were collected from eight sites. Samples were taken from animals belonging mostly to Maasai herdsmen in the Ngorongoro Crater Conservation Area (NCA) and analysed for the presence of trypanosomiasis infection using PCR techniques. Those that tested positive for the *T. brucei* parasite were further tested using the SRA LAMP technique to check for *T. brucei rhodesiense*, the human infective subspecies of parasite. Our study found a high incidence of *Trypanosoma brucei* infections across species. Of animals tested, 47% of cattle, 91.7% of sheep, and 60.8% of goats were infected. Most of the infections were of the *T. brucei* species. We also identified sheep and goats as carriers of the *T. brucei rhodesiense* subspecies, which causes acute human trypanosomiasis. Together, these results point toward the need for stricter control strategies in the area to prevent disease outbreaks.
A cross-sectional study was conducted in nine selected districts of the southern part of the Rift Valley, Ethiopia to estimate the dry period prevalence of bovine trypanosomosis as well as assessment of *Glossina* species. From a total of 1,838 cattle examined for trypanosomosis by the buffy coat technique, 133 (7.2%) were found to be infected by trypanosome species. From the total positive animals 66.9 and 33.1% of them were due to *Trypanosoma congolense* and *Trypanosoma vivax*, respectively. A significantly higher prevalence (19.4%, p < 0.05) was recorded in Arba-Minch district. Black coloured cattle were the most highly affected animals ($\chi^2 = 79.35$, $p < 0.05$). The overall average PCV values for parasitaemic and aparasitaemic animals were 22.2% (95% CI = 21.6–22.7) and 27% (95% CI = 26.8–27.2), respectively. The number of flies caught per trap per day were 1.4 for *Glossina* species and 2.8 for other biting flies. Two species of *Glossina* were identified, namely *Glossina pallidipes* and *Glossina fuscipes*.

The Bafia sleeping sickness focus of Cameroon is considered as "silent" with no case reported for about 20 years despite medical surveys performed during the last decades. In this focus, all epidemiological factors that can contribute to trypanosomes transmission are present. To update our knowledge on the current risks of human and animal African trypanosomiases, different trypanosome species were identified in midguts of tsetse flies captured in the Bafia focus. Tsetse flies were trapped using pyramidal traps. Each tsetse fly was identified and live flies were dissected and their midguts collected. DNA was extracted from each midgut and thereafter, blood meals and different trypanosome species were identified with molecular tools. The biological data were transported onto maps in order to record their distribution. Of the 98 traps set up, 461 *Glossina palpalis palpalis* were captured; 322 (69.8 %) tsetse flies were dissected and 49 (15.2 %) tenereal flies identified. The average apparent density of tsetse flies per day was 1.18. Of the 35 (10.9 %) blood meals collected, 82 % were taken on pigs and 17.6 % on humans. Eighty-two (25.5 %) trypanosome infections were identified: 56 (17.4%) *T. congolense* savannah, 17 (5.3%) *T. congolense* forest, 5 (1.6%) *T. vivax* and 4 (1.2%) *T. brucei*.
No infections of *T. simiae* and *T. b. gambiae* were identified. Sixty-seven (81.7%) infections were single and 15 (18.3%) mixed involving one triple infection (*T. congolense* forest, *T. brucei* and *T. vivax*) and 14 double infections: 11 *T. congolense* forest and *T. congolense* savannah, two *T. congolense* savannah and *T. brucei*, and one of *T. brucei* and *T. vivax*. The generated maps show the distribution of tsetse flies and trypanosome infections across the focus. This study has shown that animal trypanosomes remain an important problem in this region. Meanwhile, it is very likely that HAT no longer seems to be a public health problem in this focus. The generated maps enabled us to define high risk transmission areas for AAT, and where disease control must be focused in order to improve animal health as well as the quantity of animal proteins.


The *gambiae* form of sleeping sickness is a neglected tropical disease, which is presumed to be anthroponotic. However, the parasite persists in human populations at levels of considerable rarity and as such the existence of animal reservoirs has been suspected. Clarifying the impact of animal host reservoirs on the feasibility of interrupting sleeping sickness transmission through interventions is a matter of urgency. We developed a mathematical model allowing for heterogeneous exposure of humans to tsetse, with animal populations that differed in their ability to transmit infections, to investigate the effectiveness of two established techniques–screening and treatment of at-risk populations, and vector control. Importantly, under both assumptions, an integrated approach of human screening and vector control was supported in high transmission areas. However, increasing the intensity of vector control was more likely to eliminate transmission, while increasing the intensity of human screening reduced the time to elimination. Animal hosts played important, but different roles in HAT transmission, depending on whether or not they contributed as reservoirs. If they did not serve as reservoirs, sensitivity analyses suggested their attractiveness may instead function as a sink for tsetse bites. These outcomes highlight the importance of understanding the ecological and environmental context of sleeping sickness in optimizing integrated interventions, particularly for moderate and low transmission intensity settings.


The gambiense form of sleeping sickness is a neglected tropical disease, which is presumed to be anthropogetic. However, the parasite persists in human populations at levels of considerable rarity and as such the existence of animal reservoirs has been suspected. Clarifying the impact of animal host reservoirs on the feasibility of interrupting sleeping sickness transmission through interventions is a matter of urgency. We developed a mathematical model allowing for heterogeneous exposure of humans to tsetse, with animal populations that differed in their ability to transmit infections, to investigate the effectiveness of two established techniques–screening and treatment of at-risk populations, and vector control. Importantly, under both assumptions, an integrated approach of human screening and vector control was supported in high transmission areas. However, increasing the intensity of vector control was more likely to eliminate transmission, while increasing the intensity of human screening reduced the time to elimination. Animal hosts played important, but different roles in HAT transmission, depending on whether or not they contributed as reservoirs. If they did not serve as reservoirs, sensitivity analyses suggested their attractiveness may instead function as a sink for tsetse bites. These outcomes highlight the importance of understanding the ecological and environmental context of sleeping sickness in optimizing integrated interventions, particularly for moderate and low transmission intensity settings.
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Tsetse and tabanid flies transmit several *Trypanosoma* species, some of which are human and livestock pathogens of major medical and socioeconomic impact in Africa. Recent advances in molecular techniques and phylogenetic analyses have revealed a growing diversity of previously unidentified tsetse-transmitted trypanosomes potentially pathogenic to livestock and/or other domestic animals as well as wildlife, including African great apes. To map the distribution, prevalence and co-occurrence of known and novel trypanosome species, we analysed tsetse and tabanid flies collected in the primary forested part of the Dzanga-Sangha Protected Areas, Central African Republic, which hosts a broad spectrum of wildlife including primates and is virtually devoid of domestic animals. Altogether, 564 tsetse flies and 81 tabanid flies were individually screened for the presence of trypanosomes using 18S rRNA-specific nested PCR. Here, we demonstrate that wildlife animals are parasitized by a surprisingly wide range of trypanosome species that in some cases may circulate via these insect vectors. While one-third of the examined tsetse flies harboured trypanosomes either from the *Trypanosoma theileri*, *Trypanosoma congoense* or *Trypanosoma simiae* complex, or one of the three new members of the genus *Trypanosoma* (strains “Bai”, “Ngbanda” and “Didon”), more than half of the tabanid flies exclusively carried *T. theileri*. To establish the putative vertebrate hosts of the novel trypanosome species, we further analysed the provenance of blood meals of tsetse flies. DNA individually isolated from 1 033 specimens of *Glossina* spp. and subjected to high-throughput library-based screening proved that most of the examined tsetse flies engorged on wild ruminants (buffalo, sitatunga, bongo), humans and suids. Moreover, they also fed (albeit more rarely) on other vertebrates, thus providing indirect but convincing evidence that trypanosomes can be transmitted via these vectors among a wide range of warm- and cold-blooded hosts.

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

[See also 38: 17674, 17652, 17654]


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Human African trypanosomiasis (HAT) is a neglected tropical disease affecting poor rural communities living in tsetse-infested regions of sub-Saharan Africa. In Zambia, sporadic cases of HAT have been reported mainly in the old foci along the tsetse-infested Luangwa river valley in north-eastern part of the country. In such places where malaria is the major endemic febrile disease, with possibilities of co-infections of HAT and malaria and where the levels of alertness to the presence of HAT among health care personnel (HCP) are low, there is a high chance of misdiagnosing HAT for malaria because of their similarities in clinical presentation. This study, conducted in Zambia's tsetse-infested rural health centres (RHCs) of Chama and Mambwe districts, was designed to investigate the staffing levels, the HCP levels of alertness
to the occurrence of HAT and their capacity to detect the disease. Structured questionnaires were used to collect information pertaining to HAT alertness and the capacity to detect the disease from 101 HCPs in a cross-sectional study of 23 RHCs drawn from Zambia's Chama and Mambwe districts between April and July 2013. The data collected were analysed using Stata/SE version 11.0. Participants from both Chama and Mambwe district RHCs reported similar very low levels of qualified HCPs and laboratory technicians, and that they had similar basic tools for HAT diagnosis. Although not statistically significant, respondents from Chama (~89%) tended to be more aware about the occurrence of HAT compared with their Mambwe counterparts (~78%). Whereas ~40% of the HCPs from Chama district (n = 52) claimed to have encountered at least one case of HAT, only ~4% of their Mambwe counterparts (n = 49) had similar experiences. Health care personnel in RHCs from Chama tended to be more alert to the occurrence of HAT than the HCP from Mambwe district. The extremely low levels of specialized HCPs, general absence of functional laboratories, coupled with absence of national HAT surveillance and control programmes, are among some of the serious challenges that Zambia's Chama and Mambwe districts face to control/eliminate HAT.

(b) PATHOLOGY AND IMMUNOLOGY


Apolipoprotein L1 gene (APOL1) G1 and G2 variants are strongly associated with progressive nondiabetic 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 nondiabetic ESRD, 965 African Americans with type 2 diabetes-associated ESRD, and 1029 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 nondiabetic 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 nondiabetic nephropathy susceptibility.
(c) TREATMENT


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The virulent vector-borne disease, Gambian human African trypanosomiasis (HAT), is one of several diseases targeted for elimination by the World Health Organization. This article utilises human case data from a high-endemicity region of the Democratic Republic of Congo in conjunction with a suite of novel mechanistic mathematical models to address the effectiveness of on-going active screening and treatment programmes and compute the likely time to elimination as a public health problem (i.e. < 1 case per 10 000 per year). The model variants address uncertainties surrounding transmission of HAT infection including heterogeneous risk of exposure to tsetse bites, non-participation of certain groups during active screening campaigns and potential animal reservoirs of infection. Model fitting indicates that variation in human risk of tsetse bites and participation in active screening play a key role in transmission of this disease, whilst the existence of animal reservoirs remains unclear. Active screening campaigns in this region are calculated to have been effective, reducing the incidence of new human infections by 52–53% over a 15-year period (1998–2012). However, projections of disease dynamics in this region indicate that the elimination goal may not be met until later this century (2059–2092) under the current intervention strategy. Improvements to active detection, such as screening those who have not previously participated and raising overall screening levels, as well as beginning widespread vector control in the area have the potential to ensure successful and timely elimination.

6. **ANIMAL TRYPANOSOMOSIS**

(a) **SURVEY AND DISTRIBUTION**

[See also 38: 17683, 17684, 17698]


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Livestock trypanosomiasis, transmitted mainly by tsetse flies of the genus *Glossina* is a major constraint to livestock health and productivity in sub-Saharan Africa. Knowledge of the prevalence and intensity of trypanosomiasis is important in understanding the epidemiology of the disease. The objectives of this study were to (a) assess the prevalence and intensity of trypanosome infections in cattle, and (b) to investigate the reasons for the heterogeneity of the disease in the tsetse infested districts of Amuru and Nwoya, northern Uganda. A cross-sectional study was conducted from September, 2011 to January, 2012. Blood samples were collected from 816 cattle following jugular vein puncture, and screened for trypanosomes by HCT and ITS-PCR. A Pearson chi-squared test and logistic regression analyses were performed to determine the association between location, age, sex, and prevalence of trypanosome infections. Out of the 816 blood samples examined, 178 (22%) and 338 (41%) tested positive for trypanosomiasis by HCT and ITS-PCR, respectively. *Trypanosoma vivax* infection accounted for 77% of infections detected by ITS-PCR, *T. congolense* (16%), *T. brucei* s.l (4%) and mixed (*T. vivax/ T. congolense/T. brucei*) infections (3%). The risk of trypanosome infection was significantly associated with cattle age (chi$^2 = 220.4$, df = 3, $p < 0.001$). The highest proportions of infected animals were adult males (26.7%) and the least infected were the less than one year old calves (2.0%). In addition, the risk of trypanosome infection was significantly associated with sex (chi$^2 = 16.64$, df = 1, $p < 0.001$), and males had a significantly higher prevalence of infections (26.8%) than females (14.6%). Our results indicate that the prevalence and intensity of trypanosome infections are highly heterogeneous being associated with cattle age, location and sex.


