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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 (FAO) of the United Nations, the International Atomic Energy Agency (IAEA), the Inter-African Bureau for Animal Resources of the African Union (AU-IBAR), the World Health Organization (WHO), the Research Department for Livestock Production and Veterinary Medicine of the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD-EMVT) and the British Government's Department for International Development (DFID).

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Since the value of this information service depends to a great extent on the receipt of relevant material from research workers, campaign planners and organizers and field workers themselves, readers are requested to submit news items and copies of scientific papers and reports to the Editor: Dr James Dargie, Brunnstubengasse 43, 2102 Bisamberg, Austria (tel. +43 2262 61735; e-mail j.dargie@aon.at).

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

Distribution dates and copy deadlines

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ABBREVIATIONS USED IN TTI

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

Organizations

ANDE	Agence Nationale de Développement de l'Elevage
AU	African Union
AU/STRC	African Union/Scientific, Technical and Research Commission
BICOT	Biological Control of Tsetse by the Sterile Insect Technique
CEBV	Communauté Economique du Bétail et de la Viande
CEMV	Centre Universitaire de Formation en Entomologie Médicale et Vétérinaire
CGIAR	Consultative Group on International Agricultural Research
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CIRAD-EMVT	Département d'Elevage et de Médecine Vétérinaire des Pays Tropicaux du CIRAD
CIRDES	Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide
CNERV	Centre National d'Elevage et de Recherches Vétérinaires
CNRS	Centre National de Recherche Scientifique
CREAT	Centre de Recherche et d'Elevage, Avétonou, Togo
CRSSA	Centre de Recherches du Service de Santé des Armées Emile Pardé
CTVM	Centre for Tropical Veterinary Medicin
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
GTZ	Deutsche Gesellschaft für Technische Zusammenarbeit
IAEA	International Atomic Energy Agency
IBAR	Interafrican Bureau for Animal Resources
ICIPE	International Centre of Insect Physiology and Ecology
ICPTV	Integrated Control of Pathogenic Trypanosomes and their Vectors
IFAD	International Fund for Agricultural Development
ILRI	International Livestock Research Institute
INRA	Institut National de Recherche Agronomique
IPR	Institut Pierre Richet
IRD	Institut de Recherche et de Développement (formerly ORSTOM)
ISCTRC	International Scientific Council for Trypanosomiasis Research and Control
ISRA	Institut Sénégalais de Recherches Agricoles
ITC	International Trypanotolerance Centre
KARI	Kenya Agricultural Research Institute
KETRI	Kenya Trypanosomiasis Research Institute
LCV	Laboratoire Central Vétérinaire
LNERV	Laboratoire National de l'Elevage et de Recherches Vétérinaires
LSHTM	London School of Hygiene and Tropical Medicine
MRC	Medical Research Council
MRU	Mano River Union
NITR	Nigerian Institute for Trypanosomiasis Research
NRI	Natural Resources Institute
OCCGE	Organisation de Coopération et de Coordination pour la Lutte contre les Grande Endémies
OCEAC	Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale
OGAPROV	Office Gabonais pour l'Amélioration de la Production de la Viande
OIE	Office International des Epizooties
OMVG	Organisation pour la Mise en Valeur du Fleuve Gambie
PAAT	Programme against African 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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SECTION A – NEWS

FROM THE AFRICAN UNION

32ND CONFERENCE OF THE INTERNATIONAL SCIENTIFIC COUNCIL FOR TRYPANOSOMIASIS RESEARCH AND CONTROL (ISCTRC) 8-12 SEPTEMBER 2013, KHARTOUM, SUDAN

Held at the Friendship Hall, Khartoum, the theme of the Conference was "Tsetse and Trypanosomiasis Research and Control for Sustainable Agricultural and Rural Development: Promoting Partnership and Learning Agenda in the Context of African Renaissance". Over 200 participants attended the meeting, drawn from 22 African Member States, the African Union Commission, universities and research Institutions from all over the world, the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the International Atomic Energy Agency (IAEA), the Foundation for Innovative New Diagnostics (FIND), the Drugs for Neglected Diseases (DNDi) and the Global Alliance for Livestock Veterinary Medicine (GALVmed).

The Conference was officially opened by the First Vice President of the Republic of the Sudan, His Excellency Ali Osman Muhammed Taha. Other dignitaries who also graced the Conference included the Minister for Livestock, Fisheries and Rangeland, Dr Faysal Hassan Ibrahim; The African Union Commission Commissioner for Rural Economy and Agriculture, Madam Rhoda Peace Tumusiime; the Director of African Union Inter-African Bureau for Animal Resources (AU-IBAR), Prof. Ahmed Elsawalhy; and the Coordinator of the African Union Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), Dr Hassane Mahamat.

The keynote speaker, His Excellency, Ali Osman Muhammed Taha, underscored the importance of the joint African Cooperation and the role of Pan Africanism in the development of Africa. He accentuated the contribution of livestock to food security and consequently the obligation of African countries to protect livestock from animal diseases. He encouraged the ISCTRC platform to continue providing the vital information that would aid the fight against T&T. The Minister for Livestock, Fisheries and Rangeland, Dr Faysal Hassan Ibrahim, highlighted the negative impact of trypanosomiasis on rural development of the Sudan and urged scientists and development partners to harness their energies and resources towards a sustainable solution to the problem. The Director of AU-IBAR Prof. Ahmed El-Sawalhy, outlined the five broad objectives of the Conference as being: information sharing, review of T&T control technologies, strategies and policy options, identification of research gaps, and to propose recommendations for the next two years. He also expressed his sincere appreciation for the excellent preparations made by the Government of the Sudan in hosting the Conference.

The Conference was further inspired through the keynote address headlined "Tsetse and Trypanosomiasis Research and Control for Sustainable Agricultural and Rural Development: Promoting Partnership and Learning Agenda in the Context of African Renaissance" made by the African Union Commission, Commissioner for Rural Economy, Madam Rhoda Peace Tumusiime. It was appreciated that the theme of the Conference was perfectly aligned with the on-going initiative of developing the Africa Agenda 2063 on a Shared Strategic Framework for Inclusive Growth and Sustainable Development. The Conference was informed that through this agenda, Africa sought to achieve three objectives: to agree on the

Africa we want; to set milestones and concrete strategies on how we will get there; and to define the role each of us should play to achieve this agenda.

It was observed that tsetse and trypanosomosis was costing the continent an estimated US$5 billion annually resulting from losses attributable to animal African typanosomosis (AAT). In addition, despite a decline in the number of human sleeping sickness cases reported, the condition continued to cause a strain on public health services in many areas of rural Africa. Participants were petitioned to leverage the opportunity to reflect on the progress made so far in the fight against the scourge of trypanosomosis. In this regard, AU-PATTEC was commended for initiating projects that had yielded significant benefits to rural communities.

To sustain this momentum, African countries were persuaded to commit to two initiatives:

- Sign up to the National and Regional Comprehensive Africa Agriculture Development Programme (CAADP) to ensure that T&T receives enough attention and support;
- To include T&T control in their poverty reduction strategy papers.

The Conference was also motivated through deliberations on 95 papers and posters that encompassed T&T research and control activities in Member States, reports from international organizations, and sessions on: human African trypanosomiasis, animal African trypanosomosis, *Glossina* biology, control and eradication, and socio-economics, environment and land use. These reports and papers are available on the AU-IBAR website at: http://www.au-ibar.org/events/au-ibar/232-32nd-general-conference-of-the-isctrc.

The meeting appreciated that despite the progress made, capacity gaps still exist for the control and elimination of tsetse and trypanosomosis. It was also observed that the number of young African scientists that had taken keen interest in T&T research and publications had increased. It was encouraging to note the increasing synergy between actors in endemic countries, international organizations, research institutions, development partners and the private sector in the fight against T&T. This state of affairs had been facilitated by the good advocacy carried out by AU-PATTEC.

The meeting noted the progress that was being made in the development of new diagnostic tools and new therapeutics such as tsetse genetic profiling as a step towards the development of area-wide tsetse control and/or eradication. Interest in the use of non-invasive methods for the early detection of second stage HAT continued to take a centre stage. The meeting noted with appreciation the contribution of countries towards the elimination of HAT as evidenced by the declining incidence of the disease. Endemic countries were, however, cautioned not to relent on HAT surveillance, and to ensure proper combination of drugs and dosages for the treatment of HAT.

The Conference noted a general trend towards the adoption of an integrated approach to the management of T&T using methods that are appropriate for given circumstances. Some of the methods include the use of the sequential aerosol technique (SAT), ground spraying, insecticide impregnated targets and screens, sterile insect technique (SIT), and chemotherapy. These efforts had been targeted at improving both human and animal health. Suggestions were made to involve the United Nations Environment Programme (UNEP) in the formulation and implementation of tsetse programmes. Participants also called on AU-PATTEC to play a leading role in identifying management and resource challenges facing member countries in the fight against T&T and to propose sustainable ways of addressing these challenges.

The closing ceremony was officiated by The Minister for Livestock, Fisheries and Rangeland, Dr Faysal Hassan Ibrahim, the Director of AU-IBAR, Prof. Ahmed Elsawalhy, the Coordinator of AU-PATTEC, Dr Hassane Mahamat, and the incoming Chairman of ISCTRC, Prof. Ahmed Hussien Abdel Rahman.

Speaking at the closing ceremony, the incoming Chairman of ISCTRC, thanked the outgoing Chairman Dr Sadou Maiga for steering the ISCTRC over the last two years. He said that ISCTRC Conferences provided an environment for sharing knowledge on T&T and he equated the ISCTRC Conference to an intensive training course on T&T. He further said that ISCTRC provided the PATTEC initiative with a strong scientific input. He thanked the First Vice President of the Sudan, His Excellency Ali Osman Muhammed Taha and the Minister for Livestock, Fisheries and Rangeland, Dr Faysal Hassan Ibrahim for their support. He further thanked the National Organizing Committee Members for their hard work and everyone who contributed to the success of the meeting.

On his part, Prof. Ahmed Elsawalhy thanked the Government and people of the Sudan for hosting the 32nd ISCTRC General Conference. He also thanked the National Organizing Committee and the ISCTRC Executive Committee for working tirelessly to make the event possible. He appreciated the participation of Member States, universities and research institutions, and of regional and international organizations. He observed that the Conference had achieved its set objectives and said that AU-IBAR would work with all the stakeholders in addressing the recommendations made during the Conference.

While closing the meeting, the Minister for Livestock, Fisheries and Rangeland, Dr Faysal Hassan Ibrahim thanked all the dignitaries for participating in the Conference. He paid special tributes to the scientists who were awarded certificates for having displayed winning posters. He said that he was pleased to note that women were in the forefront in the fight against T&T as witnessed by the number of women who received the certificates. He said that the recommendations made at the meeting should be implemented at the grassroots as the stakeholders expected fruits from the Conference. He further said that the T&T plague required national and international unity to address and was pleased to note that scientists working on T&T were unified as demonstrated during the Conference. He said that socio-economic development goes beyond political borders and political obstacles. In thanking the ISCTRC for having honoured the Sudan to host its 32nd Conference, he reiterated his country's commitment to jointly with other Arab countries, support AU-PATTEC to mobilize the necessary resources and to work with neighbouring countries on T&T control.