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Bovine African trypanosomosis (BAT) remains one of the major vector-borne diseases with serious impediment to cattle production and economic advancement in sub-Saharan Africa. The present study evaluated the performance of the trypanosome-species-specific loop-mediated isothermal amplification (LAMP), using parasite DNA obtained from 295 indigenous Tanzanian short horn Zebu (TSHZ) and Boran crosses in Monduli district within northern Tanzania, against routine microscopy on Giemsa-stained blood films. Compared with parasitological data in which the prevalence of BAT was estimated at 2.4% (95% CI 0.7-4.1%), LAMP increased the prevalence to 27.8% (95% CI 22.3-32.5%), of which 11.9% (95% CI 8.2-15.6%) were monolytic infections with *Trypanosoma vivax*, while 13.6% (95% CI 9.7-17.5%) were coinfections of either *T. vivax* and *Trypanosoma brucei* subspecies or *T. vivax* and *Trypanosoma congolense*, respectively. Among the *T. brucei* subspecies detected, 0.7% (95% CI 0-1.7%) were human-infective *Trypanosoma brucei rhodesiense*. Our study is in concordance with previous reports and suggests that LAMP is a potential tool for routine diagnosis of trypanosomes in domestic animals in BAT endemic regions. According to LAMP, *T. vivax* seems to be the predominant trypanosome species circulating among the indigenous Monduli cattle. Importantly, the detection of *T. b. rhodesiense* in cattle in such wildlife-domestic animal-human-interface areas poses a risk of contracting human African trypanosomiasis (HAT) by local communities and tourists. Continuous trypanosome
surveillances in domestic animals, humans, and tsetse flies using sensitive and specific tests such as LAMP are recommended.


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The present study, conducted in Zambia's Luangwa valley where both animal African trypanosomiasis (AAT) and human African trypanosomiasis (HAT) are endemic, combined the use of microscopy and molecular techniques to determine the presence of trypanosome species in cattle, goats and tsetse flies. This study was conducted between 2008 and 2010 in Petauke, Chama and Isoka districts, north-eastern Zambia. A total of 243 cattle, 36 goats and 546 tsetse flies, were examined for the presence of trypanosome species using microscopy, PCR and loop-mediated isothermal amplification (LAMP). There was poor agreement among the test methods used for detection of trypanosome species in animal blood and tsetse flies. Trypanosomes were observed in 6.1% (95% CI: 3.3-8.9%) of the animals sampled by microscopy, 7.5% (95% CI: 4.4-10.6%) by PCR and 18.6% (95% CI: 13.6-23.6%) by PFR-LAMP. PFR-LAMP was more sensitive for detecting Trypanozoon than KIN-PCR. The highest occurrence of AAT was recorded in cattle from Petauke (58.7%, 95% CI: 44.7-72.7%) while the lowest was from Isoka (5.4%, 95% CI: 0.8-10.0%). Infection of both cattle and goats with Trypanosoma congolense and T. vivax was associated with clinical AAT. When selecting molecular techniques for AAT surveillance in endemic regions, the KIN-PCR and species-specific PCR may be recommended for screening animal or tsetse fly samples for T. congolense and T. vivax, respectively. On the other hand, species-specific PCR and/or LAMP might be of greater value in the screening of animal and human body fluids as well as tsetse fly samples for Trypanozoon.


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A study was conducted in Tororo District in eastern Uganda to assess the socio-economic contribution of draft cattle to rural livelihoods. The aim of the study was to empirically quantify the economic value of draft cattle thus contributing to understanding the impact of endemic parasitic diseases of cattle on livestock productivity and subsequently household income, labor and food security. A total of 205 draft cattle keeping households were randomly selected and structured household questionnaires were administered, focusing on work oxen use, productivity, inputs and outputs. The data obtained was analyzed using standard statistical methods and used to calculate the gross margin from the draft cattle enterprise. Secondary data were obtained from focus group discussions and key informant interviews and these were analyzed using Bayesian methods. The study showed that, apart from being labor saving, the use of animal traction is highly profitable with the gross margin per year from the use of draft cattle amounting to US$ 245 per work oxen owning household. The cash obtained from hiring out draft animals was equivalent to nearly a quarter of the average local household’s monetary receipts. It also revealed that endemic bovine parasitic diseases such as trypanosomiasis and tick-borne diseases reduced draft cattle output by 20.9% and potential household income from the use of draft oxen by 32.2%. The presence of endemic cattle diseases in rural Uganda is adversely affecting the productivity of draft cattle, which in turn affects household income, labour and ultimately food security. This study highlights the contribution of draft cattle to rural livelihoods, thus increasing the expected impact of cost-effective control strategies of endemic production limiting livestock diseases in Uganda.


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A study was made to determine the prevalence of camel trypanosomosis (surra) and its associated risk factors in Borena zone, southern Ethiopia during 2013-2014. A total of 2 400 blood samples were collected and examined by the buffy coat and thin blood film laboratory methods, and data were analysed using the SPSS statistical software. The overall prevalence of camel trypanosomosis in the area was found to be 2.33%. Prevalence was significantly different among the surveyed districts (p = 0.000), the pastoral associations (F = 6.408, p = 0.000), altitudinal divisions (p = 0.000), age groups (p = 0.034), and between animals possessing packed cell volume (PCV) values greater than 25% and less than 25% (p = 0.000); whereas, prevalence of the disease was not statistically significantly different between the sexes (p = 0.311) and among the body condition score groups (p = 0.739). The PCV of trypanosome positive and trypanosome negative camels differed significantly (p = 0.001), and prevalence of trypanosomosis was seen to be negatively correlated with packed cell volume (r = -0.069, p = 0.000) revealing the effect of camel trypanosomosis on the anaemic state of parasitized animals. In conclusion, camel trypanosomosis is a serious and economically important disease hampering camel production and productivity in southern Ethiopia. Further studies involving more sensitive molecular techniques to reveal the precise magnitude of the disease and to identify the vector species of the parasite are recommended.
In Senegal, several areas provide great potential for agriculture and animal production, but African animal trypanosomosis (AAT) is one of the major constraints to the development of more effective livestock production systems. A study was conducted to assess the current situation of AAT in this country. Surveys were carried out between June 2011 and September 2012 in four different areas: Dakar, Sine Saloum, Kedougou region and Basse Casamance in several animal species: dogs (152), donkeys (23), horses (63), sheep (43), goats (52) and cattle (104), distributed in the four sites. Molecular tools (PCR) indicated 3.4% positive animals including dogs, donkeys, a goat and cattle. The savannah type of *Trypanosoma congolense* Broden, 1904 (53% of positive cases) and the forest type of *T. congolense* (subgenus Nannomonas Hoare, 1964) were predominant. *Trypanosoma vivax* Ziemann, 1905 (subgenus Duttonella Chalmers, 1918) was only present in one animal and no trypanosome of the subgenus *Trypanozoon* Luhe, 1906 was found. Half of the positive cases were detected in Sine Saloum, where *T. congolense* savannah-type was predominant, and the other half in Basse Casamance, where *T. congolense* forest-type was predominant; no cases were found in Dakar or in the Kedougou region. A high risk of infection in dogs with *T. congolense* savannah-type was shown in Sine Saloum, requiring prevention and control of dogs in this area. The involvement of tsetse flies in the transmission of *T. congolense* in Sine Saloum and Basse Casamance is discussed.

The Brazilian semiarid region is the home of the largest herd of donkeys in South America and of outbreaks of *Trypanosoma vivax* infection of high mortality in dairy cattle and sheep. For a comprehensive understanding of the underlying mechanisms of these outbreaks and the epidemiological role of donkeys, we surveyed for *T. vivax* in wandering donkeys and followed experimental infections of donkeys and sheep with a highly virulent isolate from the semiarid region. Blood samples from 180 randomly selected wandering donkeys from the...
Brazilian semiarid region were employed for PCV and parasitaemia assessments and tested using the *T. vivax*-specific TviCATL-PCR assay. PCR-amplified cathepsin L (CATL) sequences were employed for genotyping and phylogenetic analyses. Four wandering donkeys were experimentally infected with a *T. vivax* isolate obtained during an outbreak with high mortality in the semiarid region; the control group consisted of two non-inoculated donkeys. We detected *T. vivax* in 30 of 180 wandering donkeys (16.6%) using TviCATL-PCR. The prevalence was higher during the dry (15.5%) than the wet season (1.1%) and more females (23.1%) than males (8.9%) were infected. All the PCR-positive donkeys lacked patent parasitaemia and showed normal values of body condition score (BCS) and packed cell volume (PCV). To evaluate the probable tolerance of donkeys to *T. vivax*, we inoculated five donkeys with a highly virulent isolate (TviBrRp) from the semiarid region. All inoculated donkeys became PCR-positive, but their parasitaemia was always subpatent. A control goat inoculated with TviBrRp showed increasing parasitaemia concurrently with fever, declining PCV, tachycardia, mucous membrane pallor, enlarged lymph nodes and anorexia. None of these signs was observed in donkeys. However, *T. vivax* from wandering donkeys shared identical or highly similar genotypes (identified by cathepsin L sequences) with isolates from cattle and sheep outbreaks of acute disease in the semiarid region. This is the first report of *T. vivax* in donkeys in Brazil and, to our knowledge, the first experimental infection of donkeys with *T. vivax*. The symptomless field and experimental infections indicated that donkeys are more tolerant to *T. vivax* than other livestock species as shown in African countries. Therefore, farmers, veterinaries and those involved in control programmes should be aware of healthy carrier donkeys as a possible source of *T. vivax* for susceptible livestock species in the Brazilian semiarid region.

(b) PATHOLOGY AND IMMUNOLOGY

[See also 38: 17684, 17695, 17697]


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The measure of anaemia status using packed cell volume (PCV) can be a reliable indicator of African trypanosomosis (AT) in the absence of other anaemia-causing conditions. However, studies that have estimated prevalence of anaemia in cattle from AT endemic areas have rarely reported the prevalence of the disease in the anaemic cattle. Therefore we investigated the prevalence of AT in anaemic cattle at sites that had recently reported the disease in Itezhi-Tezhi district of central Zambia. During a survey, blood samples were collected from 564 randomly selected cattle for anaemia determination from seven crush pens (Mutenda, Kapulwe, Banachoongo, Itumbi, Iyanda, New Ngoma and Shinampamba). At a PCV value cutoff of 26%, all samples positive for anaemia were subjected to both parasitological examination on thick and thin blood smears and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for detection of trypanosome DNA. Fisher's exact test and a mixed
effect logistic regression analysis were used to determine and measures associations, respectively. Of 564 cattle screened, 58 (10.3%; 95% CI: 7.8-12.8%) had anaemia. PCR-RFLP results showed that 17 (29.3%; 95% CI: 17.2-41.4%) anaemic cattle were positive for pathogenic trypanosomes compared with 1 (1.7%; 95% CI: 0.0-5.2%) on parasitological examination using thick smears. The infections were caused by *Trypanosoma congolense* and *Trypanosoma vivax*. Fisher's exact test showed a strong association between PCV and pathogenic trypanosome infection ($p = 0.004$). A mixed effect multivariate logistic regression showed that a one unit increase in PCV reduced the likelihood of detecting AT with PCR-RFLP by 24.7% (95% CI: 4.6-40.6%; $p = 0.019$) in anaemic cattle, taking into account their age and sex, with random effects for crush pen. These results suggest that *T. congolense* and *T. vivax* could be important causes of anaemia in cattle reared in AT endemic areas of Itezhi-Tezhi in Central Zambia. This also suggests that even though pathogenic trypanosomal infection was strongly associated with PCV, it could only account for up to 41% of the anaemia in cattle. Therefore further investigation to ascertain other factors responsible for anaemia in AT endemic areas of Itezhi tezhi in Central Zambia is needed.