12TH PATTEC COORDINATORS MEETING. 25–27 NOVEMBER 2013, DAKAR, SENEGAL

Within the framework of its mandate and role in initiating action and providing technical support for implementing the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), the PATTEC Coordination Office in collaboration with the Government of Senegal organized the 12th PATTEC Coordinators and Focal Points' meeting and a field visit to the PATTEC project sites to meet members of the rural communities benefiting from the project's activities in Senegal.

About 70 National PATTEC Coordinators and Focal Points from 29 African countries, representatives of international organizations, research institutions and private and public partners attended the meeting.

Participants of the PATTEC Coordinators Meeting

The specific objectives of the meeting included: harmonizing country level programme performance indicators; building synergy among country results; sharing experiences/elaborating on challenges; sharing best practices to boost implementation; identifying global problems; and tracking of cross-cutting issues and revised work plans and budgets.

In his opening remarks, Dr Hassane H. Mahamat, AU-PATTEC Coordinator conveyed messages of best wishes from the African Union Commission Chairperson, H. E. Dr Nkosazana Dlamini-Zuma and the Commissioner for Rural Economy and Agriculture, H. E. Mrs Tumusiime Rhoda Peace. He hailed the collective efforts being made to address the T&T challenge and thanked the Government of the Republic of Senegal for hosting the meeting and resource partners for their continued support. He reminded delegates that the objectives of the meeting were to review progress towards the PATTEC Initiative and to highlight and discuss lessons learned and challenges being faced. He also reiterated that the T&T challenge requires African solutions and that this meeting is timely as it comes during the period when Africa is commemorating the 50th Anniversary of the founding of the OAU/AU. He also noted that as Africa is developing the 2063 agenda, this meeting should be aiming at developing strategies that contribute to sustainable agricultural development and growth of which livestock is a key component. Dr Hassane further noted that despite the numerous successes that have been registered to date, more needs to be done to address the T&T challenge. He urged T&T affected countries to integrate T&T interventions into their national development policies. Dr Hassane also thanked the African Development Bank for taking a leading role in supporting the six countries that were identified in the first phase of the multinational project to create tsetse free areas. He also thanked the IAEA for the support being rendered to Senegal. Dr Hassane underscored the importance of the new strategic alliances being promoted with the Arab Bank for Economic Development in Africa (BADEA).

In his remarks, the representative from the African Development Bank expressed his gratitude for the invitation extended to the Bank to participate in the meeting. He pledged the Bank's continued support for the fight against T&T and wished delegates successful deliberations.

The meeting was officially opened by the Honourable Papa Abdoulaye Seck, Ministère de l'agriculture et de l'équipement rural on behalf of Madame Aminata Mbengue Ndiaye, Ministre de l'elevage et des productions animales. He informed delegates that livestock deserves special attention in view of the role it plays in national economies. He also recognized the role of the AU-PATTEC Coordination Office in coordinating the PATTEC Initiative, and with specific reference to Senegal, he informed delegates that the national programme to support the PATTEC Initiative started in 2005 with the objective of progressively eliminating tsetse through the use of integrated approaches. He also observed that the programme is currently being extended to other areas. He proposed that this meeting should, among other things, use the opportunity to discuss lessons learned in order to inform future programmes. He thanked resource partners, notably the IAEA and the US Government for their support to Senegal.

Following the opening ceremony, the new office bearers of the meeting were elected as follows:

Dr Baba Sall, National Coordinator PATTEC Senegal was elected Chairman and was assisted by Dr Hassane H. Mahamat, AU-PATTEC Coordinator.
Dr. Seck Ismaila from Senegal, Mrs. Joyce Daffa from Tanzania, Dr. Lisette Kohagne from Cameroon and Dr. Gift Wanda from the PATTEC Coordination Office were elected rapporteurs.

The agenda was adopted after announcing the chairpersons of the various sessions of the meeting.

Dr Rafael Argiles Harrero, Joint FAO/IAEA Division, chaired the session covering presentations by the PATTEC Coordination Office and from T&T affected countries**.** The AU-PATTEC Coordination Office presented a report of the previous meeting and its progress report for 2013, followed by the PATTEC Senegal. Country progress reports were then received from Angola, Burundi, Equatorial Guinea, Ethiopia, Ghana, Guinea, Kenya, Gabon, Cameroon, Mali, Malawi, Nigeria, Senegal, South Sudan, Sudan, Tanzania, Uganda, Zambia, Zimbabwe, Niger in that order.

The country presentations revealed wide disparities with respect to the status of implementation of activities contributing to the PATTEC Initiative. The disparities ranged from countries that have no T&T Coordination Office and no project on the ground to those which have made big gains and are in the process of undertaking M&E activities in the areas where tsetse flies have either been suppressed to very low densities or completely eliminated. Some countries that have made big gains are planning to expand T&T activities to new areas. Notable examples of countries that have registered substantial gains include Kenya which has established a tsetse eradication council to oversee tsetse operations and sustaining T&T freedom where the vector and the disease were eliminated; Nigeria which has registered substantial suppression gains using its own internal resources; Ghana which is planning to expand the use of the Zero Fly Nets; and Ethiopia which is currently continuing with SIT operations in the Deme Valley. Others are Uganda which is following up on a funding request to BADEA for a follow–up project to one which drew to a close in December 2011; funding is anticipated for the Gabon national proposal to secure support by 2014; Tanzania has drawn up a new tsetse distribution map in readiness for a large scale tsetse operation; and Zimbabwe has demonstrated long term successes in protecting tsetse cleared areas from reinvasion while progressively expanding the area of coverage.

A common approach in border areas is being encouraged by a number of countries. In general, the majority of countries are seeking external support to initiate and sustain tsetse operations. To this effect, a number of countries have prepared national proposals, some of which have been validated by the AU-PATTEC Coordination Office. From the presentations, it was evident that those countries that have committed their own resources are generally more advanced in tsetse control than those solely dependent on external funding. All in all, resource mobilization remains the biggest challenge as it is evident that effective technologies do exist to control/eliminate tsetse.

Professor Josenando chaired the next session in which experts and partners presented case studies and activities in support of the PATTEC initiative. The following presentations were made: Kenya Tsetse and Trypanosomiasis Eradication Council (KENTTEC), Global Alliance for Livestock Veterinary Medicine (GaLVmed), WHO, Joint FAO/IAEA Programme, AU-IBAR/ISCTRC, OCEAC, FAO, OIE, Rapid Simplified Test, CIRDES, Bayer, and Chad.

A subsequent session on regional approaches to T&T strategies was chaired by Dr. James Wabacha, AU-IBAR. The following presentations were made during this session: Ethio-Sudan Joint T&T project, EAC Regional T&T Strategy, Ethio-Kenya Joint T&T project, Multinational project for the creation of tsetse free zone in West Africa, the Regional Project for Central Africa + Nigeria, and resource mobilization opportunities. It was noted that the regional programmes were at different stages of operationalization and in terms of levels of resources available.

The final session on special presentations was chaired by Dr Florencia Cipriano, Deputy Regional Representative of the OIE Regional Commission for Africa. Here, presentations included: development of standards for T&T interventions; ISCTRC Conference report; role of EANETT in support of PATTEC; lessons learned at Kality Mass Rearing Facility; SWOT analysis of the PATTEC/ISCTRC questionnaire; PATTEC training manual and the AU-PATTEC Work Plan 2014.

It was evident that there is goodwill towards the PATTEC Initiative and that most of the activities being undertaken are consistent with its ultimate objective.

At the conclusion of the meeting, participants made recommendations on a number of themes, notably, on the need to adhere to the harmonized report format regarding presentations on progress of national T&T interventions, and to develop clear follow up mechanisms on resource mobilization pledges made in previous conferences to avoid loss of programme momentum. In addition, participants agreed that the PATTEC Coordinators' meetings should not be held during the same year as the ISCTRC Conference to give time to gather more information and to make strategic use of resources. Regarding sustainable land management plans in tsetse freed areas, participants recommended that all T&T intervention strategies should have validated plans for sustainable land management and sustainable protection measures against re-invasion. The meeting also recommended that African OIE delegates should propose to the OIE General Session that a Technical Working Group be established to develop guidelines for the declaration of freedom from T&T.

2ND STEERING COMMITTEE MEETING OF AU-PATTEC

The second Steering Committee (SC) of the AU-PATTEC was held on 28 November 2013 back to back with the 12th PATTEC Coordinators' meeting at the Hotel des Almadies in Dakar. The key PATTEC Steering Committee members were present and the agenda was

fully discussed. The meeting was chaired by Dr Assefa Mebrate on behalf of the AUC Commissioner for Rural Economy and Agriculture, H. E. Mrs. Tumusiime Rhoda Peace.

The PATTEC Coordinator briefed Members of the Steering Committee (SC) on actions undertaken on specific issues. In his remarks, the PATTEC Coordinator informed the SC Members that while action had been taken on a number of issues, the inclusion of progress registered in individual T&T affected countries in the progress report of the PATTEC Coordination Office was still underway and would be ready at a later date. Further, he informed SC Members that the T&T budgets for countries would be included in next year's report. The SC Members were also informed that the ten years' progress report after the inception of the PATTEC Initiative was presented during the 32nd Conference of the ISCTRC. Presentations were also made from the PATTEC Coordination Office on:

- Report of the 12th Coordinators Meeting
- AU-PATTEC progress report, 2013
- AU-PATTEC Work Plan for 2014
- The PATTEC Training Manual
- Concepts on the development of international standards on T&T

SC members acknowledged the report on the outcome of the 12th Coordinators Meeting and endorsed the recommendations that had a direct bearing on the ToRs of the SC. The SC commended the AU-PATTEC Coordination Office for the remarkable achievements registered in spite of the lean team. The SC welcomed the positive developments demonstrated by the Coordinators Meeting in their resolve to support the development of international standards on tsetse and non-tsetse transmitted trypanosomiasis. In addition, the SC welcomed the recommendation on avoiding overlap between activities of the ISCTRC and the Coordinators Meeting. The SC also endorsed the long term vision of eradicating T&T and commended the PATTEC Coordination Office for initiating formal collaboration with the Regional Economic Communities (RECs) in the fight against T&T.

4TH AND 5TH TRAINING SESSIONS ON PATTEC MANAGEMENT

Since its establishment, the PATTEC Coordination Office initiated a number of activities in collaboration with affected countries and in cooperation with various partners. These included efforts to increase awareness about the cause and purposes of the PATTEC Initiative through the development and dissemination of publicity and public information materials; training to build the necessary technical capacities and competence to carry out activities in implementing PATTEC; development of tsetse eradication project proposals for specific areas; and seeking financial and technical support for executing such projects in the affected countries.