(c) TRYPANOTOOLERANCE

(d) TREATMENT


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*Trypanosoma evansi* (*T. evansi*), the protozoan parasitic cause of camel trypanosomosis (Surra), constitutes one of the major veterinary problems worldwide. An infectious disease model of camel trypanosomosis (Surra) was adopted from one developed for buffalo and applied to study the impact of *T. evansi* infection on camel production. The model contained deterministic and stochastic components and the seroprevalence based on a survey conducted in Somaliland in 2011 and 2012 to simulate and estimate the economic benefits of four different control options against *T. evansi* infection in camels (1, 2, 3 and 4 regimens). The mean benefit per animal of controlling surra was calculated at US$354 (the treatment of all camels biannually), US$426 (the monthly targeted treatment of clinically sick camels) and US$287 (biannual targeted treatment of seropositive camels), respectively, compared with US$137 for untreated camels. Consequently, the model predicted that the total net benefit loss to a camel herd or to a village that was not applying the recommended effective surra control strategy was US$115 605.


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African trypanosomiasis of humans and animals can be controlled by attacking the vectors, various species of tsetse fly. Treatment of cattle with pyrethroids to kill tsetse as they feed is the most cost-effective method. However, such treatments can contaminate cattle dung, thereby killing the fauna which disperse the dung and so play an important role in soil fertility. Hence there is a need to identify cost-effective methods of treating cattle with minimal impact on dung fauna. We used dung beetles to field bioassay the levels of dung contamination following the use of spray and pour-on formulations of deltamethrin, applied to various parts of the body of cattle in Zimbabwe. Results suggested that dung was contaminated by contact with insecticide on the body surface as the cattle defaecated, and by ingestion of insecticide as the cattle licked themselves. Death of dung beetles was reduced to negligible levels by using only the spray and applying it to the legs and belly or legs alone, i.e. places where most tsetse feed. The restricted applications suitable for minimising the impact on dung fauna have the collateral benefits of improving the economy and convenience of cattle treatments for tsetse control. The demonstration of collateral benefits is one of the surest ways of promoting environmentally friendly procedures.

7. EXPERIMENTAL TRYPANOSOMOSIS

(a) DIAGNOSTICS


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*Trypanosoma vivax* affects cattle herds in Africa and the Americas and has been spreading rapidly in Brazil through introduction of animals with subclinical infections and without apparent parasitaemia, which makes its diagnosis challenging. PCR and LAMP are effective in detecting the presence of *T. vivax* DNA in situations of low parasitaemia. LAMP is a simpler and faster technique than PCR, and can be performed in the field with limited resources. In this study, the capacities of conventional PCR and LAMP for detecting *T. vivax* in bovine blood samples classified as aparasitaemic were evaluated. The capacity of conventional PCR (56.25%) for detecting positive samples was lower than that of LAMP (93.73%). This may influence the choice of screening tests for cattle herds infected with *T. vivax*.


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The present immuno-diagnostic method using soluble antigens from whole cell lysate antigen for trypanosomosis have certain inherent problems like lack of standardized and reproducible antigens, as well as ethical issues due to in vivo production, that could be alleviated by in vitro production. In the present study we have identified heat shock protein 70 (HSP70) from the *T. evansi* proteome. The nucleotide sequence of *T. evansi* HSP70 was 2 116 bp, which encodes 690 amino acid residues. The phylogenetic analysis of *T. evansi* HSP70 showed that *T. evansi* occurred within the *Trypanosoma* clade and is most closely related to *T. brucei brucei* and *T. brucei gambiense*, whereas *T. congolense* HSP70 lies in a separate clade. The two partial HSP70 sequences (HSP-1 from the N-terminal region and HSP-2 from the C-terminal region) were expressed and evaluated as diagnostic antigens using experimentally infected equine serum samples. Both recombinant proteins detected antibody in immunoblots using serum samples from donkeys experimentally infected with *T. evansi*. Recombinant HSP-2 showed comparable antibody response to whole cell lysate (WCL) antigen in immunoblot and ELISA tests. These initial results indicate that HSP70 has potential to detect *T. evansi* infection and needs further validation on a large set of equine serum samples.


Human African trypanosomiasis (HAT) remains a major neglected tropical disease in sub-Saharan Africa. As clinical symptoms are usually non-specific, new diagnostic and prognostic markers are urgently needed to enhance the number of identified cases and optimise treatment. This is particularly important for disease caused by *Trypanosoma brucei rhodesiense*, where indirect immunodiagnostic approaches have to date been unsuccessful. We have conducted global metabolic profiling of plasma from *T. b. rhodesiense* HAT patients and endemic controls, using 1H nuclear magnetic resonance (NMR) spectroscopy and ultra-performance liquid chromatography, coupled with mass spectrometry (UPLC-MS) and identified differences in the lipid, amino acid and metabolite profiles. Altogether 16 significantly disease discriminatory metabolite markers were found using NMR, and a further 37 lipid markers via UPLC-MS. These included significantly higher levels of phenylalanine, formate, creatinine, N-acetylated glycoprotein and triglycerides in patients relative to controls. HAT patients also displayed lower concentrations of histidine, sphingomyelins, lysophosphatidylcholines, and several polyunsaturated phosphatidylcholines. While the disease metabolite profile was partially consistent with previous data published in experimental rodent infection, we also found unique lipid and amino acid profile markers highlighting subtle but important differences between the host response to trypanosome infections between animal models and natural human infections. Our results demonstrate the potential of metabolic profiling in the identification of novel diagnostic biomarkers and the elucidation of pathogenetic mechanisms in this disease.
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A third of African Americans with sporadic focal segmental glomerulosclerosis (FSGS) or HIV-associated nephropathy (HIVAN) do not carry APOL1 renal risk genotypes. This raises the possibility that other APOL1 variants may contribute to kidney disease. To address this question, we sequenced all APOL1 exons in 1437 Americans of African and European descent, including 464 patients with biopsy-proven FSGS/HIVAN. Testing for association with 33 common and rare variants with FSGS/HIVAN revealed no association independent of strong recessive G1 and G2 effects. Seeking additional variants that might have been under selection by pathogens and could represent candidates for kidney disease risk, we also sequenced an additional 1112 individuals representing 53 global populations. Except for G1 and G2, none of the 7 common codon-altering variants showed evidence of selection or could restore lysis against trypanosomes causing human African trypanosomiasis. Thus, only APOL1 G1 and G2 confer renal risk, and other common and rare APOL1 missense variants, including the archaic G3 haplotype, do not contribute to sporadic FSGS and HIVAN in the US population. Hence, in most potential clinical or screening applications, our study suggests that sequencing APOL1 exons is unlikely to bring additional information compared with genotyping only APOL1 G1 and G2 risk alleles.

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Animal trypanosomosis is a disease that is distributed worldwide and results in huge economic losses due to reduced animal productivity. Endemic regions are often located in the countryside where laboratory diagnosis is costly or inaccessible. The establishment of simple, effective, and accurate field tests is therefore of great interest to the farming and veterinary sectors. Our study aimed to develop a simple, rapid, and sensitive immunochromatographic test
(ICT) for animal trypanosomosis utilizing the recombinant tandem repeat antigen TeGM6-4r, which is conserved amongst salivarian trypanosome species. In the specificity analysis, TeGM6-4r/ICT detected all Trypanosoma evansi-positive controls from experimentally infected water buffaloes. As expected, uninfected controls tested negative. All sera samples collected from Tanzanian and Ugandan cattle that were Trypanosoma congolense- and/or Trypanosoma vivax-positive by microscopic examination of the buffy coat were found to be positive by the newly developed TeGM6-4r/ICT, which was comparable to results from TeGM6-4r/ELISA (kappa coefficient = 0.78). TeGM6/ICT also showed substantial agreement with ELISA using Trypanosoma brucei brucei (kappa = 0.64) and T. congolense (kappa = 0.72) crude antigen, suggesting the high potential of TeGM6-4r/ICT as a field diagnostic test, both for research purposes and on-site diagnosis of animal trypanosomosis.


The development of rapid serodiagnostic tests for sleeping sickness and other diseases caused by kinetoplastids relies on the affordable production of parasite-specific recombinant antigens. Here, we describe the production of recombinant antigens from Trypanosoma brucei gambiense (T. b. gambiense) in the related species Leishmania tarentolae (L. tarentolae), and compare their diagnostic sensitivity and specificity to native antigens currently used in diagnostic kits against a panel of human sera. A number of T. b. gambiense protein antigen candidates were chosen for recombinant expression in L. tarentolae based on current diagnostics in field use and recent findings on immunodiagnostic antigens found by proteomic profiling. In particular, the extracellular domains of invariant surface glycoprotein 65 (ISG65), variant surface glycoproteins VSG LiTat 1.3 and VSG LiTat 1.5 were fused with C-terminal histidine tags and expressed as soluble proteins in the medium of cultured, recombinant L. tarentolae. Using affinity chromatography, on average 10 mg/L of recombinant protein was purified from cultures and subsequently tested against a panel of sera from sleeping sickness patients and from controls, i.e. persons without sleeping sickness living in HAT endemic countries. The evaluation on sera from 172 T. b. gambiense human African trypanosomiasis (HAT) patients and from 119 controls showed very high diagnostic potential of the two recombinant VSG and the rISG65 fragments with areas under the curve between 0.97 and 0.98 compared with 0.98 and 0.99 with native VSG LiTat 1.3 and VSG LiTat 1.5 (statistically not different). Evaluation of sera from 78 T. b. rhodesiense HAT patients and from 100 controls showed an acceptable diagnostic potential of rISG65 with an area under the curve of 0.83. These results indicate that a combination of these recombinant antigens has the potential to be used in next generation rapid serodiagnostic tests. In addition, the L. tarentolae expression system enables simple, cheap and efficient production of recombinant kinetoplastid proteins for use in diagnostic, vaccine and drug discovery research that does not rely on animal use to generate materials.
(b) PATHOLOGY AND IMMUNOLOGY

[See also 38: 17651, 17655]


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Trypanosoma evansi is an important pathogen that causes changes in nitric oxide (NO) levels and antioxidant enzymes, as well as oxidative stress. The present study evaluated the in vivo effect of T. evansi infection on the frequency and index of DNA damage in liver, heart, spleen and total blood of rats. Twenty rats were assigned into two groups with ten rats each, being subdivided into four subgroups (A1 and A2, 5 animals/group; and B1 and B2, 5 animals/group). Rats in the subgroups A1 and A2 were used as control (uninfected) and animals in the subgroups B1 and B2 were inoculated with T. evansi (infected). NO in serum and the comet assay were used to measure DNA damage index (DI) and damage frequency (DF) in liver, heart, spleen and total blood of infected rats. Increased NO levels on days 3 and 9 post-infection (PI) were observed (p < 0.001). Also, increases in DI and DF were recorded in the evaluated organs on days 3 and 9 PI (p < 0.001). Our data show that T. evansi infection causes genotoxicity due to the production of NO, causing not only the death of the protozoan, but also inducing DNA damage in the host.


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Trypanosomes infect humans and animals throughout the African continent. These parasites maintain chronic infections by various immune evasion strategies. While antigenic variation of their surface coat is the most studied strategy linked to evading the host humoral response, African trypanosomes also induce impaired B-cell lymphopoiesis, the destruction of the splenic B-cell compartment and abrogation of protective memory responses. Here we investigate the mechanism of follicular B-cell destruction. We show that during infection follicular B cells undergo apoptosis, correlating to enhanced Fas death receptor surface expression. Investigation of various type 1 cytokine knockout mice indicates a crucial role of
IFN-gamma in the early onset of FoB cell destruction. Indeed, both IFN-gamma(-/-) and IFN-gammaR(-/-) mice are protected from trypanosomosis-associated FoB cell depletion, exhibiting an inhibition of B-cell apoptosis as well as a reduced activation of FoB cells during the first week post-infection. The data presented here offer new insights into B-cell dysfunction during experimental African trypanosome infections.