One of the most important pillars of the PATTEC initiative is the training of project personnel supporting PATTEC project activities without which the objective of eradicating tsetse and trypanosomiasis from Africa will not be possible. For this reason, the PATTEC Coordination Office has organised three training sessions since 2011 in both French and English.

The 4th training session on project planning and execution was held at the École de Lutte Anti Tsetse (ELAT) in Bobo-Dioulasso, Burkina Faso. Twenty-three participants from 12 countries attended this course.

FROM FAO

WORLD LIVESTOCK 2013. CHANGING DISEASE LANDSCAPES

Changing disease landscapes offers a comprehensive approach for the promotion of global health and gives greater emphasis on agro-ecological resilience, protection of biodiversity and efficient use of natural resources to ensure safer food supply chains. The publication examines why and how pathogens of animal origin have become a major global public health threat, and what might be done to mitigate this threat. The increasing dynamics of disease at the human–animal–ecosystem interface are explored against the backdrop of changing biophysical and social landscapes. Based on a Pressure–State–Response analysis framework, disease events are described in their agro-ecological and socio-economic contexts. Human demographic and economic developments are resulting in increased pressure on the earth's natural resources. Both play important roles in the ongoing transformation of farming and natural landscapes. A major feature is the expanding demand for milk, meat and eggs from the rapidly growing middle-income class across the globe. Changes in major land-use systems are assessed for the period 2000–2030, with particular attention to the main land-use dynamics where cropland is being converted to human settlements and related infrastructure; cropland is replacing pastoral systems and forested areas; and pastoral and cropland systems are encroaching onto forested areas. Areas prone to deforestation are highlighted as potential hotspots for the emergence in humans and livestock of pathogens originating from wildlife. The dynamics of food and agriculture are described as the main drivers of disease emergence, spread and persistence in both extensive and intensive livestock systems and in food supply chains. Livestock biomass distributions are assessed in conjunction with farming systems and land pressures to identify areas with enhanced human-livestock interfaces. Developments in South and East Asia – two areas of dynamic change in the livestock sector – are described in detail, focusing on the important smallholder dairy subsector in South Asia and the prominent poultry and pig subsectors are analysed in different geographic areas and for several livestock commodities, to trace possible animal and veterinary public health risks. Separate chapters discuss changes in the international trade of animals and animal products, and the ways in which this trade may have affected disease occurrence. The implications of climate change and the effects of globalization are also discussed. The evolution of animal health systems is assessed to identify failures and successes in disease control. Tentative livestock disease impact profiles are drawn up to illustrate how disease may interfere with the achievement of sustainable development targets, and to argue for a people-centred approach to health protection. The main impact domains considered are human health, livelihoods, economics and the environment. Particular attention is given to endemic disease burdens in humans and livestock, both in densely populated areas with very high land pressures and in remote dry lands and other harsh environments. The publication suggests the need for a paradigm shift in risk assessment, with more attention to a health-in-development approach that engages society at large and is built on analysis of the drivers of disease dynamics. Such analysis will be instrumental in defining preventive measures for countering disease emergence, spread and persistence. Four distinct driver disease complexes need to be addressed: poverty-related endemic disease burdens in humans and livestock; biological threats and biosafety challenges posed by globalization and climate change; food and agriculture-related veterinary public health threats; and the risk of disease agents jumping species from wildlife to livestock and humans. The preventive approach suggested relates disease dynamics and pathogen evolution

directly to human behaviour at all points of animal-source food value chains. The book can be downloaded at: http://www.fao.org/docrep/019/i3440e/i3440e.pdf.

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

This FAO project is funded by the Government of Italy and aims to assist trypanosomosis-affected countries and other stakeholders in their efforts to reduce the burden of the disease. The project hinges on four pillars: (i) capacity development, (ii) technical assistance, (iii) partnership, networking and knowledge sharing, and (iv) streamlining of the gender dimension. Focus is placed on data management and analysis, with an emphasis on Geographic Information Systems (GIS).

Six priority countries benefit from the project: Burkina Faso, Ethiopia, Ghana, Kenya, Mali and Uganda (i.e. those that first started implementing field interventions in the framework of the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), an initiative of the African Union (AU)). However, a number of additional countries are also being assisted. Other key beneficiaries include the World Health Organization (WHO), the International Atomic Energy Agency (IAEA), and the AU-PATTEC Coordination Unit. In particular, in order to better support the latter, the Project Officer was based in Addis Ababa (Ethiopia).

The project has already organized demand-driven courses on GIS and data management in Mali, Sudan and Zimbabwe (the latter in collaboration with IAEA), thereby training 40 people. Gender-balance has been actively promoted in all training activities. Requests for this type of training from other countries are welcome, and they should be addressed to Raffaele Mattioli (Raffaele.Mattioli@fao.org) and Giuliano Cecchi (Giuliano.Cecchi@fao.org).



Group photos of training courses held in Sudan, Mali, and Zimbabwe

The continental Atlas of tsetse and African animal trypanosomosis is being assembled, and technical assistance is being provided to countries that have an interest in developing national Atlases by adopting the FAO methodology. The project is also enabling FAO to continue assisting WHO in the upgrade, update and dissemination of the Atlas of human African trypanosomiasis (HAT).

FROM THE JOINT FAO/IAEA PROGRAMME

SENEGAL NEARS FIRST VICTORY IN WAR ON TSETSE FLY

A campaign against the tsetse fly, a pest that transmits a disease that devastates livestock, in the Niayes area near the capital Dakar has radically reduced the fly population and is paving the way for complete eradication. "Since the project started, there is already less disease. It has not only reduced the tsetse but also ticks, which cause lots of other diseases in the area. We have noticed over all better health of the herds," said Baba Sall, project manager and Head of the Animal Health Section in the Ministry of Livestock.

A multi-year programme of the Government of Senegal, with financial help from the United States and technical support from the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France, is slowly eradicating the tsetse fly using the sterile insect technique (SIT). The programme is supported by FAO through its joint division with the International Atomic Energy Agency in Vienna.

The Niayes area has a coastal micro-climate suitable for exotic breeds of cattle, the kind that produces more milk and meat than local stock. Unfortunately, the area is also home to the tsetse fly, which transmits trypanosomosis. Often lethal, the disease reduces fertility, weight gain, meat and milk production and makes the cattle too weak to be used for ploughing or transport. A government study estimates that when the tsetse fly is completely eradicated in the Niayes farmers will earn €1.2 million ($1.6 million) more per year because they will spend less on treating animals and earn more profit from selling milk and meat. The government has an ambitious plan to introduce exotic livestock breeds and create a modern meat and dairy sector in the area, which is near the capital Dakar.

The SIT is a form of pest control that uses radiation to sterilize male flies that are mass-produced in special rearing facilities. The sterile males are released systematically by air on a sustained and area-wide basis over tsetse-infested areas, where they mate with wild females. These do not produce offspring and, as a result, this technique can eventually eradicate populations of wild flies. Before the SIT can be used, the wild fly population needs to be suppressed to very low levels using other control methods. In the Senegal project area, this was achieved through applying pesticide directly to livestock, and the use of fly traps and netted fences around pig pens. Ground release of sterile males flies started in 2012 and air release in 2013 after three years of feasibility assessments, capacity building, preparation and testing. After six months, the fly population was suppressed by more than 99 percent. A further advantage of the technique is the fact that - after an initial phase of insecticide-based suppression - it does not require the use of pesticides and reduces environmental contamination.

"We expect to announce the tsetse fly eradicated in the first block in mid-2014. We have not captured any wild flies in our traps since March 2012 so they are almost finished there," said Sall. "After that we are going to attack blocks number 2 and 3 where we expect to achieve eradication in 2015 or early 2016. Two other zones will come next to continue the work we have started to eradicate the tsetse fly and trypanosomosis in Senegal," he said.

Jorge Hendrichs, chief of the FAO/IAEA SIT programme, says his team at the Insect Pest Control Laboratory at the FAO/IAEA Agriculture and Biotechnology Laboratories in Seibersdorf, Austria, offers a full service of applied research, training, field validation and operations and laboratory backup, with activities reinforcing each other in a type of "feedback loop." "For example, for the Senegal operation the sterile flies are mass-produced at the facility of the Centre International de Recherche-Développement sur l'Élevage en zone Subhumide (CIRDES) in Burkina Faso and shipped to Senegal", he said. "At first the flies arrived in bad condition. We had to improve quality control, make adjustments to shipment and logistics, establish back-up colonies of the fly and so on. Once we had it working properly, we fed that experience back into our overall programme and we can use the lessons learned elsewhere."

The FAO/IAEA SIT programme currently supports 14 African nations in their efforts to eradicate the tsetse fly.

Glossina pallidipes COLONY IN KALITY FACILITY ETHIOPIA FINALLY WITHOUT SALIVARY GLAND HYPERTROPHY

Since the first attempt in 1999 to establish a mass-rearing colony of *G. pallidipes* tsetse flies collected from Arba Minch, Ethiopia at the Insect Pest Control Laboratory (IPCL), Seibersdorf, Austria to support the STEP project, salivary gland hypertrophy (SGH) has been a major challenge for colony establishment and sustainability. One colony was lost at the IPCL and several colonies failed in the Kality facility. Over the last eight years the Joint FAO/IAEA Division conducted pioneering research to develop an effective SGH management strategy based on the use of an antiviral drug (valacyclovir) combined with modifications to the feeding system (“clean feeding” system reducing the number of times flies are exposed to the same batch of blood). The virus management package was transferred to the Kality facility and has been implemented since 2012. This has resulted in a significant decrease in the SGH prevalence in the *G. pallidipes* colony such that this no longer poses a problem to colony maintenance. Nevertheless, research is continuing to develop alternative strategies to be used in case the virus develops resistance to the antiviral drug.

THE FAO/IAEA TUTORIAL DVD ON USING OPEN SOURCE GIS TECHNIQUES IN INSECT PEST CONTROL PROGRAMMES

Area-wide insect pest control programmes rely on updated geospatial data for efficiently conducting and evaluating baseline data surveys and progress monitoring. Soft- and hardware available as geographic information system (GIS) packages are applied to analyse and understand these data for planning and implementing optimised pest intervention strategies. Many developing countries need to address major insect pest problems and face difficulties to fund licence extensions for commercial GIS-software. In recent years, the software development known as free open source software (FOSS) has made great strides in producing high quality software applications, and GIS is no exception.

Insect pest control programmes can now take advantage of the development of the GIS FOSS. The tutorial "Using Open Source GIS Techniques in Insect Pest Control Programmes", developed by the Joint FAO/IAEA Division, offers a practical hands-on learning experience. The video lessons include an introduction to GIS and provide special applied chapters for the use of GIS in programmes against fruit fly pests, screwworm flies and tsetse flies. The self-contained *LiveDVD* also includes a broad collection of pre-installed GIS software, ready to use in any computer without further installations.