Ethiopia, particularly in the northwest region, is affected by both tsetse and non-tsetse fly transmitted trypanosomosis, with significant impacts on livestock productivity. The aim of this study was to determine and compare clinical findings and haematological values between experimental infections induced by Trypanosoma vivax isolates from areas of either transmission mode. Sixteen young (aged between 6 and 12 months) Zebu cattle (Bos indicus), purchased from a trypanosome-free area and confirmed to be trypanosome-negative, were randomly assigned into four groups each of four animals. Groups 1, 2 and 3 were infected with an isolate from a tsetse infested or one of two isolates from a non-tsetse infested area, and group 4 was a non-infected control. All animals in the infected groups were inoculated intravenously with 2 x 10^6 trypanosomes from donor animals. The experimental animals were monitored for eight consecutive weeks post infection for clinical signs, parasitaemia and haematological changes in packed cell volume (PCV), haemoglobin concentration (Hb), total red blood cell (RBC) and white blood cell (WBC) counts, differential WBC count and blood indices (mean corpuscular volume [MCV], and mean corpuscular haemoglobin concentration). Infection was characterized by reduced feed intake, weakness, pyrexia, parasitaemia, rough hair coat, enlarged prescapular lymph nodes, lacrimation, weight loss, pallor of the mucus membrane and dehydration. Body weight loss in all infected groups was significantly higher than in the non-infected control. Similarly, body weight loss was higher (p < 0.001) in animals infected with the tsetse infested isolate than with the non-tsetse infested isolates. The mean PCV, Hb, total RBC and WBC counts were lower (p < 0.001), and the mean MCV was higher (p = 0.01) in all infected groups than in non-infected control animals at different time points during the study period. Except for minor variations in haematological values, the overall changes were similar in all infected groups. Clinical signs and significant reduction in haematological values in the infected groups indicated the pathogenicity of the T. vivax parasites. The pathogenicity of T. vivax from the non-tsetse infested area can be considered as nearly as important as that of its counterpart derived from the tsetse infested area.


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The northwest region of Ethiopia is affected by both tsetse and non-tsetse transmitted trypanosomosis with a huge impact on livestock productivity. The objective of this experimental study was to determine clinical and pathological findings in young Zebu cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of northwest Ethiopia. A total of 18 cattle (*Bos indicus*) aged between 6 and 12 months, purchased from a trypanosome-free area and confirmed to be trypanosome negative and divided into three groups of six animals were used. Animals in the first two groups (Group TT: tsetse infested isolate infected and Group NT: non-tsetse infested isolate infected) received 2 mL of infected blood from donor animals at 10^6 trypanosomes/mL, and the remaining group was a non-infected control (NIC). Each group was observed daily for a period of eight consecutive weeks for clinical signs and once per week for parasitaemia. Post-mortem examinations were done on euthanized animals, and tissue samples were taken for histopathological analysis. The prepatent period of the disease was earlier in the NT group – 6 days post infection (dpi) than in the TT group 12 dpi. The infection was characterized by reduced feed intake, intermittent pyrexia and parasitaemia, enlarged lymph nodes, lacrimation, and emaciation. Less frequently diarrhoea, oedema and nervous signs were observed in both groups of infected animals. At necropsy, infected animals had enlarged spleens, enlarged lymph nodes, pneumonic and emphysematous lungs, enlarged livers, and haemorrhages on the brain and intestine. Histopathological analysis revealed lymphoid hyperplasia of the spleen, necrosis of the liver, encephalitis and hyperplasia of lymph nodes. In conclusion, *Trypanosoma vivax* isolates from both tsetse infested and non-tsetse areas showed a variety of virulence factors leading to the development of acute clinical signs and gross and histopathological lesions. However, the parasitaemia and clinical signs appeared earlier in the NT compared with the TT infected groups.


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Anaemia is an important complication of trypanosomiasis. The mechanisms through which trypanosomal infection leads to anaemia are poorly defined. A number of studies have implicated inflammatory cytokines, but these data are limited and inconsistent. In this article,
we reviewed the published literature on cytokines associated with *Trypanosoma brucei* infections and their role in the immunopathology leading to anaemia. Articles were searched in PubMed through screening of titles and abstracts with no limitation on date of publishing and study design. Articles in English were searched using keywords "African trypanosomiasis", "sleeping sickness", "Trypanosoma brucei", in all possible combinations with "anaemia" and/or "cytokines". Twelve articles examining cytokines and their role in trypanosome induced anaemia were identified out of 1 095 originally retrieved from PubMed. None of the articles identified was from human-based studies. A total of eight cytokines were implicated, with four cytokines (IFN-gamma, IL-10, TNF-alpha, IL-12) showing an association with anaemia. These articles reported that mice lacking TNF-alpha were able to control anaemia, and that IFN-gamma was linked to severe anaemia given its capacity to suppress erythropoiesis, while IL-10 was shown to regulate IFN-gamma and TNF-alpha, providing a balance that was associated with the severity of anaemia. IFN-gamma and TNF-alpha have also been reported to work in concert with other factors such as nitric oxide and iron in order to induce anaemia. In conclusion, IFN-gamma, IL-10, and TNF-alpha were the three major cytokines identified to be heavily involved in anaemia caused by *Trypanosoma brucei* infection. The anti-inflammatory cytokine, IL-10, was shown to counter the effects of pro-inflammatory cytokines in order to reduce the severity of anaemia. The mechanism of anaemia is multifactorial and therefore requires further, more elaborate research. Data from human subjects would also shed more light.


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Invasion of the central nervous system (CNS) by African trypanosomes represents a critical step in the development of human African trypanosomiasis. In both clinical cases and experimental mouse infections it has been demonstrated that predisposition to CNS invasion is associated with a type 1 systemic inflammatory response. Using the *Trypanosoma brucei brucei* GVR35 experimental infection model, we demonstrate that systemic delivery of the counter-inflammatory cytokine IL-10 lowers plasma IFN-gamma and TNF-alpha concentrations, CNS parasitosis and ameliorates neuro-inflammatory pathology and clinical symptoms of disease. The results provide evidence that CNS invasion may be susceptible to immunological attenuation.


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Sleeping sickness (human African trypanosomiasis - HAT) is a deadly neglected tropical disease affecting mainly rural communities in sub-Saharan Africa. This parasitic
disease is caused by the *Trypanosoma brucei* (*T. b.*) parasite, which is transmitted to the human host through the bite of the tsetse fly. Two parasite sub-species, *T. b. rhodesiense* and *T. b. gambiense*, are responsible for two clinically different and geographically separated forms of sleeping sickness. The objective of the present study was to characterise and compare the cerebrospinal fluid (CSF) proteome of stage 2 (meningo-encephalitic stage) HAT patients suffering from *T. b. gambiense* or *T. b. rhodesiense* disease using high-throughput quantitative proteomics and the tandem mass tag (TMT®) isobaric labelling. In order to evaluate the CSF proteome in the context of HAT pathophysiology, the protein dataset was then submitted to gene ontology and pathway analysis. Two significantly differentially expressed proteins (C-reactive protein and orosomucoid 1) were further verified on a larger population of patients (n = 185) by ELISA, confirming the mass spectrometry results. By showing a predominant involvement of the acute immune response in *rhodesiense* HAT, the proteomics results obtained in this work will contribute to further understand the mechanisms of pathology occurring in HAT and to propose new biomarkers of potential clinical utility. The mass spectrometry raw data are available in the Pride Archive via ProteomeXchange through the identifier PXD001082.

(c) CHEMOTHERAPEUTICS


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The synthesized compounds showed weak potency against TbrPDEB1.


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Neuroblastoma is a childhood tumour in which MYC oncogenes are commonly activated to drive tumour progression. Survival for children with high-risk neuroblastoma remains poor despite treatment that incorporates high-dose chemotherapy, stem cell support, surgery, radiation therapy and immunotherapy. More effective and less toxic treatments are sought and one approach under clinical development involves re-purposing the anti-protozoan drug difluoromethylornithine (DFMO; Efornithine) as a neuroblastoma therapeutic. DFMO is an irreversible inhibitor of ornithine decarboxylase (Odc), a MYC target gene, *bona fide* oncogene, and the rate-limiting enzyme in polyamine synthesis. DFMO is approved for the treatment of *Trypanosoma brucei gambiense* encephalitis ("African sleeping sickness") since polyamines are essential for the proliferation of these protozoa. However, polyamines are also
critical for mammalian cell proliferation and the finding that MYC coordinately regulates all aspects of polyamine metabolism suggests polyamines may be required to support cancer promotion by MYC. Pre-emptive blockade of polyamine synthesis is sufficient to block tumour initiation in an otherwise fully penetrant transgenic mouse model of neuroblastoma driven by MYCN, underscoring the necessity of polyamines in this process. Moreover, polyamine depletion regimens exert potent anti-tumour activity in pre-clinical models of established neuroblastoma as well, in combination with numerous chemotherapeutic agents and even in tumours with unfavourable genetic features such as MYCN, ALK or TP53 mutation. This has led to the testing of DFMO in clinical trials for children with neuroblastoma. Current trial designs include testing lower dose DFMO alone (2,000 mg/m²/day) starting at the completion of standard therapy, or higher doses combined with chemotherapy (up to 9,000 mg/m²/day) for patients with relapsed disease that has progressed. In this review we will discuss important considerations for the future design of DFMO-based clinical trials for neuroblastoma, focusing on the need to better define the principal mechanisms of anti-tumour activity for polyamine depletion regimens. Putative DFMO activities that are both cancer cell intrinsic (targeting the principal oncogenic driver, MYC) and cancer cell extrinsic (altering the tumour microenvironment to support anti-tumour immunity) will be discussed. Understanding the mechanisms of DFMO activity is critical in determining how it might be best leveraged in upcoming clinical trials. This mechanistic approach also provides a platform by which iterative pre-clinical testing using translational tumour models may complement our clinical approaches.


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The largest continuous bacterial nonribosomal peptide synthetase discovered so far is described. It consists of 15 consecutive modules arising from an uninterrupted, fully functional gene in the entomopathogenic bacterium Photobacterium luminescens. The identification of its cryptic biosynthesis product was achieved by using a combination of genome analysis, promoter exchange, isotopic labelling experiments, and total synthesis of a focused collection of peptide candidates. Although it belongs to the growing class of D-/L-peptide natural products, the encoded metabolite kolossin A was found to be largely devoid of antibiotic activity and is likely involved in interspecies communication. A stereoisomer of this peculiar natural product displayed high activity against Trypanosoma brucei rhodesiense, a recalcitrant parasite that causes the deadly disease African sleeping sickness.


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In response to reports of *Trypanosoma brucei* resistance to the nitroaromatic drug nifurtimox, we evaluated the potential of antituberculosis nitrofuran isoxazolines as inhibitors of trypanosome growth. The susceptibility of *T. brucei brucei* was assessed *in vitro*. The lowest effective concentration to inhibit growth (EC$_{90}$) against drug-susceptible and -resistant parasites, time-to-kill kinetics, reversibility of inhibition and propensity for P-glycoprotein-mediated exclusion from the blood-brain barrier were determined. Nitrofuran isoxazolines were potent inhibitors of *T. brucei brucei* proliferation at nanomolar concentrations, with pentacyclic nitrofurans being 100-fold more potent than nifurtimox. Activity was sustained against nifurtimox-resistant parasites, suggesting the possibility of a unique mechanism of activation and potential for use in the treatment of drug-resistant infections. Exposure of parasites to the maximum concentrations of Compound 15 achieved *in vivo* with oral dosing yielded >2 logs of irreversible killing in <4 hours, indicating rapid trypanocidal activity. Pentacyclic nitrofuran isoxazolines warrant further development for the treatment of drug-susceptible and nifurtimox-resistant trypanosome infections.


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There is an urgent need for new, brain penetrant small molecules that target the central nervous system second stage of human African trypanosomiasis (HAT). We report that a series of novel indoline-2-carboxamides have been identified as inhibitors of *Trypanosoma brucei* from screening of a focused protease library against *Trypanosoma brucei brucei* in culture. We describe the optimization and characterization of this series. Potent antiproliferative activity was observed. The series demonstrated excellent pharmacokinetic properties, full cures in a stage 1 mouse model of HAT, and a partial cure in a stage 2 mouse model of HAT. Lack of tolerability prevented delivery of a fully curative regimen in the stage 2 mouse model and thus further progress of this series.

Human African trypanosomiasis (HAT), Chagas disease and leishmaniasis, which are caused by the trypanosomatids *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* species, are among the most deadly neglected tropical diseases. The development of drugs that are active against several trypanosomatids is appealing from a clinical and economic viewpoint, and seems feasible as these parasites share metabolic pathways and hence might be treatable by common drugs. From benzonapthyridine 1, an inhibitor of acetylcholinesterase (AChE) for which we have found a remarkable trypanocidal activity, we have designed and synthesized novel benzo[h][1,6]naphthyridines, pyrrolo[3,2-c]quinolines, azepino[3,2-c]quinolines, and pyrano[3,2-c]quinolines through 2-4-step sequences featuring an initial multicomponent Povarov reaction as the key step. To assess the therapeutic potential of the novel compounds, we have evaluated their *in vitro* activity against *T. brucei*, *T. cruzi*, and *Leishmania infantum*, as well as their brain permeability, which is of specific interest for the treatment of late-stage HAT. To assess their potential toxicity, we determined their cytotoxicity against rat myoblast L6 cells and their AChE inhibitory activity. Several tricyclic heterofused quinoline derivatives displayed an interesting multi-trypanosomatid profile, with one-digit μM potencies against two of these parasites and two-digit μM potency against the other. Pyranoquinoline 39, which displayed IC_{50} values of 1.5 μM, 6.1 μM and 29.2 μM against *T. brucei*, *L. infantum* and *T. cruzi*, respectively, brain permeability, better drug-like properties (lower lipophilicity and molecular weight and higher CNS MPO desirability score) than hit 1, and the lowest AChE inhibitory activity of the series (IC_{50} > 30 μM), emerges as an interesting multi-trypanosomatid lead, amenable to further optimization particularly in terms of its selectivity index over mammalian cells.


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Nifurtimox is a 5-nitrofuran derived antiprotozoal drug used to treat diseases caused by trypanosomes including Chagas’ disease and sleeping sickness (African trypanosomiasis). Available methods for the determination of nifurtimox in plasma are tedious and of low sensitivity. For the first time, an isotope dilution HPLC/MS/MS method for the sensitive quantitation of nifurtimox down to 10.0 μg/L in plasma is described. Protein precipitation was used for sample preparation. Samples were analysed on a standard triple quadrupole tandem mass spectrometer. The validated concentration range covers 10.0 μg/L to 5 000 μg/L. Inter-assay accuracy and precision (%CV) ranged from 98.4 to 101%, and 2.61 to 10.1%, respectively. The method consists of very simple sample preparation and provides unmatched sensitivity, high reproducibility and robustness enabling analysis of large sample numbers. Method performance met current guidelines on bioanalytical method validation.


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As part of our ongoing efforts to identify natural products with activity against pathogens causing neglected tropical diseases, we are currently performing an extensive screening of natural product (NP) databases against a multitude of protozoan parasite proteins. Within this project, we screened a database of NPs from a commercial supplier, AnalytiCon Discovery (Potsdam, Germany), against Trypanosoma brucei glyceraldehyde-3-phosphate dehydrogenase (TbGAPDH), a glycolytic enzyme whose inhibition deprives the parasite of energy supply. NPs acting as potential inhibitors of the mentioned enzyme were identified using a pharmacophore-based virtual screening and subsequent docking of the identified hits into the active site of interest. In a set of 700 structures chosen for the screening, 13 (1.9%) were predicted to possess significant affinity towards the enzyme and were therefore tested in an in vitro enzyme assay using recombinant TbGAPDH. Nine of these in silico hits (69%) showed
significant inhibitory activity at 50 μM, of which two geranylated benzophenone derivatives proved to be particularly active with IC₅₀ values below 10 μM. These compounds also showed moderate in vitro activity against *T. brucei rhodesiense* and may thus represent interesting starting points for further optimization.


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Current treatment options for human African trypanosomiasis (HAT) are ineffective, and they have several well-known clinical limitations. In our continued efforts to identify chemotypes that can be developed into clinically useful drugs, we screened a targeted compound library against the major cathepsin L (rhodesain) in *T. brucei*. We report the antirhodesain activity and antitrypanosomal activity of the compounds in this letter. The identified compounds can serve as starting points for structure- and/or phenotype-based lead optimization strategy against *Trypanosoma brucei*.


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The screening of a focused library identified FTY720 (Fingolimod; Gilenya) as a potent selective antitrypanosomal compound active against *Trypanosoma brucei gambiense* and *T. brucei rhodesiense*, the causative agents of human African trypanosomiasis (HAT). This is the first report of trypanocidal activity for FTY720, an oral drug registered for the treatment of relapsing multiple sclerosis, and the characterization of sphingolipids as a potential new class of compounds for HAT.


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Trypanosoma brucei, the causal agent for sleeping sickness, depends on ergosterol for growth. Here, we describe the effects of a mechanism-based inhibitor, 26-fluorolanosterol (26FL), which converts in vivo to a fluorinated substrate of the sterol C24-methyltransferase essential for sterol methylation and function of ergosterol, and missing from the human host. These results demonstrate that poisoning of ergosterol biosynthesis by a 26-fluorinated Delta (24)-sterol is a promising strategy for developing a new treatment for trypanosomiasis.


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Trypanosomatids are the causative agents of African sleeping sickness, Chagas' disease, and the different forms of leishmaniasis. This family of protozoan parasites possesses a trypanothione-based redox metabolism that provides the reducing equivalents for various vital processes such as the biosynthesis of DNA precursors and the detoxification of hydroperoxides. Almost all enzymes of the redox pathway proved to be essential and therefore fulfil one crucial prerequisite for a putative drug target. Trypanothione synthetase and trypanothione reductase are present in all trypanosomatids but absent from the mammalian host which, in addition to the essentiality, renders them highly specific. Chemotherapy research on both enzymes is further supported by the availability of high-throughput screening techniques and crystal structures. In this review we focus on the recent advances and limitations in the development of lead compounds targeting trypanothione synthetase and trypanothione reductase. We present an overview of the available inhibitors and discuss future perspectives including other components of the parasite-specific redox pathway.


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Most potent activity was found against *T. brucei*, the causative agent of human African trypanosomiasis, and involved targeting of the mitochondrial membrane potential with 15 SQ109 analogues being more active than was SQ109 in cell growth inhibition, having IC$_{50}$ values as low as 12 nM (5.5 ng/mL) and a selectivity index of ~300.

Sleeping sickness, Chagas disease, leishmaniasis, and malaria are infectious diseases caused by unicellular eukaryotic parasites ("protozoans"). The three first mentioned are classified as Neglected Tropical Diseases (NTDs) by the World Health Organization and together threaten more than one billion lives worldwide. Due to the lack of research interest and the high increase of resistance against the existing treatments, the search for effective and safe new therapies is urgently required. In view of the large tradition of natural products as sources against infectious diseases, the aim of the present study is to investigate the potential of legally approved and marketed herbal medicinal products (HMPs) as antiprotozoal agents. Fifty-eight extracts from 53 HMPs on the German market were tested by a multiple-target-screening (MTS) against parasites of the genera *Leishmania*, *Trypanosoma*, and *Plasmodium*. Sixteen HMPs showed *in vitro* activity against at least one of the pathogens (IC$_{50}$ < 10 μg/mL). Six extracts from preparations of *Salvia*, *Valeriana*, *Hypericum*, *Silybum*, *Arnica*, and *Curcuma* exhibited high activity (IC$_{50}$ < 2.5 μg/mL). They were analytically characterized by UHPLC/ESI-QqTOF-MSMS, and the activity-guided fractionation of the extracts with the aim to isolate and identify the active compounds is in progress.


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Isolona hexaloba (Pierre) Engl. and Diels (Annonaceae) is traditionally used in D.R. Congo against parasitic diseases including malaria. Two crude aqueous extracts, 3 crude methanol extracts and 3 crude 80% ethanol extracts from the leaves, root bark and stem bark together with 12 subfractions from the crude 80% ethanol extracts were evaluated in vitro for their antiprotozoal activity against Trypanosoma brucei brucei, T. cruzi, Leishmania infantum and the chloroquine and pyrimethamine resistant K1 strain of Plasmodium falciparum. Their cytotoxic effects against MRC-5 cell lines were also assessed. Results indicated that the most pronounced activities against T. b. brucei were recorded for the crude methanol extracts of root bark (IC50 = 1.97 μg/mL; SI > 32.49) and leaves (IC50 = 2.65 μg/mL; SI > 24.15). None of the tested crude extracts and fractions was found to be cytotoxic against MRC-5 cell lines except the petroleum ether soluble fraction from the leaves which displayed a cytotoxic effect (CC50 = 21.40 μg/mL). Overall, extracts of I. hexaloba tested here, showed good results concerning parasitic infections such as sleeping sickness without considerable toxicity.


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Cyclic nucleotide phosphodiesterases (PDEs) have been identified as important enzyme targets for drug development in both humans and Trypanosoma brucei, the causative agent of human African trypanosomiasis. With this in mind, we recently reported the profiling of a range of human phosphodiesterase inhibitors, showing that human PDE4 inhibitors tend to display the best potency against the trypanosomal phosphodiesterase TbrPDE1. Among these was GSK-256066, a potent inhibitor of human PDE4 and a weak inhibitor of TbrPDE1. In this report, we describe the results of a structure-activity relationship study of this chemotype, leading to the discovery of analogues with improved potency against TbrPDE1 and μM inhibition of T. brucei cellular growth. We rationalize the potency trends via molecular docking of the new inhibitors into a recently reported apo structure of TbrPDE1. The studies in this
article will inform future efforts in repurposing human PDE inhibitors as antitypansomal agents.


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The growth-inhibitory properties of a 5-nitrothiazole series were evaluated against *Trypanosoma brucei*. A subset of related compounds displayed the greatest potency toward the parasite while exhibiting little cytotoxic effect on mammalian cells, with this antiparasitic activity dependent on expression of a type I nitroreductase by the trypanosome. We conclude that the 5-nitrothiazole class of nitroheterocyclic drugs may represent a new lead in the treatment of human African trypanosomiasis.


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From a whole-organism high throughput screen of approximately 87,000 compounds against *Trypanosoma brucei*, we recently identified eight new unique compounds for the treatment of human African trypanosomiasis. In an effort to understand the structure-activity relationships around these compounds, we report for the first time our results on a new class of trypanocides, the pyrazine carboxamides. Attracted by the low molecular weight (270 g.mol⁻¹) of our starting hit (9) and its potency (0.49 μM), the SAR around the core was explored, leading to compounds having an EC₅₀ as low as 25 nM against *T. b. brucei* and being more than 1,500 times less toxic against mammalian L6 and HEK293 cell lines. The most potent compounds in the series were exquisitely selective for *T. brucei* over a panel of other protozoan parasites, showing an excellent correlation with the human infective parasite *Trypanosoma brucei rhodesiense*, the most potent compound (65) having an EC₅₀ of 24 nM. The compounds are highly drug-like and are able to penetrate the CNS, their only limitation currently being their rate of microsomal metabolism. To that effect, efforts to identify potential metabolites of selected compounds are also reported.


the ligand on the basis of the designed pharmacophore. The docking has been performed for the resultant seventeen approved drugs and two known inhibitors. Two approved drugs have negative binding energy and their pKa values are similar to the selected known inhibitors. The result of this study suggests that the approved drugs Ethambutol (DB00330) and Metaraminol (DB00610) may prove useful in the treatment of African sleeping sickness.


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The human and veterinary disease complex known as African trypanosomiasis continues to inflict significant global morbidity, mortality, and economic hardship. Drug resistance and toxic side effects of old drugs call for novel and unorthodox strategies for new and safe treatment options. We designed methyltriazenyl purine prodrugs to be rapidly and selectively internalized by the parasite, after which they disintegrate into a nontoxic and naturally occurring purine nucleobase, a simple triazene-stabilizing group, and the active toxin: a methyldiazonium cation capable of damaging DNA by alkylation. We identified 2-(3-acetyl-3-methyltriazen-1-yl)-6-hydroxypurine (compound 1) as a new lead compound, which showed submicromolar potency against *Trypanosoma brucei*, with a selectivity index of > 500, and it demonstrated a curative effect in animal models of acute trypanosomiasis. We investigated the mechanism of action of this lead compound and showed that this molecule has significantly higher affinity for parasites over mammalian nucleobase transporters, and it does not show cross-resistance with current first-line drugs. Once selectively accumulated inside the parasite, the prodrug releases a DNA-damaging methyldiazonium cation. We propose that ensuing futile cycles of attempted mismatch repair then lead to G2/M phase arrest and eventually cell death,
as evidenced by the reduced efficacy of this purine analog against a mismatch repair-deficient (MSH2(-/-)) trypanosome cell line. The observed absence of genotoxicity, hepatotoxicity, and cytotoxicity against mammalian cells revitalizes the idea of pursuing parasite-selective DNA alkylators as a safe chemotherapeutic option for the treatment of human and animal trypanosomiasis.