The tutorial package is intended for use by senior personnel tasked with insect pest control and consists of an introductory pamphlet, a tutorial DVD with the video lessons and the GIS software, and a USB flash drive to be used throughout the tutorial for saving the work done.

DVDs are available upon request from r.argiles-herrero@iaea.org.

FROM WHO

CONTROL AND SURVEILLANCE OF HUMAN AFRICAN TRYPANOSOMIASIS. REPORT OF A WHO EXPERT COMMITTEE. TECHNICAL REPORT SERIES NO. 984

In the 1960s, it appeared that human African trypanosomiasis (HAT) could be effectively controlled, but by the beginning of the twenty-first century several decades of neglect had led to alarming numbers of reported new cases, with an estimated 300 000 people infected. The World Health Organization (WHO) responded with a series of initiatives aimed at bringing HAT under control again. Since 2001, the pharmaceutical companies that produce drugs for HAT have committed themselves to providing them free of charge to WHO for distribution for the treatment of patients. In addition, funds have been provided to WHO to support national sleeping sickness control programmes to boost control and surveillance of the disease. That, coupled with bilateral cooperation and the work of non-governmental organizations, helped reverse the upward trend in HAT prevalence. By 2012, the number of reported cases was fewer than 8 000. This success in bringing HAT under control led to its inclusion in the WHO Roadmap for eradication, elimination and control of neglected tropical diseases, with a target set to eliminate the disease as a public health problem by 2020. A further target has been set, by countries in which HAT is endemic, to eliminate *gambiense* HAT by reducing the incidence of infection to zero in a defined geographical area.

This 237-page report, with its eight Chapters and a set of Recommendations supported by 14 Annexes is quite simply a "must have" for all interested both in the specific topic of the human disease and in animal trypanosomosis, providing after an introduction, truly comprehensive and state of the art coverage of all relevant aspects by acknowledged authorities in their respective fields. Topics covered include information about the epidemiology of HAT, the parasite, vector and the disease, new diagnostic approaches, new therapeutic regimens and better understanding of the distribution of the disease with high-quality mapping. The roles of human and animal reservoirs and the tsetse fly vectors that transmit the parasites are emphasized. This new information has formed the basis for an integrated strategy with which it is hoped that elimination of *gambiense* HAT will be achieved. The report also contains recommendations on the approaches that will lead to elimination of the disease. It can be downloaded at http://apps.who.int/iris/bitstream/10665/95732/1/9789241209847_eng.pdf?ua=1.

FROM FIND

REPUBLIC OF GUINEA LAUNCHES NEW PROJECT TO IMPROVE DETECTION OF SLEEPING SICKNESS CASES IN THE COUNTRY'S COASTAL REGION

An innovative project to intensify surveillance of sleeping sickness in Guinea was launched on 15 January 2014 after the Ministry of Health of the Republic of Guinea signed a collaborative agreement with the Foundation for Innovative New Diagnostics (FIND). The aim of the partnership is to strengthen diagnostic capacity by introducing new tools in health centres located in the most endemic areas of the country, thus increasing the chances for early diagnosis of the disease. With more than 800 cases reported between 2000 and 2009, Guinea has the largest sleeping sickness (human African trypanosomiasis, HAT) burden in West Africa. Despite significant efforts over the last years that resulted in improved disease control, HAT remains endemic in the coastal region of the country. At present, most HAT cases are being diagnosed through mass screening of the population. However, due to limited funding and manpower, annual active screening campaigns can only cover a fraction of endemic areas, and passive screening is possible in only three health centres. Thus, most sleeping sickness patients remain undiagnosed until they are in the late stage of the disease. The Ministry of Health, with support from FIND, will strengthen the capacity of health centres in the districts of Forécariah, Dubréka and Boffa in the littoral part of the country, where the most active disease foci are located. Novel diagnostic tools and strategies will be implemented which will reduce the time taken between infection and confirmatory diagnosis of the disease, thereby increasing the chances of detecting cases in the early stage, when treatment is safer and more effective.

The project includes the introduction of three new diagnostic tests that work well in field conditions and require only minimal training to be used. The first one is a rapid diagnostic test (RDT) developed by Standard Diagnostics and FIND. The SD BIOLINE HAT RDT, which detects host antibodies to parasites, is cheap (US$0.50, ex-works) and easy to use for screening. Tests are packed individually and are stable at 40°C for at least 25 months; they are performed on fresh blood obtained from a finger prick, and no instrument or electricity is required. Individuals who are positive with the RDT will be further tested using a new LED fluorescence microscope that was co-developed by FIND and Carl Zeiss GmbH. The microscope does not require a dark room and can be operated using solar power. The sensitivity of detecting parasites using the microscope is greatly improved when blood samples are concentrated and stained with acridine orange, then examined using fluorescent light. A third test, developed by FIND and Eiken Chemical Co of Japan, is a simple molecular method known as LAMP that detects parasite DNA in blood samples. The Loopamp™ *Trypanosoma brucei* Detection Kit can be implemented in a simple laboratory by technicians who have no previous training in molecular biology. Individuals who are found positive by this method are identified as strong suspects, and will have to undergo parasitological confirmation. The LAMP equipment can also be operated using solar energy, and on blood samples that are dried and stored on filter papers.

The Ministry of Health and FIND will equip four main laboratories with facilities for confirmatory diagnosis of HAT with the new diagnostic tools. In addition, all health facilities in the three coastal HAT foci will be geo-referenced and characterized. Based on this information, facilities will be selected for introduction of the RDT in order to maximize accessibility of this screening test to the population at risk. HAT suspects identified by a positive RDT result at the peripheral level will be referred to the nearest of the four main

laboratories for confirmatory diagnosis and receive treatment according to national guidelines. Communities will be informed of the possibility of being tested with the RDT at the level of health centres using various advocacy tools, such as national TV and local radio broadcasting. The project will also include research activities to evaluate the performance and cost of using the new tests developed by FIND and its partners in comparison with the methods currently used in Guinea, in order to establish the optimal conditions under which these tests should be implemented, both in passive and in active surveillance.

This three-year project, which is complementary to other efforts by the Guinean Ministry of Health and its partners (including the World Health Organization, the Bill and Melinda Gates Foundation and the Institut de Recherche pour le Développement) to strengthen HAT case detection and vector control, is expected to dramatically improve surveillance and early detection of sleeping sickness patients, and to eventually interrupt transmission of the disease. Similar projects have recently been initiated in Uganda and in Malawi by FIND and partners. This initiative is supported by the government of the Republic of Guinea and FIND, and through FIND, by the Bill and Melinda Gates Foundation, the German Federal Ministry of Education and Research, the Department for International Development (DFID) of the United Kingdom and the Swiss Agency for Development and Cooperation (SDC).

FROM THE STEPS CENTRE

THE POLITICS OF TRYPANOSOMOSIS CONTROL IN AFRICA

This new working paper by Professor Ian Scoones of the Institute of Development Studies, UK, explores the science and policy debates surrounding trypanosomosis control. The paper focuses in particular on Zambia and Zimbabwe where Professor Scoones is undertaking research with the Dynamic Drivers of Disease in Africa Consortium (www.driversofdisease.org), a multi-disciplinary research programme exploring the links between ecosystems, poverty and disease.

Based on an extensive review of documentary material and interviews with scientists and policymakers, Professor Scoones's paper offers an assessment of the changing institutional politics associated with tsetse and trypanosomosis control. It investigates the controversies surrounding different control methods and considers how the focus on particular methods has meant that alternatives have often been overlooked and the perspectives of livestock keepers ignored. In addition, it explores how competition for dwindling research and operational funds, combined with a lack of institutional coordination, has resulted in the failure to develop an integrated approach linking ecological and disease dynamics with socio-economic conditions. The conclusion discusses why such a "One Health" approach is required and why addressing the politics of science and policy is essential.

The paper is published in the STEPS (Social, Technological and Environmental Pathways to Sustainability) Working Paper series, and can be downloaded from the STEPS Centre website (http://steps-centre.org/publication/politics-trypanosomiasis-control-africa/).

SECTION B – ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

16765**. Avery, V., Buckner, F., Baell, J., Fairlamb, A., Michels, P.A. & Tarleton, R., 2013.** Drug discovery for the treatment of leishmaniasis, African sleeping sickness and Chagas disease. *Future Medicinal Chemistry*, **5** (15): 1709-1718.

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The trypanosomatid protozoa *Leishmania*, *Trypanosoma brucei* and *Trypanosoma cruzi* are the causative agents of the human diseases respectively, leishmaniasis, African sleeping sickness and Chagas disease. Among the 17 "neglected tropical diseases" highlighted by WHO, progress towards the treatment of these diseases has improved in recent decades as a result of increased awareness, the emergence of public-private research partnerships and advances in drug-discovery technologies and techniques. Despite this, the current therapies for these diseases have serious shortcomings and, as such, the need to develop novel drugs, improve diagnosis and control the spread of disease is of paramount importance. This article describes the changing face of drug discovery in the pursuit of treatments for trypanosomatid-based diseases.

16766. **Blum, J. & Pletscher, M., 2013.** Skin diseases in travellers returning from tropical countries. *Therapeutische Umschau Revue thérapeutique,* **70** (6): 335-342.

Swiss Tropical and Public Health Institute, Basel, Switzerland. [johannes.blum@unibas.ch].

The most frequently observed skin lesions in travellers returning from tropical countries are insect bite reactions, bacterial skin diseases, creeping eruption and allergic reactions. This article describes these most relevant diseases and their differential diagnosis, focussing on the diseases which are potentially dangerous and which should not be missed such as resistant staphylococci, chancre of rickettsia or sleeping sickness, cutaneous leishmaniasis or worms, which are not limited to the skin.

16767. **Cecchi, G., Paone, M., Feldmann, U., Vreysen, M. J. B., Diall, O. & Mattioli, R. C. 2014**. Assembling a geospatial database of tsetse-transmitted animal trypanosomosis for Africa. Parasites & Vectors, 7: 39.

Food and Agriculture Organization of the United Nations (FAO), Sub-regional Office for Eastern Africa, Addis Ababa, Ethiopia; Food and Agriculture Organization of the United Nations (FAO), Animal Production and Health Division, Rome, Italy; Joint Food and Agriculture Organization/International Atomic Energy Agency Programme, Vienna, Austria. [giuliano.cecchi@fao.org].

African animal trypanosomosis (AAT), or nagana, is widespread within the tsetse-infested belt of sub-Saharan Africa. Although a wealth of information on its occurrence and prevalence is available in the literature, synthesized and harmonized data at the regional and continental scales are lacking. To fill this gap the Food and Agriculture Organization of the

United Nations (FAO) launched the Atlas of tsetse and AAT, jointly implemented with the International Atomic Energy Agency (IAEA) in the framework of the Programme Against African Trypanosomosis (PAAT). The Atlas aims to build and regularly update a geospatial database of tsetse species occurrence and AAT at the continental level. The present paper focuses on the methodology to assemble a dynamic database of AAT, which hinges on herd-level prevalence data as estimated using various diagnostic techniques. A range of ancillary information items is also included (e.g. trypanosome species, survey period, species and breed of animals, husbandry system, etc.). Input data were initially identified through a literature review. Preliminary results are presented for Ethiopia, Kenya and Uganda in East Africa: 122 papers were identified and analysed, which contained field data collected from January 1990 to December 2013. Information on AAT was extracted and recorded for 348 distinct geographic locations. The distribution maps presented exemplify the range of outputs that can be directly generated from the AAT database.