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Marine natural products are an important source of lead compounds against many pathogenic targets. Herein, we report the discovery of lobosamides A-C from a marine actinobacterium, Micromonospora sp., representing three new members of a small but growing family of bacterially produced polyene macrolactams. The lobosamides display growth inhibitory activity (lobosamide A IC50 = 0.8 µM) against the protozoan parasite Trypanosoma brucei, the causative agent of human African trypanosomiasis (HAT). The biosynthetic gene cluster of the lobosamides was sequenced and suggests a conserved cluster organization among the 26-membered macrolactams. While determination of the relative and absolute configurations of many members of this family is lacking, the absolute configurations of the lobosamides were deduced using a combination of chemical modification, detailed spectroscopic analysis, and bioinformatics. We implemented a "molecules-to-genes-to-molecules" approach to determine the prevalence of similar clusters in other bacteria, which led to the discovery of two additional macrolactams, mirilactams A and B from Actinosynnema mirum. These additional analogues have allowed us to identify specific structure-activity relationships that contribute to the antitrypanosomal activity of this class. This approach illustrates the power of combining chemical analysis and genomics in the discovery and characterization of natural products as new lead compounds to target neglected diseases.
The enzyme N-myristoyltransferase (NMT) from *Trypanosoma brucei* has been validated both chemically and biologically as a potential drug target for human African trypanosomiasis. We previously reported the development of some very potent compounds based around a pyrazole sulphonamide series, derived from a high-throughput screen. Herein we describe work around thiazolidinone and benzomorpholine scaffolds that were also identified in the screen. An X-ray crystal structure of the thiazolidinone hit in *Leishmania major* NMT showed the compound bound in the previously reported active site, utilising a novel binding mode. This provides potential for further optimisation. The benzomorpholinone was also found to bind in a similar region. Using an X-ray crystallography/structure-based design approach, the benzomorpholinone series was further optimised, increasing activity against *T. brucei* NMT by >1 000-fold. A series of trypanocidal compounds were identified with suitable in vitro drug metabolism and pharmacokinetic properties, including CNS exposure for further development. Further work is required to increase selectivity over the human NMT isoform and activity against *T. brucei*.

African trypanosomiasis affects both humans and livestock in sub-Saharan countries including Ethiopia. Due to limitations to current chemotherapy, there is an urgent need for the development of new, safe, cheap and effective drugs. In the present study, the leaf of *Dovyalis abyssinica* (Salicaceae) was tested for its in vivo antitrypanosomal activity against *Trypanosoma congolense* field isolate on mice. The leaf of *D. abyssinica* was macerated using dichloromethane and methanol. The extracts at doses of 250, 200, 150 and 100 mg/kg body weight were administered intraperitoneally daily for 7 days to mice infected with *T. congolense*. Following administration, parasitaemia, packed cell volume, rectal temperature, body weight and survival time were monitored. Administration of dichloromethane and methanol extracts at 250 and 200 mg/kg reduced (p < 0.05) parasitaemia and rectal temperature, and improved (p < 0.05) PCV, mean body weight, and mean survival time compared with dimethylsulfoxide treatment. To conclude, crude dichloromethane and methanol leaf extracts of *D. abyssinica* displayed anti-trypanosomal activity that may serve as lead for the development of effective alternative antitrypanosomal drugs.

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A screen of a focused kinase inhibitor library against *Trypanosoma brucei rhodesiense* led to the identification of seven series, totalling 121 compounds, which showed > 50% inhibition at 5 μM. Screening of these hits in a *T. b. brucei* proliferation assay highlighted three compounds with a 1H-imidazo[4,5-b]pyrazin-2(3H)-one scaffold that showed sub-μM activity and excellent selectivity against the MRC5 cell line. Subsequent rounds of optimisation led to the identification of compounds that exhibited good *in vitro* drug metabolism and pharmacokinetics (DMPK) properties, although in general this series suffered from poor solubility. A scaffold-hopping exercise led to the identification of a 1H-pyrazolo[3,4-b]pyridine scaffold, which retained potency. A number of examples were assessed in a *T. b. brucei* growth assay, which could differentiate static and cidal action. Compounds from the 1H-imidazo[4,5-b]pyrazin-2(3H)-one series were found to be either static or growth-slowing and not cidal. Compounds with the 1H-pyrazolo[3,4-b]pyridine scaffold were found to be cidal and showed an unusual biphasic nature in this assay, suggesting they act by at least two mechanisms.


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Overall, the results suggest that it may be possible to develop multi-target drug leads against *T. brucei* that act by inhibiting both k-DNA replication and isoprenoid biosynthesis.

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Eflopnithine (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 eflopnithine in rat brain. To improve chromatographic retention and MS detection, eflopnithine was derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate for 5 min at room temperature prior to injection. Derivatized eflopnithine was separated on a reverse-phase C18 UPLC column with a 6-min gradient; elution occurred at approximately 1.5 min. Prior to derivatization, eflopnithine was reproducibly extracted from rat brain homogenate by methanol protein precipitation (~70% recovery). Derivatized eflopnithine was stable in the autosampler (6 degrees C) for at least 24 hours. 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 eflopnithine assay was applied successfully to determine eflopnithine distribution in different regions of rat brain in an in situ rat brain perfusion study.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY, CHARACTERISATION OF ISOLATES


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The African tsetse-transmitted trypanosomes are considered to be a well-known group of parasitic protozoa, but in 2008 a novel and distinctive trypanosome related to *Trypanosoma brucei* was discovered among tsetse isolates from Msugwwe in Tanzania. The host range, distribution and potential pathogenicity of this new trypanosome remain to be elucidated; such studies would be facilitated by a sensitive and specific identification method. Here, we identified two highly repetitive elements in the genome of the new trypanosome: a 177 bp repeat, which was located predominantly on the highly abundant minichromosomes, and a 138 bp repeat, which was widely dispersed in the genome. A PCR test based on each repeat was specific for the new trypanosome and sensitive to < 0.1 trypanosome equivalent. These PCR tests were used to identify trypanosomes in archival pig blood smears from the 1950's, confirming the identity of the Msugwwe trypanosome as *Trypanosoma (Pycnomonas) suis*. We also present data on the molecular karyotype and spliced leader (SL, miniexon) repeat of the new trypanosome, both of which distinguish *T. suis* from other, better-known African tsetse-
transmitted trypanosomes. The rediscovery of *T. suis* opens new lines of research into the evolution and biology of the African trypanosomes.


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Livestock trypanosomoses, caused by three species of the *Trypanozoon* subgenus, *Trypanosoma brucei brucei*, *T. evansi* and *T. equiperdum* are widely distributed throughout the world and constitute important limitations for the production of animal protein. *T. evansi* and *T. equiperdum* are morphologically indistinguishable parasites that evolved from a common ancestor but acquired important biological differences, including host range, mode of transmission, distribution, clinical symptoms and pathogenicity. At a molecular level, *T. evansi* is characterized by the complete loss of the maxicircles of the kinetoplastic DNA, while *T. equiperdum* has retained maxicircle fragments similar to those present in *T. brucei*. *T. evansi* causes the disease known as surra, derregnadera or "mal de cadeiras", while *T. equiperdum* is the aetiologic agent of dourine or "mal du coit", characterized by venereal transmission and white patches in the genitalia. Nine Venezuelan *Trypanosoma* spp. isolates, from horse, donkey or capybara were genotyped and classified using microsatellite analyses and maxicircle genes. The variables from the microsatellite data and the procyclin PE repeats matrices were combined using the Hill-Smith method and compared to a group of *T. evansi*, *T. equiperdum* and *T. brucei* reference strains from South America, Asia and Africa using co-inertia analysis. Four maxicircle genes (cytb, cox1, a6 and nd8) were amplified by PCR from TeAp-N/D1 and TeGu-N/D1, the two Venezuelan isolates that grouped with the *T. equiperdum* STIB841/OVI strain. These maxicircle sequences were analysed by nucleotide BLAST and aligned to orthologous genes from the *Trypanozoon* subgenus by MUSCLE tools. Phylogenetic trees were constructed using maximum parsimony (MP) and maximum likelihood (ML) with the MEGA5.1® software. We characterized microsatellite markers and procyclin PE repeats of nine Venezuelan *Trypanosoma* spp. isolates with various degrees of virulence in a mouse model, and compared them to a panel of *T. evansi* and *T. equiperdum* reference strains. Coinertia analysis of the combined repeats and previously reported *T. brucei brucei* microsatellite genotypes revealed three distinct groups. Seven of the Venezuelan isolates grouped with globally distributed *T. evansi* strains, while TeAp-N/D1 and TeGu-N/D1 strains clustered in a separate group with the *T. equiperdum* STIB841/OVI strain isolated in South Africa. A third group included *T. brucei brucei*, two strains previously classified as *T. evansi* (GX and TC) and one as *T. equiperdum* (BoTat-1.1). Four maxicircle genes, cytochrome b, cytochrome oxidase subunit 1, ATP synthase subunit 6 and NADH dehydrogenase subunit 8, were identified in the two Venezuelan strains clustering with the *T. equiperdum* STIB841/OVI strain. Phylogenetic analysis of the *cox1* gene sequences further separated these two Venezuelan *T. equiperdum* strains: TeAp-
N/D1 grouped with *T. equiperdum* strain STIB818 and *T. brucei brucei*, and TeGu-N/D1 with the *T. equiperdum* STIB841/OVI strain. Based on the coiner tia analysis and maxicircle gene sequence phylogeny, TeAp-N/D1 and TeGu-N/D1 constitute the first confirmed *T. equiperdum* strains described from Latin America.

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

[See also 38: 17705, 17706, 17713, 17716, 17722, 17725, 17726, 17728, 17730, 17736, 17745, 17746, 17749, 17750, 17752, 17753, 17754, 17757]


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Mitochondrial ribosomes of *Trypanosoma brucei* are composed of 9S and 12S rRNAs, eubacterial-type ribosomal proteins, polypeptides lacking discernible motifs and approximately 20 pentatricopeptide repeat (PPR) RNA binding proteins. Several PPRs also populate the polyadenylation complex; among these, KPAF1 and KPAF2 function as general mRNA 3’ adenylcation/uridylation factors. The A/U-tail enables mRNA binding to the small ribosomal subunit and is essential for translation. The presence of A/U-tail also correlates with requirement for translation of certain mRNAs in mammalian and insect parasite stages. Here, we inquired whether additional PPRs activate translation of individual mRNAs. Proteomic analysis identified KRIPP1 and KRIPP8 as components of the small ribosomal subunit in mammalian and insect forms, but also revealed their association with the polyadenylation complex in the latter. RNAi knockdowns demonstrated essential functions of KRIPP1 and KRIPP8 in the actively-respiring insect stage, but not in the mammalian stage. In the KRIPP1 knockdown, A/U-tailed mRNA encoding cytochrome c oxidase subunit 1 declined concomitantly with the *de novo* synthesis of this subunit whereas polyadenylation and translation of cyb mRNA were unaffected. In contrast, the KRIPP8 knockdown inhibited A/U-tailing and translation of both CO1 and cyb mRNAs. Our findings indicate that ribosome-associated PPRs may selectively activate mRNAs for translation.


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Antigenic variation in *Trypanosoma brucei* relies on periodic switching of variant surface glycoproteins (VSGs), which are transcribed monoallelically by RNA polymerase I from one
of about 15 bloodstream expression sites (BES). Chromatin of the actively transcribed BES is depleted of nucleosomes, but it is unclear if this open conformation is a mere consequence of a high rate of transcription, or whether it is maintained by a transcription-independent mechanism. Using an inducible BES-silencing reporter strain, we observed that chromatin of the active BES remains open for at least 24 hours after blocking transcription. This conformation is independent of the cell-cycle stage, but dependent upon TDP1, a high mobility group box protein. For two days after BES silencing, we detected a transient and reversible derepression of several silent BESs within the population, suggesting that cells probe other BESs before commitment to one, which is complete by 48 hours. FACS sorting and subsequent subcloning confirmed that probing cells are switching intermediates capable of returning to the original BES, switch to the probed BES or to a different BES. We propose that regulation of BES chromatin structure is an epigenetic mechanism important for successful antigenic switching.


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Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are among the most abundant phospholipids in biological membranes. In many eukaryotes, the CDP-ethanolamine and CDP-choline branches of the Kennedy pathway represent major and often essential routes for the production of PE and PC, with ethanolamine and choline/ethanolamine phosphotransferases (EPT and CEPT, respectively), catalysing the last reactions in the respective pathways. Although the site of PE and PC synthesis is commonly known to be the endoplasmic reticulum (ER), detailed information on the localization of the different phosphotransferases is lacking. In the unicellular parasite, *Trypanosoma brucei*, both branches of the Kennedy pathway are essential for cell growth in culture. We have previously reported that *T. brucei* EPT (TbEPT) catalyses the production of ether-type PE molecular species while *T. brucei* CEPT (TbCEPT) synthesizes diacyl-type PE and PC molecular species. We now show that the two enzymes localize to different sub-compartments of the ER. By expressing a series of tagged forms of the two enzymes in *T. brucei* parasites, in combination with sub-cellular fractionation and enzyme activity measurements, TbEPT was found exclusively in the perinuclear ER, a distinct area located close to but distinct from the nuclear membrane. In contrast, TbCEPT was detected in the bulk ER.

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Incubation of African trypanosomes with the lectin concanavalin A (conA) leads to alteration in cellular DNA content, DNA degradation, and surface membrane blebbing. Here, we report the generation and characterization of a conA-refractory Trypanosoma brucei line. These insect stage parasites were resistant to conA killing, with a medium lethal dose at least 50-fold greater than the parental line. Fluorescence-based experiments revealed that the resistant cells bound less lectin when compared to the parental line. Western blotting and mass spectrometry confirmed that the resistant line lacked an N-glycan required for conA binding on the cellular receptors, EP procyclin proteins. The failure to N-glycosylate the EP procyclins was not the consequence of altered N-glycan precursor biosynthesis, as another glycosylated protein (Fla1p) was normally modified. These findings support the likelihood that resistance to conA was a consequence of failure to bind the lectin trigger.


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The potent trypanolytic properties of human apolipoprotein L1 (APOL1) can be neutralized by the trypanosome variant surface antigen gene product known as serum resistance-associated protein. However, two common APOL1 haplotypes present uniquely in individuals of West African ancestry each encode APOL1 variants resistant to serum resistance-associated protein, and each confers substantial resistance to human African sleeping sickness. In contrast to the dominantly inherited anti-trypanosomal activity of APOL1, recessive inheritance of these two trypanoprotective APOL1 alleles predisposes to kidney disease. Proposed mechanisms of APOL1 toxicity have included BH3 domain-dependent autophagy and/or ion channel activity. We probed these potential mechanisms by expressing APOL1 in Xenopus laevis oocytes. APOL1 expression in oocytes increased ion permeability and caused profound morphological deterioration (toxicity). Coexpression of BCL2 family members rescued APOL1-associated oocyte toxicity in the order MCL1 approximately BCLW > BCLXL approximately BCL2A1 >> BCL2. Deletion of nine nominal core BH3 domain residues abolished APOL1-associated toxicity, but missense substitution of the same residues abolished neither oocyte toxicity nor its rescue by coexpressed MCL1. The APOL1 BH3 domain was similarly dispensable for the ability of APOL1 to rescue intact mice from lethal trypanosome challenge. Replacement of most extracellular Na⁺ by K⁺ also reduced APOL1-associated oocyte toxicity.
toxicity, allowing demonstration of APOL1-associated increases in Ca(2+) and Cl(-) fluxes and oocyte ion currents, which were similarly reduced by MCL1 coexpression. Thus APOL1 toxicity in *Xenopus* oocytes is BH3-independent, but can nonetheless be rescued by some BCL2 family proteins.


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The polo-like kinase (PLK) in *Trypanosoma brucei* plays multiple roles in basal body segregation, flagellum attachment, and cytokinesis. However, the mechanistic role of TbPLK remains elusive, mainly because most of its substrates are not known. Here, we report a new substrate of TbPLK, SPBB1, and its essential roles in *T. brucei*. SPBB1 was identified through yeast two-hybrid screening with the kinase-dead TbPLK as the bait. It interacts with TbPLK *in vitro* and *in vivo*, and is phosphorylated by TbPLK *in vitro*. SPBB1 localizes to both the mature basal body and the probasal body throughout the cell cycle, and co-localizes with TbPLK at the basal body during early cell cycle stages. RNAi against SPBB1 in procyclic trypanosomes inhibited basal body segregation, disrupted the new flagellum attachment zone filament, detached the new flagellum, and caused defective cytokinesis. Moreover, RNAi of SPBB1 confined TbPLK at the basal body and the bilobe structure, resulting in constitutive phosphorylation of TbCentrin2 at the bilobe. Altogether, these results identified a basal body protein as a TbPLK substrate and its essential role in promoting basal body segregation and flagellum attachment zone filament assembly for flagellum adhesion and cytokinesis initiation.

The unicellular parasite *Trypanosoma brucei* shuttles between its definitive host, the tsetse fly, and various mammals including humans. In the fly digestive tract, *T. brucei* must first migrate to the ectoperitrophic space, establish a persistent infection of the midgut and then migrate to the salivary glands before being transmitted to a new mammalian host. In 2010, it was shown that insect stages of the parasite (procyclic forms) exhibit social motility (SoMo) when cultured on a semi-solid surface, and it was postulated that this behaviour might reflect a migration step in the tsetse fly. Now, almost 5 years after the initial report, several new publications shed some light on the biological function of SoMo and provide insights into the underlying signalling pathways.


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The parasitic flagellate *Trypanosoma vivax* is a cause of animal trypanosomiasis across Africa and South America. The parasite has a digenetic life cycle, passing between mammalian hosts and insect vectors, and a series of developmental forms adapted to each life cycle stage. Each point in the life cycle presents radically different challenges to parasite metabolism and physiology and distinct host interactions requiring remodelling of the parasite cell surface. Transcriptomic and proteomic studies of the related parasites *T. brucei* and *T. congolense* have shown how gene expression is regulated during their development. New methods for *in vitro* culture of the *T. vivax* insect stages have allowed us to describe global gene expression
throughout the complete *T. vivax* life cycle for the first time. We combined transcriptomic and proteomic analysis of each life stage using RNA-seq and mass spectrometry respectively, to identify genes with patterns of preferential transcription or expression. While *T. vivax* conforms to a pattern of highly conserved gene expression found in other African trypanosomes, (e.g. developmental regulation of energy metabolism, restricted expression of a dominant variant antigen, and expression of “Fam50” proteins in the insect mouthparts), we identified significant differences in gene expression affecting metabolism in the fly and a suite of *T. vivax*-specific genes with predicted cell-surface expression that are preferentially expressed in the mammal (“Fam29, 30, 42”) or the vector (“Fam34, 35, 43”). *T. vivax* differs significantly from other African trypanosomes in the developmentally-regulated proteins likely to be expressed on its cell surface and thus, in the structure of the host-parasite interface. These unique features may yet explain the species differences in life cycle and could, in the form of bloodstream-stage proteins that do not undergo antigenic variation, provide targets for therapy.


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SCYX-7158, an oxaborole, is currently in Phase I clinical trials for the treatment of human African trypanosomiasis. Here we investigate possible modes of action against *Trypanosoma brucei* using orthogonal chemo-proteomic and genomic approaches. SILAC-based proteomic studies using an oxaborole analogue immobilised onto a resin were used either in competition with a soluble oxaborole or an immobilised inactive control to identify thirteen proteins common to both strategies. Cell-cycle analysis of cells incubated with sub-lethal concentrations of an oxaborole identified a subtle but significant accumulation of G2 and > G2 cells. Given the possibility of compromised DNA fidelity, we investigated long-term exposure of *T. brucei* to oxaboroles by generating resistant cell lines in vitro. Resistance proved more difficult to generate than for drugs currently used in the field, and in one of our three cell lines was unstable. Whole-genome sequencing of the resistant cell lines revealed single nucleotide polymorphisms in 66 genes and several large-scale genomic aberrations. The absence of a simple consistent mechanism among resistant cell lines and the diverse list of binding partners from the proteomic studies suggest a degree of polypharmacology that should reduce the risk of resistance to this compound class emerging in the field. The combined genetic and chemical
biology approaches have provided lists of candidates to be investigated for more detailed information on the mode of action of this promising new drug class.


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Trypanosomatidae are a dangerous family of Euglenobionta parasites that threaten the health and economy of millions of people around the world. More precisely describing the population biology and reproductive mode of such pests is not only a matter of pure science, but can also be useful for understanding parasite adaptation, as well as how parasitism, specialization (parasite specificity), and complex life cycles evolve over time. Studying this parasite's reproductive strategies and population structure can also contribute key information to the understanding of the epidemiology of associated diseases; it can also provide clues for elaborating control programs and predicting the probability of success for control campaigns.
Tsetse and Trypanosomosis Information

(such as vaccines and drug therapies), along with emergence or re-emergence risks. Population genetics tools, if appropriately used, can provide precise and useful information in these investigations. In this paper, we revisit recent data collected during population genetics surveys of different Trypanosoma species in sub-Saharan Africa. Reproductive modes and population structure depend not only on the taxon but also on the geographical location and data quality (absence or presence of DNA amplification failures). We conclude on issues regarding future directions of research, in particular vis-a-vis genotyping and sampling strategies, which are still relevant yet, too often, neglected issues.


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Trypanosoma brucei, the causative agent of the African sleeping sickness of 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 comparative and evolutionary interesting 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.


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KREPB5 is an essential component of approximately 20S editosomes in Trypanosoma brucei which contains a degenerate, noncatalytic RNase III domain. To explore the function of this protein, we used a novel approach to make and screen numerous conditional null T. brucei bloodstream form cell lines that express randomly mutagenized KREPB5 alleles. We identified nine single amino acid substitutions that could not complement the conditional loss of wild-type KREPB5. Seven of these were within the RNase III domain, and two were in the C-terminal region that has no homology to known motifs. Exclusive expression of these mutated KREPB5 alleles in the absence of wild-type allele expression resulted in growth inhibition, the loss of approximately 20S editosomes, and inhibition of RNA editing in BF cells. Eight of these mutations were lethal in bloodstream form parasites but not in procyclic-form parasites, showing that multiple domains function in a life cycle-dependent manner. Amino acid changes at a substantial number of positions, including up to 7 per allele, allowed complementation and thus did not block KREPB5 function. Hence, the degenerate RNase III domain and a newly
identified domain are critical for KREPB5 function and have differential effects between the life cycle stages of *T. brucei* that differentially edit mRNAs.


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Rhodesain, the major cathepsin L-like cysteine protease in the protozoan *Trypanosoma brucei rhodesiense*, the causative agent of African sleeping sickness, is a well-validated drug target. In this work, we used a fragment-based approach to identify inhibitors of this cysteine protease, and identified inhibitors of *T. brucei*. To discover inhibitors active against rhodesain and *T. brucei*, we screened a library of covalent fragments against rhodesain and conducted preliminary SAR studies. We envision that *in vitro* enzymatic assays will further expand the use of the covalent tethering method, a simple fragment-based drug discovery technique to discover covalent drug leads.


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*Trypanosoma brucei* is a uniflagellated protist and the causative agent of African trypanosomiasis, a neglected tropical disease. The single flagellum of *T. brucei* is essential to a number of cellular processes such as motility, and has been a longstanding focus of scientific enquiry. A number of cytoskeletal structures are associated with the flagellum in *T. brucei*, and one such structure—a multiprotein complex containing the repeat motif protein TbMORN1—is the focus of this review. The TbMORN1-containing complex, which was discovered less than ten years ago, is essential for the viability of the mammalian-infective form of *T. brucei*. The complex has an unusual asymmetric morphology, and is coiled around the flagellum to form a hook shape. Proteomic analysis using the proximity-dependent biotin identification (BioID) technique has elucidated a number of its components. Recent work has uncovered a role for TbMORN1 in facilitating protein entry into the cell, thus providing a link between the cytoskeleton and the endomembrane system. This review summarises the extant data on the complex, highlights the outstanding questions for future enquiry, and provides speculation as to its possible role in a size-exclusion mechanism for regulating protein entry. The review additionally clarifies the nomenclature associated with this topic, and proposes the adoption of the term "hook complex" to replace the former name "bilobe" to describe the complex.

The parasite *Trypanosoma brucei* lives in the bloodstream of infected mammalian hosts, fully exposed to the adaptive immune system. It relies on a very high rate of endocytosis to clear bound antibodies from its cell surface. All endo- and exocytosis occur at a single site on its plasma membrane, an intracellular invagination termed the flagellar pocket. Coiled around the neck of the flagellar pocket is a multiprotein complex containing the repeat motif protein *T. brucei* MORN1 (TbMORN1). In this study, the phenotypic effects of TbMORN1 depletion in the mammalian-infective form of *T. brucei* were analysed. Depletion of TbMORN1 resulted in a rapid enlargement of the flagellar pocket. Dextran, a polysaccharide marker for fluid phase endocytosis, accumulated inside the enlarged flagellar pocket. Unexpectedly, however, the proteins concanavalin A and bovine serum albumin did not do so, and concanavalin A was instead found to concentrate outside it. This suggests that TbMORN1 may have a role in facilitating the entry of proteins into the flagellar pocket.