Activities are on-going to map the distribution of AAT in all affected countries and to develop the tsetse component of the Atlas. The methodology presented is also being transferred to partners in affected countries, with a view to developing capacity and strengthening data management, harmonization and sharing. In the future, geospatial modelling will enable predictions to be made within and beyond the range of AAT field observations. This variety of information layers will inform decisions on the most appropriate, site-specific strategies for intervention against AAT. Data on the occurrence of human-infective trypanosomes in non-human hosts will also provide valuable information for sleeping sickness control and elimination.

16768. **Ferrins, L., Rahmani, R. & Baell, J. B., 2013.** Drug discovery and human African trypanosomiasis: a disease less neglected? *Future Medicinal Chemistry*, **5** (15): 1801-1841.

Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), 381 Royal Parade, Parkville, VIC 3052, Australia. [Jonathan.Baell@monash.edu].

Human African trypanosomiasis (HAT) has been neglected for a long time. The most recent drug to treat this disease, eflornithine, was approved by the US FDA in 2000. Current treatments exhibit numerous problematic side effects and are often ineffective against the debilitating CNS resident stage of the disease. Fortunately, several partnerships and initiatives have been formed over the last 20 years in an effort to eradicate HAT, along with a number of other neglected diseases. This has led to an increasing number of foundations and research institutions that are currently working on the development of new drugs for HAT and tools with which to diagnose and treat patients. New biochemical pathways as therapeutic targets are emerging, accompanied by increasing numbers of new antitrypanosomal compound classes. The future looks promising that this collaborative approach will facilitate eagerly awaited breakthroughs in the treatment of HAT.

16769. **Gilbert, I. H., 2013.** Drug discovery for neglected diseases: molecular target-based and phenotypic approaches. *Journal of Medicinal Chemistry,* **56** (20)**:** 7719-26.

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Drug discovery for neglected tropical diseases is carried out using both target-based and phenotypic approaches. In this paper, target-based approaches are discussed, with a particular focus on human African trypanosomiasis. Target-based drug discovery can be successful, but careful selection of targets is required. There are still very few fully validated drug targets in neglected diseases, and there is a high attrition rate in target-based drug discovery for these diseases. Phenotypic screening is a powerful method in both neglected and non-neglected diseases and has been very successfully used. Identification of molecular targets from phenotypic approaches can be a way to identify potential new drug targets.

16770. **Gilbert, I. H., 2013.** Target-based drug discovery for human African trypanosomiasis: selection of molecular target and chemical matter. *Parasitology,* **141** (1)**:** 28-36.

Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK. [i.h.gilbert@dundee.ac.uk].

Target-based approaches for human African trypanosomiasis (HAT) and related parasites can be a valuable route for drug discovery for these diseases. However, care needs to be taken in selection of both the actual drug target and the chemical matter that is developed. In this article, potential criteria to aid target selection are described. Then the physiochemical properties of typical oral drugs are discussed and compared to those of known anti-parasitics.

16771. **Goyard, S., Dutra, P. L., Deolindo, P., Autheman, D., D'Archivio, S. & Minoprio, P., 2014.** *In vivo* imaging of trypanosomes for a better assessment of host-parasite relationships and drug efficacy. *Parasitology International*, **63** (1): 260-268.

Institut Pasteur, Laboratoire des Processus Infectieux à Trypanosomatidés, Département Infection et Epidémiologie, 25 Rue du Dr. Roux, 75724 Paris, France. [paola.minoprio@pasteur.fr].

The advances in microscopy combined with the invaluable progress made through the utilization of molecular, immunological or immunochemical markers and the implementation of more powerful imaging technologies have yielded great improvements to the knowledge of the interaction between microorganisms and their hosts, notably a better understanding of the establishment of infectious processes. Still today, the intricacies of the dialogue between parasites, cells and tissues remain limited. Some improvements have been attained with the stable integration and expression of the green fluorescence protein or firefly luciferase and other reporter genes, which have allowed better approaches for monitoring of gene expression and protein localization *in vivo*, *in situ* and in real time. Aiming at better exploring the well-established models of murine infections with characterized strains of *Trypanosoma cruzi* and *Trypanosoma vivax*, we revisited in the present report the state of the art about the tools for the imaging of trypanosomatids *in vitro* and *in vivo* and show the latest transgenic parasites that we have engineered in our laboratory using conventional transfection methods. The targeting of trypanosomes presented in this study is a promising tool for approaching the biology of parasite interactions with host cells, the progression of the diseases they trigger and the screening of new drugs *in vivo* or *in vitro*.

16772. **Holzmuller, P., Grebaut, P., Semballa, S., Gonzatti, M. I. & Geiger, A., 2013.** Proteomics: a new way to improve human African trypanosomiasis diagnosis? *Expert Review of Proteomics*, **10** (3): 289-301.

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African trypanosomiases, including the human disease referred to as "sleeping sickness" and the animal diseases such as nagana, surra and dourine, are neglected vector-borne diseases that after years of research still need improved diagnosis and chemotherapy. Advances in proteomics offer new tools to define biomarkers, whose expression may reflect host-parasite interactions occurring during the infection. In this review, the authors first describe the current diagnostic tools used to detect a trypanosome infection during field surveys, and then discuss their interests, limits and further evolutions. The authors also report on the contribution of molecular diagnostics, and the recent advances and developments that make them suitable for field work. The authors then explore the recent uses of proteomics technology to define host and parasite biomarkers that allow detection of the infection, and the power and constraints of the technology. They conclude by discussing the urgent need to use the biomarkers discovered in order to develop tools to improve trypanosomiasis control in the near future.

16773. **Horn, D. & Duraisingh, M. T., 2013.** Antiparasitic chemotherapy: from genomes to mechanisms. *Annual Review of Pharmacology & Toxicology*, **54**: 71-94.

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

Owing to the absence of antiparasitic vaccines and the constant threat of drug resistance, the development of novel antiparasitic chemotherapies remains of major importance for disease control. A better understanding of drug transport (uptake and efflux), drug metabolism and the identification of drug targets, and mechanisms of drug resistance would facilitate the development of more effective therapies. Here, we focus on malaria and African trypanosomiasis. We review existing drugs and drug development, emphasizing high-throughput genomic and genetic approaches, which hold great promise for elucidating antiparasitic mechanisms. We describe the approaches and technologies that have been influential for each parasite and develop new ideas for future research directions, including mode-of-action studies for drug target deconvolution.

16774. **Jones, A. J., Grkovic, T., Sykes, M. L. & Avery, V. M., 2013.** Trypanocidal activity of marine natural products. *Marine Drugs*, **11** (10): 4058-4082.

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Marine natural products are a diverse, unique collection of compounds with immense therapeutic potential. This has resulted in these molecules being evaluated for a number of different disease indications including the neglected protozoan diseases, human African trypanosomiasis and Chagas disease, for which very few drugs are currently available. This

article reviews the marine natural products for which activity against the kinetoplastid parasites *Trypanosoma brucei brucei*, *T. b. rhodesiense* and *T. cruzi* has been reported. As it is important to know the selectivity of a compound when evaluating its trypanocidal activity, this article will only cover molecules which have simultaneously been tested for cytotoxicity against a mammalian cell line. Compounds have been grouped according to their chemical structure and representative examples from each class were selected for detailed discussion.

16775. **Kiwou, M. K., Lanetti, R., Sladeckova, V., Kalavska, A., Benca, G. J., Sokolova, J., Kulkova, N., Dobrodenkova, S., Mikolasova, G., Mzwan, J. M., Muli, J. M., Bukovinova, P., Kralova, J. & Mamova, A., 2013.** Tropical neuroinfections in South Sudanese rural hospitals - analysis of 8 709 patients. *Neuro Endocrinology Letters*, **34** (Suppl. 1): 24-27.

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Infections involving the central nervous system have very serious consequences and affect thousands of people in Africa. Despite the availability of new antibiotics and vaccines, neuroinfections are dangerous and life-threatening conditions. The most frequent neuroinfections which are of the greatest importance for public health systems are viral diseases (such as HIV, encephalitis, poliomyelitis, rabies), bacterial diseases (bacterial meningitis, neurological complications of leprosy and tuberculosis) and parasitic infections (cerebral malaria, sleeping sickness, schistosomiasis, toxoplasmosis etc.). A descriptive study to assess the occurrence of neuroinfections in two rural hospitals in Sudan (Mapuordit in Yirol and Gordim in Aweil) was performed over two periods of two years: (i) 2005-2006 and (ii) 2010-2011. We obtained data on patients from Mapuordit and from Gordim by studying their medical records. Several cases of neuroinfections were observed during both periods; these were represented by tetanus, meningococcal meningitis, leprosy with neuropathy (altogether 442 patients) in Mapuordit. Also in Gordim, severe neuroinfections such as cerebral malaria were very rare (1 case), as well as tetanus (1 case), meningococcal meningitis (8 cases) and sleeping sickness (9 cases). However, the incidence of neuroinfections decreased from 44/1 000 in 2005-2006 to 2/1 000 in 2010-2011. Decreased incidence of serious neuroinfections (cerebral malaria, sleeping sickness, meningococcal meningitis) in Sudan may be related to improvement of effective therapeutic options, represented by (i) intermittent preventive therapy (IPT) for malaria, (ii) by suppression of sleeping sickness vectors and (iii) by better accessibility of antibiotics.

16776. **Leder, K., Torresi, J., Libman, M. D., Cramer, J. P., Castelli, F., Schlagenhauf, P., Wilder-Smith, A., Wilson, M. E., Keystone, J. S., Schwartz, E., Barnett, E. D., von Sonnenburg, F., Brownstein, J. S., Cheng, A. C., Sotir, M. J., Esposito, D. H. & Freedman, D. O., 2013.** GeoSentinel surveillance of illness in returned travellers, 2007-2011. *Annals of Internal Medicine*, **158** (6): 456-468.

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International travel continues to increase, particularly to Asia and Africa and clinicians are increasingly likely to be consulted for advice before travel or by returning ill travellers. This paper describes typical diseases in returned travellers according to region, travel reason,

and patient demographic characteristics; the pattern of low-frequency travel-associated diseases; and refines key messages for care before and after travel. The study design used GeoSentinel records from 53 tropical or travel disease units in 24 countries. The patients were 42 173 ill returned travellers seen between 2007 and 2011, and measurements included frequencies of demographic characteristics, regions visited, and illnesses reported. The results showed that Asia (32.6 percent) and sub-Saharan Africa (26.7 percent) were the most common regions where illnesses were acquired. Three quarters of travel-related illness were due to gastrointestinal (34.0 percent), febrile (23.3 percent), and dermatologic (19.5 percent) diseases. Only 40.5 percent of all ill travellers reported pre-travel medical visits. The relative frequency of many diseases varied with both travel destination and reason for travel, with travellers visiting friends and relatives in their country of origin having both a disproportionately high burden of serious febrile illness and very low rates of advice before travel (18.3 percent). Life-threatening diseases, such as *Plasmodium falciparum* malaria, melioidosis, and African trypanosomiasis, were reported. Sentinel surveillance data collected by specialist clinics do not reflect healthy returning travellers or those with mild or self-limited illness, and the data cannot be used to infer quantitative risk for illness. It is concluded that many illnesses may have been preventable with appropriate advice, chemoprophylaxis, or vaccination. Clinicians can use these 5-year GeoSentinel data to help tailor more efficient pre-travel preparation strategies and evaluate possible differential diagnoses of ill returned travellers according to destination and reason for travel.

16777. **Lejon, V., Jacobs, J. & Simarro, P. P., 2013.** Elimination of sleeping sickness hindered by difficult diagnosis. *Bulletin of the World Health Organization*, **91** (10): 718.

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Sleeping sickness or human African trypanosomiasis (HAT) is a fatal disease caused by *Trypanosoma brucei gambiense* and *T. b. rhodesiense* and transmitted by tsetse flies occurring in sub-Saharan Africa. Almost 80 percent of cases are detected in the Democratic Republic of the Congo. Control of infection by *T. b. gambiense*, which causes chronic disease, relies primarily on case detection followed by treatment. The prevalence of this form of HAT has been greatly reduced through intensive campaigns based on active screening by mobile teams that travel to high-incidence settings and test the population. This "vertical" approach is no longer sustainable or cost-effective in light of decreased incidence, and a proposed alternative has been to integrate HAT control activities into the "horizontal" health system. However, organizational, logistical and technical difficulties, especially related to diagnosis, may jeopardize elimination, which is now an established goal. The signs and symptoms of sleeping sickness are diverse and non-specific and resemble those of many other diseases, including malaria, human immunodeficiency virus (HIV) infection, tuberculosis, toxoplasmosis, viral encephalitis, brucellosis, lymphoma and typhoid fever. Because of this, diagnosis must rely on laboratory tests, yet none of the available techniques for the laboratory diagnosis of HAT has the features of the ideal diagnostic test: affordable, user-friendly, fast and accurate; requiring no special equipment; and available where needed. The card agglutination test for trypanosomiasis, which detects antibodies to *T. b. gambiense*, is particularly suited to active mass screening campaigns, but it is not an individual test and has

poor thermal stability. Since specificity is limited, parasitological confirmation is required. Although wet preparations of lymph node aspirates and Giemsa-stained thick blood films for parasite detection can be easily examined microscopically at the primary care level, these tests lack the necessary sensitivity. Concentration techniques have higher sensitivity but are costly and rely on electricity and specialized equipment often unavailable in primary care facilities, where most patients are tested by thick blood film examination alone. As a result, HAT may remain undiagnosed.

Rapid diagnostic tests (RDTs) are increasingly being used to diagnose HIV infection and malaria. Although their sensitivity and specificity for HIV infection usually exceed 98 percent, in patients with HAT, specificity can be as low as 39 percent and false positives cannot be entirely excluded by serial testing algorithms. Similarly, RDTs for malaria can have a specificity as low as 11 percent in patients with HAT. This poses an additional risk, since RDTs for malaria are assumed to be accurate enough to substitute for microscopy. In addition, the replacement of microscopy by RDTs for the diagnosis of malaria eliminates the opportunity to incidentally detect trypanosomes in blood. The risk of misdiagnosing sleeping sickness as HIV infection or malaria is thus considerable and even higher in co-infected HAT patients. The health system's competence for laboratory testing represents an additional problem, as suggested by reports for thick blood film microscopy. During external quality assessments of malaria microscopy in diagnostic laboratories of the Democratic Republic of the Congo, only 49 percent of laboratories recognized trypanosomes and fewer than 20 percent produced good Giemsa stains. This illustrates the difficulty of procuring quality *in vitro* diagnostics and reagents. In health centres, routine thick blood film examination for malaria had a false positivity rate of 66 percent. Similar or poorer diagnostic quality can be expected in the case of HAT. Integration of sleeping sickness control into the health system is hindered by various factors, including the limitations of current diagnostic tests. Health systems must be strengthened to reduce diagnostic delays. A supply of basic quality consumables should be assured and refresher training in microscopy and laboratory management needs to be organized, along with regular external quality assessments to maintain competency and help monitor test and end-user performance. Furthermore, when RDTs are used to diagnose other diseases in areas where HAT is endemic, the decrease in specificity observed in HAT patients and the risk of misdiagnosis should be kept in mind. Diagnostic algorithms should be adapted accordingly. Finally, individual RDTs for the detection of antibodies specific for *T. b. gambiense* are being developed. Such tests will facilitate HAT screening in primary health centres, although parasite confirmation will be required, especially since current drugs for the treatment of HAT have side-effects and are difficult to administer.

16778. **MacLean, L., Myburgh, E., Rodgers, J. & Price, H. P., 2013**. Imaging African trypanosomes. *Parasite Immunology*, **35**: 283-294.

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Trypanosoma brucei are extracellular kinetoplastid parasites transmitted by the blood-sucking tsetse fly. They are responsible for the fatal disease human African trypanosomiasis (HAT), also known as sleeping sickness. In late-stage infection, trypanosomes cross the blood-brain barrier (BBB) and invade the central nervous system (CNS) invariably leading to coma and death if untreated. There is no available vaccine and current late-stage HAT

chemotherapy consists of either melarsoprol, which is highly toxic causing up to 8 percent of deaths, or nifurtimox-eflornithine combination therapy (NECT), which is costly and difficult to administer. There is therefore an urgent need to identify new late-stage HAT drug candidates. Here, we review how current imaging tools, ranging from fluorescent confocal microscopy of live immobilized cells in culture to whole-animal imaging, are providing insight into *T. brucei* biology, parasite-host interplay, trypanosome CNS invasion and disease progression. We also consider how imaging tools can be used for candidate drug screening purposes that could lead to new chemotherapies.

16779. **Mountford, A. P., 2013. "**Seeing is Believing": the use of novel imaging approaches towards creating a greater understanding of parasite-host interactions. *Parasite Immunology*, **35** (9-10): 245-247.

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This editorial highlights some of the key points made in the six invited reviews in this special issue of *Parasite Immunology* on the use of contemporary imaging technologies to investigate the parasite: host interface. Three of the reviews deal with the protozoa *Trypanosoma*, *Leishmania*, and *Plasmodium*, whilst the remainder focus on helminth parasites particularly *Schistosoma*. The reviews cover aspects related to how the development of transgenic parasites has vastly advanced our understanding of how parasites interact with host cells, particularly as a cause of pathology. Imaging technologies have also been exploited in revealing parasite localisation within host tissues and identifying novel therapeutic targets. Combined the reviews show how "state of the art" imaging technologies have resulted in a seismic advance in our understanding of parasite biology and how this has the potential to develop new, and improved, strategies to combat disease caused by parasite infections.

16780. **Sassera, D., Epis, S., Pajoro, M. & Bandi, C., 2013.** Microbial symbiosis and the control of vector-borne pathogens in tsetse flies, human lice, and triatomine bugs. *Pathogens & Global Health*, **107** (6): 285-292.

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Symbiosis is a widespread biological phenomenon, and is particularly common in arthropods. Bloodsucking insects are among the organisms that rely on beneficial bacterial symbionts to complement their unbalanced diet. This review is focused on describing symbiosis, and possible strategies for the symbiont-based control of insects and insect-borne diseases in three bloodsucking insects of medical importance: the flies of the genus *Glossina*, the lice of the genus *Pediculus*, and triatomine bugs of the subfamily Triatominae. *Glossina* flies are vector of *Trypanosoma brucei*, the causative agent of sleeping sickness and other pathologies. They are also associated with two distinct bacterial symbionts, the primary symbiont *Wigglesworthia* spp., and the secondary, culturable symbiont *Sodalis glossinidius*. The primary symbiont of human lice, *Riesia pediculicola*, has been shown to be fundamental for the host, due to its capacity to synthesize B-group vitamins. An antisymbiotic approach, with antibiotic treatment targeted on the lice symbionts, could represent an alternative strategy to control these ectoparasites. In the case of triatominae bugs, the genetic modification of their symbiotic *Rhodococcus* bacteria, for production of anti-*Trypanosoma* molecules, is an

example of paratransgenesis, i.e. the use of symbiotic microorganism engineered in order to reduce the vector competence of the insect host.

16781. **Shaw, A. P. M., Cecchi G., Wint, G. R. W., Mattioli, R. C. & Robinson, T. P., 2014.** Mapping the economic benefits to livestock keepers from intervening against bovine trypanosomosis in Eastern Africa. *Preventive Veterinary Medicine,* **113:** 197–210.

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Endemic animal diseases such as tsetse-transmitted trypanosomosis are a constant drain on the financial resources of African livestock keepers and on the productivity of their livestock. Knowing where the potential benefits of removing animal trypanosomosis are distributed geographically would provide crucial evidence for prioritising and targeting cost-effective interventions as well as a powerful tool for advocacy. To this end, a study was conducted on six tsetse-infested countries in Eastern Africa: Ethiopia, Kenya, Somalia, South Sudan, Sudan and Uganda. First, a map of cattle production systems was generated, with particular attention to the presence of draught and dairy animals. Second, herd models for each production system were developed for two scenarios: with or without trypanosomosis. The herd models were based on publications and reports on cattle productivity (fertility, mortality, yields, sales), from which the income from, and growth of cattle populations were estimated over a twenty-year period. Third, a step-wise spatial expansion model was used to estimate how cattle populations might migrate to new areas when maximum stocking rates are exceeded. Last, differences in income between the two scenarios were mapped, thus providing a measure of the maximum benefits that could be obtained from intervening against tsetse and trypanosomosis. For this information to be readily mappable, benefits were calculated per bovine and converted to US$ per square kilometre. Results indicate that the potential benefits from dealing with trypanosomosis in Eastern Africa are both very high and geographically highly variable. The estimated total maximum benefit to livestock keepers for the whole of the study area amounts to nearly US$2.5 billion, discounted at 10 percent over twenty years – an average of approximately US$3 300 per square kilometer of tsetse-infested area – but with great regional variation from less than US$500 per square kilometer to well over US$10 000. The greatest potential benefits accrue to Ethiopia, because of its very high livestock densities and the importance of animal traction, but also to parts of Kenya and Uganda. In general, the highest benefit levels occur on the fringes of the tsetse infestations. The implications of the models' assumptions and generalisations are discussed.