During natural *Trypanosoma brucei* infections, the parasites differentiate spontaneously into a non-dividing "stumpy" form when a certain level of parasitaemia is attained. This form is metabolically adapted for rapid further differentiation into procyclic forms upon uptake by tsetse flies. We describe here four central Ugandan isolates of *Trypanosoma brucei rhodesiense* that have undergone only three rodent passages since isolation from human patients. As expected, SNP analysis shows that these isolates are more closely related to each other than to the commonly used strains Lister 427, Antat1.1, and TREU927. TREU927 generally has smaller copy numbers of repeated genes than the other strains, while Lister 427 trypanosomes with a 30-year history of *in vitro* culture and cloning have more histone genes than the other isolates. The recently isolated trypanosomes were grown in rats, and their transcriptomes characterised. In comparison with cultured procyclic and bloodstream forms, there were increases in mRNAs encoding the stumpy-form markers ESAG9 and PIP39, with coordinated alterations in the levels of over 600 additional mRNAs. Numerous mRNAs encoding proteins of no known function were either increased or decreased. The products of the mRNAs that were increased in parallel with PIP39 included not only enzymes of procyclic-form metabolism, but also components of the translational and RNA control machineries. Many of the mRNAs that were decreased in cells with elevated PIP39 reflected reduced cell division. These transcriptomes suggest new avenues for research into the regulation of trypanosome differentiation.
Sphingolipids are important constituents of cell membranes and also serve as mediators of cell signalling and cell recognition. Sphingolipid metabolites such as sphingosine-1-phosphate and ceramide regulate signalling cascades involved in cell proliferation and differentiation, autophagy, inflammation, and apoptosis. Little is known about how sphingolipids and their metabolites function in single-celled eukaryotes. In the present study, we investigated the role of sphingosine kinase (SPHK) in the biology of the protozoan parasite Trypanosoma brucei, the agent of African sleeping sickness. T. brucei SPHK (TbSPHK) is constitutively but differentially expressed during the life cycle of T. brucei. Depletion of TbSPHK in procyclic-form T. brucei causes impaired growth and attenuation in the G1/S phase of the cell cycle. TbSPHK-depleted cells also develop organelle positioning defects and an accumulation of tyrosinated alpha-tubulin at the elongated posterior end of the cell, known as the "nozzle" phenotype, caused by other molecular perturbations in this organism. Our studies indicate that TbSPHK is involved in G1-to-S cell cycle progression, organelle positioning, and maintenance of cell morphology. Cytotoxicity assays using TbSPHK inhibitors revealed a favourable therapeutic index between T. brucei and human cells, suggesting TbSPHK to be a novel drug target.


Malaria and African trypanosomiasis are tropical diseases caused by the protozoa Plasmodium and Trypanosoma, respectively. The parasites undergo complex life cycles in the mammalian host and insect vector, during which they are exposed to oxidative and nitrosative challenges induced by the host immune system and endogenous processes. Attacking the parasite's redox metabolism is a target mechanism of several known antiparasitic drugs and a promising approach to novel drug development. Apart from this aspect, oxidation of cysteine residues plays a key role in protein-protein interaction, metabolic responses to redox events, and signalling. Understanding the role and dynamics of reactive oxygen species and thiol switches in regulating cellular redox homeostasis is crucial for both basic and applied biomedical approaches. Numerous techniques have therefore been established to detect redox changes in parasites including biochemical methods, fluorescent dyes, and genetically encoded probes. In this review, we aim to give an insight into the characteristics of redox networks in...
the pathogens *Plasmodium* and *Trypanosoma*, including a comprehensive overview of the consequences of specific deletions of redox-associated genes. Furthermore, we summarize mechanisms and detection methods of thiol switches in both parasites and discuss their specificity and sensitivity.


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**Abstract not provided.**


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African trypanosomes express three virtually identical glutathione peroxidase (Px)-type enzymes that occur in the cytosol (Px I and II) and mitochondrion (Px III) and detoxify fatty acid-derived hydroperoxides. Selective deletion of the genes revealed that procyclic *Trypanosoma brucei* lacking either the cytosolic or mitochondrial enzyme proliferate nearly as wild-type parasites, whereas the knockout of the complete genomic locus is lethal. Flow cytometry and immunofluorescence analyses revealed that the Px I-III-deficient parasites lose their mitochondrial membrane potential, which is followed by a loss of the lysosomal signal but not the glycosomal one. Mitochondrial damage and cell lysis are prevented by Trolox, ubiquinone derivatives and the iron chelator deferoxamine, whereas starch-deferoxamine is inefficient. In glucose-rich medium, cell death is attenuated suggesting that oxidants generated by the respiratory chain contribute to the lethal phenotype. Thus, the Px-type peroxidases protect procyclic cells from an iron-mediated oxidative membrane damage that originates at the mitochondrion. This contrasts with the situation in bloodstream cells, where the lysosome is the primarily affected organelle. Strikingly, either the cytosolic or the mitochondrial form of the peroxidases is required and sufficient to protect the mitochondrion and prevent cell lysis.


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Trypanosoma brucei, the causative agent of African sleeping sickness, is transmitted to its mammalian host by the tsetse. In the fly, the parasite's surface is covered with invariant procyclin, while in the mammal it resides extracellularly in its bloodstream form (BF) and is densely covered with highly immunogenic variant surface glycoprotein (VSG). In the BF, the parasite varies this highly immunogenic surface VSG using a repertoire of ~2500 distinct VSG genes. Recent reports in mammalian systems point to a role for histone acetyl-lysine recognizing bromodomain proteins in the maintenance of stem cell fate, leading us to hypothesize that bromodomain proteins may maintain the BF cell fate in trypanosomes. Using small-molecule inhibitors and genetic mutants for individual bromodomain proteins, we performed RNA-seq experiments that revealed changes in the transcriptome similar to those seen in cells differentiating from the BF to the insect stage. This was recapitulated at the protein level by the appearance of insect-stage proteins on the cell surface. Furthermore, bromodomain inhibition disrupts two major BF-specific immune evasion mechanisms that trypanosomes harness to evade mammalian host antibody responses. First, monoallelic expression of the antigenically varied VSG is disrupted. Second, rapid internalization of antibodies bound to VSG on the surface of the trypanosome is blocked. Thus, our studies reveal a role for trypanosome bromodomain proteins in maintaining bloodstream stage identity and immune evasion. Importantly, bromodomain inhibition leads to a decrease in virulence in a mouse model of infection, establishing these proteins as potential therapeutic drug targets for trypanosomiasis. Our 1.25Å resolution crystal structure of a trypanosome bromodomain in complex with I-BET151 reveals a novel binding mode of the inhibitor, which serves as a promising starting point for rational drug design.


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Variations on the statement "the variant surface glycoprotein (VSG) coat that covers the external face of the mammalian bloodstream form of *Trypanosoma brucei* acts a physical barrier" appear regularly in research articles and reviews. The concept of the impenetrable VSG coat is an attractive one, as it provides a clear model for understanding how a trypanosome population persists; each successive VSG protects the plasma membrane and is immunologically distinct from previous VSGs. What is the evidence that the VSG coat is an impenetrable barrier, and how do antibodies and other extracellular proteins interact with it? In this review, the nature of the extracellular surface of the bloodstream form trypanosome is described, and past experiments that investigated binding of antibodies and lectins to trypanosomes are analysed using knowledge of VSG sequence and structure that was unavailable when the experiments were performed. Epitopes for some VSG monoclonal antibodies are mapped as far as possible from previous experimental data, onto models of VSG structures. The binding of lectins to some, but not to other, VSGs is revisited with more recent knowledge of the location and nature of N-linked oligosaccharides. The conclusions are: (i) much of the variation observed in earlier experiments can be explained by the identity of the individual VSGs. (ii) much of an individual VSG is accessible to antibodies, and the barrier that prevents access to the cell surface is probably at the base of the VSG N-terminal domain,
approximately 5 nm from the plasma membrane. This second conclusion highlights a gap in our understanding of how the VSG coat works, as several plasma membrane proteins with large extracellular domains are very unlikely to be hidden from host antibodies by VSG.


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In trypanosomatids, the RNA polymerase I (RNAPI)-dependent promoters controlling the ribosomal RNA (rRNA) genes have been well identified. Although the RNAPI transcription machinery recognizes the DNA conformation instead of the DNA sequence of promoters, no conformational study has been reported for these promoters. Here we present the *in silico* analysis of the intrinsic DNA curvature of the rRNA gene core promoters in *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania major*. We found that, in spite of the absence of sequence conservation, these promoters hold conformational properties similar to other eukaryotic rRNA promoters. Our results also indicated that the intrinsic DNA curvature pattern is conserved within the *Leishmania* genus and also among strains of *T. cruzi* and *T. brucei*. Furthermore, we analysed the impact of point mutations on the intrinsic curvature and their impact on the promoter activity. Furthermore, we found that the core promoters of protein-coding genes transcribed by RNAPI in *T. brucei* show the same conserved conformational characteristics. Overall, our results indicate that DNA intrinsic curvature of the rRNA gene core promoters is conserved in these ancient eukaryotes and such conserved curvature might be a requirement of RNAPI machinery for transcription of not only rRNA genes but also protein-coding genes.


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Here we report that the gamma-tubulin complex in *T. brucei* is composed of gamma-tubulin and three GCP proteins, GCP2-GCP4, and is primarily localized in the basal body throughout the cell cycle. Depletion of GCP2 and GCP3, but not GCP4, disrupted the axonemal central pair microtubules, but not the subpellicular microtubules and the spindle microtubules. Furthermore, we showed that the gammaTuSC is required for assembly of two central pair proteins and that gammaTuSC subunits are mutually required for stability. Together, these results identified an unusual gamma-tubulin complex in *T. brucei*, uncovered an essential role of gammaTuSC in central pair protein assembly, and demonstrated the interdependence of individual gammaTuSC components for maintaining a stable complex.
The protist parasite *Trypanosoma brucei* causes Human African trypanosomiasis (HAT), which threatens millions of people in sub-Saharan Africa. Without treatment the infection is almost always lethal. Current drugs for HAT are difficult to administer and have severe side effects. Together with increasing drug resistance this results in urgent need for new treatments. *T. brucei* and other trypanosomatid pathogens require a distinct form of post-transcriptional mRNA modification for mitochondrial gene expression. A multi-protein complex called the editosome cleaves mitochondrial mRNA, inserts or deletes uridine nucleotides at specific positions and re-ligates the mRNA. RNA editing ligase 1 (REL1) is essential for the re-ligation step and has no close homolog in the mammalian host, making it a promising target for drug discovery. However, traditional assays for RELs use radioactive substrates coupled with gel analysis and are not suitable for high-throughput screening of compound libraries. Here we describe a fluorescence-based REL activity assay. This assay is compatible with a 384-well microplate format and sensitive, satisfies statistical criteria for high-throughput methods and is readily adaptable for other polynucleotide ligases. We validated the assay by determining kinetic properties of REL1 and by identifying REL1 inhibitors in a library of small, pharmacologically active compounds.


Recently we identified multiple suramin-sensitivity genes with a genome wide screen in *Trypanosoma brucei* that includes the invariant surface glycoprotein ISG75, the adaptin-1 (AP-1) complex and two deubiquitylating enzymes (DUBs) orthologous to ScUbp15/HsHAUSP1 and pVHL-interacting DUB1 (type I), designated TbUsp7 and TbVdu1, respectively. Here we have examined the roles of these genes in trafficking of ISG75, which appears key to suramin uptake. We found that, while AP-1 does not influence ISG75 abundance, knockdown of TbUsp7 or TbVdu1 leads to reduced ISG75 abundance. Silencing TbVdu1 also reduced ISG65 abundance. TbVdu1 is a component of an evolutionarily conserved ubiquitylation switch and responsible for rapid receptor modulation, suggesting similar regulation of ISGs in *T. brucei*. Unexpectedly, TbUsp7 knockdown also blocked endocytosis. To integrate these observations we analysed the impact of TbUsp7 and TbVdu1 knockdown on the global proteome using SILAC. For TbVdu1, ISG65 and ISG75 are the only significantly modulated proteins, but for TbUsp7 a cohort of integral membrane proteins, including the acid phosphatase MBAP1, that is required for endocytosis, and additional ISG-related proteins are down-regulated. Furthermore, we find increased expression of the ESAG6/7 transferrin receptor and ESAG5, likely resulting from decreased endocytic activity. Therefore, multiple ubiquitylation pathways, with a complex interplay with trafficking pathways, control surface proteome expression.