16782. **Simarro, P. P., Cecchi, G., Franco, J. R., Paone, M., Diarra, A., Ruiz-Postigo, J. A., Mattioli, R. C. & Jannin, J. G., 2014**. Mapping the capacities of fixed health facilities to cover people at risk of *gambiense* human African trypanosomiasis**.** *International Journal of Health Geographics.* (**In press).**

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The emphasis placed on the activities of mobile teams in the detection of *gambiense* human African trypanosomiasis (HAT) can at times obscure the major role played by fixed health facilities in the control and surveillance of the disease. The lack of consistent and detailed data on the extent and coverage of passive case-finding and treatment further constrains our ability to appreciate the full contribution of the health system to the control of HAT. This paper aims to fill this gap. A survey was made of all fixed health facilities that are active in the control and surveillance of *gambiense* HAT. Information on their diagnostic and treatment capabilities was collected, reviewed and harmonized. Health facilities were geo-referenced. Time-cost distance analysis was conducted to estimate physical accessibility and the potential coverage of the population at-risk of *gambiense* HAT. Diagnosis and treatment coverage were separately analysed. Information provided by the National Sleeping Sickness Control Programmes revealed the existence of 632 fixed health facilities that are active in the control and surveillance of *gambiense* HAT in endemic countries having reported cases or having conducted active screening activities during the period 2000-2012. Different types of diagnosis (clinical, serological, parasitological and disease staging) are available from 622 facilities. Treatment with pentamidine for first-stage disease is provided by 495 health facilities, while for second-stage disease various types of treatment are available in 206 health facilities only. Over 80 percent of the population at-risk for *gambiense* HAT lives within 5-h travel of a fixed health facility offering diagnosis and treatment for the disease. It is concluded that fixed health facilities have played a crucial role in the diagnosis, treatment and coverage of at-risk-populations for *gambiense* HAT. As the number of reported cases continues to dwindle, the role of the fixed health facilities will become increasingly important for the prospects of disease elimination. Future updates of the database here presented will regularly provide evidence to inform and monitor a rational deployment of control and surveillance efforts. Support to the development and, if successful, the implementation of new control tools (e.g. new diagnostics and new drugs) is crucial, both for strengthening and expanding the existing network of fixed health facilities by improving access to diagnosis and treatment and for securing a sustainable control and surveillance of *gambiense* HAT.

16783. **Stein, J., Mogk, S., Mudogo, C. N., Sommer, B. P., Scholze, M., Meiwes, A., Huber, M., Gray, A. & Duszenko, M., 2013.** Drug development against sleeping sickness: old wine in new bottles? *Current Medicinal Chemistry*. **E Publication ahead of print, 19 November.**

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Atoxyl, the first medicinal drug against human African trypanosomiasis (HAT), also known as sleeping sickness, was applied more than 100 years ago. Ever since, the search for more effective, more specific and less toxic drugs continued, leading to a set of compounds currently in use against this devastating disease. Unfortunately, none of these medicines fulfils modern pharmaceutical requirements and may be considered as ultimate therapeutics due to the many, often severe side effects. Starting with a historic overview on drug development against HAT, we present a selection of trypanosome specific pathways and enzymes considered as highly potent druggable targets. In addition, we describe cellular mechanisms the parasite uses for differentiation and cell density regulation and present our considerations of how interference with these steps, elementary for life cycle progression and infection, may lead to new aspects of drug development. Finally we refer to our recent work about CNS infection that offers novel insights in how trypanosomes hide in an immune privileged area to establish a chronic state of the disease, thereby considering new ways for drug application. Depressingly, HAT specific drug development has failed over the last 30 years to produce better suited medicine. However, unravelling of parasite-specific pathways and cellular behaviour together with the ability to produce high resolution structures of essential parasite proteins by X-ray crystallography, leads us to the optimistic view that development of an ultimate drug to eradicate sleeping sickness from the globe might just be around the corner.

16784. **Stich, A., Ponte-Sucre, A. & Holzgrabe, U., 2013.** Do we need new drugs against human African trypanosomiasis? *Lancet Infectious Diseases*, **13** (9): 733-734.

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Human African trypanosomiasis (sleeping sickness) is caused by the parasite *Trypanosoma brucei* and is fatal if untreated. Available drugs are at least 30 years old and have severe drawbacks, producing dangerous adverse effects and are often effective against only one of the two stages or only the chronic form (caused by *T. b. gambiense*) or the acute form (caused by *T. b. rhodesiense*) of disease. Introduction of nifurtimox-eflornithine combination treatment (NECT) in 2009 was an enormous step forward, reducing the duration of treatment with parenteral eflornithine to 1 week down from at least 14 d with eflornithine alone. NECT, a reformulation of existing drugs, is the first new treatment for human African trypanosomiasis in more than 25 years. It has not been approved in any country, but was added to the WHO list of essential medicines in 2009. However, NECT is expensive. Some other drugs are in clinical trials. The sulphone and sulphoxide metabolites of fexinidazole are effective in animal models of the disease. Fexinidazole is bioavailable orally, can cross the blood-brain barrier, and is effective in advanced stages of *T. b. rhodesiense* and *T. b. gambiense* disease. The benzoxaborole SCYX-7158 has satisfactory potency against *T. b. rhodesiens*e and *T. b. gambiense,* and has good pharmacokinetic properties *in vitro* and after oral administration in rodents and non-human primates. It can cross the blood-brain barrier and is metabolically stable, making it feasible to treat both disease stages. The pentamidine analogue pafuramidine was being tested in phase 3 clinical trials as an oral drug for first-stage human African trypanosomiasis, but these trials were stopped because of the drug's

nephrotoxicity. Finally, melarsoprol complexed with either hydroxypropyl-β-cyclodextrin or methylated β-cyclodextrin for solubility enhancement is in clinical trials. The presence of at least two potential new candidate drugs in the pipeline is an impressive achievement compared with the situation 10 years ago, but this is still not enough given that none of these drugs is in phase 3 trials and that up to 90 percent of drug candidates, like parfuramidine, do not make it to market. This drug pipeline for human African trypanosomiasis should not be allowed to run dry. In view of this weak supply of new candidate drugs from drug companies, alternative approaches are needed. Can academia and private-public partnerships provide enough resources for drug discovery and development? Can the academic research community communicate with health workers in the field to redefine the needs and necessities for an ideal human African trypanosomiasis drug and focus their efforts accordingly? And if such communication would be possible, would this spur drug companies and local governments into action? Neglected tropical diseases, including human African trypanosomiasis, need more attention. Solutions will probably come from unconventional approaches. The combined efforts of academia and the pharmaceutical industry might help to decrease the suffering of people affected by human African trypanosomiasis, and could lead to fair distribution and provision of health care.

16785. **Sykes, M. L. & Avery, V. M., 2013.** Approaches to protozoan drug discovery: phenotypic screening. *Journal of Medicinal Chemistry,* **56** (20): 7727-7740.

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Determining the activity of a compound and the potential impact on a diseased state is frequently undertaken using phenotypic or target-based approaches. Phenotypic screens have the advantage of the whole organism being exposed to the compound and thus all the targets and biological pathways associated with it. Cell penetration and access to targets in their "natural" environment are taken into account. Unless utilizing a genetically modified organism with an additional target associated indicator, elucidation of specific target(s) of active compounds is necessary. Target discovery is desirable to allow development of chemical entities based upon knowledge of the target structure. Phenotypic drug discovery has successfully identified new molecular entities for neglected protozoan disease research. In this perspective, the phenotypic approaches used to identify chemical entities for drug discovery and for use as tools against the parasites *Plasmodium falciparum*, *Trypanosoma brucei brucei*, and *Trypanosoma cruzi* are outlined.

16786. **Tediosi, F., Steinmann, P., de Savigny, D. & Tanner, M., 2013.** Developing eradication investment cases for onchocerciasis, lymphatic filariasis, and human African trypanosomiasis: rationale and main challenges. *PLoS Neglected Tropical Diseases*, **7** (11): e2446.

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This article presents the rationale, the approaches to be pursued, and the main methodological challenges of developing eradication investment cases (EICs) for three neglected tropical diseases considered as candidates for elimination and eradication:

onchocerciasis (river blindness), lymphatic filariasis (LF) (elephantiasis), and human African trypanosomiasis (HAT) (sleeping sickness). Clearly, an EIC is a broad and innovative methodology to assess the potential consequences of investing in elimination and eradication of a disease. It is aimed at going beyond the traditional reductionist approaches focusing on only one or few dimensions, e.g. the health impact or the cost-effectiveness, that are relevant for informing policy decisions. Nevertheless, developing EICs, following the approach proposed by the Guide mentioned in the article, presents several challenges.

16787. **Truc, P., Buscher, P., Cuny, G., Gonzatti, M. I., Jannin, J., Joshi, P., Juyal, P., Lun, Z. R., Mattioli, R., Pays, E., Simarro, P. P., Teixeira, M. M., Touratier, L., Vincendeau, P. & Desquesnes, M., 2013.** Atypical human infections by animal trypanosomes. *PLoS Neglected Tropical Diseases*, **7** (9): e2256.

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The two classical forms of human trypanosomiases are sleeping sickness due to *Trypanosoma brucei gambiense* or *T. brucei rhodesiense*, and Chagas disease due to *T. cruzi.* However, a number of atypical human infections caused by other trypanosome species (or sub-species) have been reported, namely due to *T. brucei brucei*, *T. vivax*, *T. congolense*, *T. evansi*, *T. lewisi*, and *T. lewisi*-like. These cases are reviewed here. Some infections were transient in nature, while others required treatments that were successful in most cases, although two cases were fatal. A recent case of infection due to *T. evansi* was related to a lack of apolipoprotein L-I, but *T. lewisi* infections were not related to immunosuppression or specific human genetic profiles. Out of 19 patients, eight were confirmed between 1974 and 2010, thanks to improved molecular techniques. However, the number of cases of atypical human trypanosomiases might be underestimated. Thus, improvement, evaluation of new diagnostic tests, and field investigations are required for detection and confirmation of these atypical cases.

16788. **Yansouni, C. P., Bottieau, E., Lutumba, P., Winkler, A. S., Lynen, L., Buscher, P., Jacobs, J., Gillet, P., Lejon, V., Alirol, E., Polman, K., Utzinger, J., Miles, M. A., Peeling, R. W., Muyembe, J. J., Chappuis, F. & Boelaert, M., 2013.** Rapid diagnostic tests for neurological infections in Central Africa. *Lancet Infectious Diseases*, **13** (6): 546-558.

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Infections are a leading cause of life-threatening neuropathology worldwide. In central African countries affected by endemic diseases such as human African trypanosomiasis, tuberculosis, HIV/AIDS and schistosomiasis, delayed diagnosis and treatment often lead to avoidable death or severe sequelae. Confirmatory microbiological and parasitological tests are essential because clinical features of most neurological infections are not specific, brain imaging is seldom feasible, and treatment regimens are often prolonged or toxic. Recognition of this diagnostic bottleneck has yielded major investment in application of advances in biotechnology to clinical microbiology in the past decade. We review the neurological

pathogens for which rapid diagnostic tests are most urgently needed in central Africa, detail the state of development of putative rapid diagnostic tests for each, and describe key technical and operational challenges to their development and implementation. Promising field-suitable rapid diagnostic tests exist for the diagnosis of human African trypanosomiasis and cryptococcal meningoencephalitis. For other infections (e.g. syphilis and schistosomiasis), highly accurate field-validated rapid diagnostic tests are available, but their role in diagnosis of disease with neurological involvement is still unclear. For others (e.g. tuberculosis), advances in research have not yet yielded validated tests for diagnosis of neurological disease.

16789. **Zhang, Y., Mi, J. Y., Rui, Y. J., Xu, Y. L. & Wang, W., 2013.** Stem cell therapy for the treatment of parasitic infections: is it far away? *Parasitology Research,* **113** (2): 607-612.

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2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

16790. **Boucias, D. G., Kariithi, H. M., Bourtzis, K., Schneider, D. I., Kelley, K., Miller, W. J., Parker, A. G. & Abd-Alla, A. M., 2013.** Transgenerational transmission of the *Glossina pallidipes* hytrosavirus depends on the presence of a functional symbiome. *PLoS One*, **8** (4): e61150.

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The vertically transmitted endosymbionts (*Sodalis glossinidius* and *Wigglesworthia glossinidia*) of the tsetse fly (Diptera: Glossinidae) are known to supplement dietary deficiencies and modulate the reproductive fitness and the defence system of the fly. Some tsetse fly species are also infected with the bacterium, *Wolbachia* and with the *Glossina* hytrosavirus (GpSGHV). Laboratory-bred *G. pallidipes* exhibit chronic asymptomatic and acute symptomatic GpSGHV infection, with the former being the most common in these colonies. However, under as yet undefined conditions, the asymptomatic state can convert to the symptomatic state, leading to detectable salivary gland hypertrophy (SGH^+) syndrome. In this study, we investigated the interplay between the bacterial symbiome and GpSGHV during development of *G. pallidipes* by knocking down the symbionts with antibiotic. Intrahaemocoelic injection of GpSGHV led to high virus titre (10^9 virus copies), but was not accompanied by either the onset of detectable SGH^+, or release of detectable virus particles into the blood meals during feeding events. When the F_1 generations of GpSGHV-challenged mothers were dissected within 24 h post-eclosion, SGH^+ was observed to increase from 4.5 percent in the first larviposition cycle to >95 percent in the fourth cycle. Despite being sterile, these F_1 SGH^+ progeny mated readily. Removal of the tsetse symbiome, however, suppressed transgenerational transfer of the virus via milk secretions and blocked the ability of GpSGHV to infect salivary glands of the F_1 progeny. Whereas GpSGHV infects and replicates in salivary glands of developing pupa, the virus is unable to induce SGH^+ within fully

differentiated adult salivary glands. The F_1 SGH^+ adults are responsible for the GpSGHV-induced colony collapse in tsetse factories. Our data suggest that GpSGHV has co-evolved with the tsetse symbiome and that the symbionts play key roles in the virus transmission from mother to progeny.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

16791 **Baumann, A. A., Benoit, J. B., Michalkova, V., Mireji, P. O., Attardo, G. M., Moulton, J. K., Wilson, T. G. & Aksoy, S., 2013.** Juvenile hormone and insulin suppress lipolysis between periods of lactation during tsetse fly pregnancy. *Molecular & Cellular Endocrinology*, **372** (1-2): 30-41.

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Tsetse flies are viviparous insects that nurture a single intrauterine progeny per gonotrophic cycle. The developing larva is nourished by the lipid-rich, milk-like secretions from a modified female accessory gland (milk gland). An essential feature of the lactation process involves lipid mobilization for incorporation into the milk. In this study, we examined roles for juvenile hormone (JH) and insulin/IGF-like (IIS) signalling pathways during tsetse pregnancy. In particular, we examined the roles for these pathways in regulating lipid homeostasis during transitions between non-lactating (dry) and lactating periods. The dry period occurs over the course of oogenesis and embryogenesis, while the lactation period spans intrauterine larvigenesis. Genes involved in the JH and IIS pathways were upregulated during dry periods, correlating with lipid accumulation between bouts of lactation. RNAi suppression of Forkhead Box Sub Group O (FOXO) expression impaired lipolysis during tsetse lactation and reduced fecundity. Similar reduction of the JH receptor Methoprene tolerant (Met), but not its paralogue germ cell expressed (gce), reduced lipid accumulation during dry periods, indicating functional divergence between Met and gce during tsetse reproduction. Reduced lipid levels following Met knockdown led to impaired fecundity due to inadequate fat reserves at the initiation of milk production. Both the application of the JH analogue (JHA) methoprene and injection of insulin into lactating females increased stored lipids by suppressing lipolysis and reduced transcripts of lactation-specific genes, leading to elevated rates of larval abortion. To our knowledge, this study is the first to address the molecular physiology of JH and IIS in a viviparous insect, and specifically to provide a role for JH signalling through Met in the regulation of lipid metabolism during insect lactation.

16792. **Chappuis, C. J., Beguin, S., Vlimant, M. & Guerin, P. M., 2013.** Water vapour and heat combine to elicit biting and biting persistence in tsetse. *Parasites & Vectors*, **6** (1): 240.

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Tsetse flies are obligatory blood feeders, accessing capillaries by piercing the skin of their hosts with the haustellum to suck blood. However, this behaviour presents a considerable risk as landing flies are exposed to predators as well as the host's own defence reactions such as tail flicking. Achieving a successful blood meal within the shortest time

span is therefore at a premium in tsetse, so feeding until replete normally lasts less than a minute. Biting in blood sucking insects is a multi-sensory response involving a range of physical and chemical stimuli. Here we investigated the role of heat and humidity emitted from host skin on the biting responses of *Glossina pallidipes*, which to our knowledge has not been fully studied in tsetse before. The onset and duration of the biting response of *G. pallidipes* was recorded by filming movements of its haustellum in response to rapid increases in temperature and/or relative humidity (RH) following exposure of the fly to two airflows. The electrophysiological responses of hygroreceptor cells in wall-pore sensilla on the palps of *G. pallidipes* to drops in RH were recorded using tungsten electrodes and the ultrastructure of these sensory cells was studied by scanning and transmission electron microscopy. Both latency and proportion of tsetse biting are closely correlated to RH when accompanied by an increase of 13.1 ^{0}C above ambient temperature but not for an increase of just 0.2 ^{0}C. Biting persistence, as measured by the number of bites and the time spent biting, also increases with increasing RH accompanied by a 13.1 ^{0}C increase in air temperature. Neurones in wall-pore sensilla on the palps respond to shifts in RH. Our results show that temperature acts synergistically with humidity to increase the rapidity and frequency of the biting response in tsetse above the levels induced by increasing temperature or humidity separately. Palp sensilla housing hygroreceptor cells, described here for the first time in tsetse, are involved in the perception of differences in RH.

16793. **Childs, S. J., 2014.** A model of teneral dehydration in *Glossina*. *Acta Tropica,* **131**: 79-91.

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The results of a long-established investigation into teneral transpiration are used as a rudimentary data set. These data are not complete in that all are at 25 ^{0}C and the temperature-dependence cannot therefore be resolved. An allowance is, nonetheless, made for the outstanding temperature-dependent data. The data are generalized to all humidities, levels of activity and, in theory, temperatures, by invoking the property of multiplicative separability. In this way a formulation which is a very simple, first order, ordinary differential equation, is devised. The model is extended to include a variety of *Glossina* species by resorting to their relative, resting water loss rates in dry air. The calculated total water loss is converted to the relevant humidity at 24 ^{0}C, that which produced an equivalent water loss in the pupa in order to exploit an adaption of an established survival relationship. The resulting computational model calculates total, teneral water loss, consequent mortality and adult recruitment. Surprisingly, the postulated race against time to feed applies more to the mesophilic and xerophilic species, in that increasing order. So much so that it is reasonable to conclude that, should *Glossina brevipalpis* survive the pupal phase, it will almost certainly survive to locate a host without there being any significant prospect of death from dehydration. With the conclusion of this work comes the revelation that the classification of species as hygrophilic, mesophylic and xerophylic is largely true only in so much as their third and fourth instars are and, possibly, the hours shortly before eclosion.

16794. **Wang, J., Weiss, B. L. & Aksoy, S., 2013.** Tsetse fly microbiota: form and function. *Frontiers in Cellular & Infection Microbiology*, **3**: 69.

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Tsetse flies are the primary vectors of African trypanosomes, which cause human and animal African trypanosomiasis in 36 countries in sub-Saharan Africa. These flies have also established symbiotic associations with bacterial and viral microorganisms. Laboratory-reared tsetse flies harbour up to four vertically transmitted organisms: obligate *Wigglesworthia*, commensal *Sodalis*, parasitic *Wolbachia* and salivary gland hypertrophy virus (SGHV). Field-captured tsetse can harbour these symbionts as well as environmentally acquired commensal bacteria. This microbial community influences several aspects of tsetse physiology, including nutrition, fecundity and vector competence. This review provides a detailed description of tsetse's microbiome, and describes the physiology underlying host-microbe and microbe-microbe interactions that occur in this fly.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

[See also **36:** 16780, 16824**]**

16795. **Geiger, A., Fardeau, M. L., Njiokou, F. & Ollivier, B., 2013.** *Glossina* spp. gut bacterial flora and their putative role in fly-hosted trypanosome development. *Frontiers in Cellular & Infection Microbiology*, **3**: 34.

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Human African trypanosomiasis (HAT) is caused by trypanosomes transmitted to humans by the tsetse fly, in which they accomplish their development into their infective metacyclic form. The crucial step in parasite survival occurs when it invades the fly midgut. Insect digestive enzymes and immune defences may be involved in the modulation of the fly's vector competence, together with bacteria that could be present in the fly's midgut. In fact, in addition to the three bacterial symbionts that have previously been characterized, tsetse flies may harbour additional bacterial inhabitants. This review focuses on the diversity of the bacterial flora in *Glossina*, with regards to the fly species and their geographical distribution. The rationale was (i) that these newly identified bacteria associated with tsetse flies may contribute to vector competence as was shown in other insects and (ii) that differences may exist according to fly species and geographic area. A more complete knowledge of the bacterial microbiota of the tsetse fly and the role these bacteria play in tsetse biology may lead to novel ways of investigation in view of developing alternative anti-vector strategies for fighting human and possibly animal trypanosomiasis.

16796. **Hoppenheit, A., Murugaiyan, J., Bauer, B., Clausen, P. H. & Roesler, U., 2013.** Analysis of *Glossina palpalis gambiensis* and *Glossina tachinoides* from two distant locations in Burkina Faso using MALDI TOF MS. *Parasitology Research,* DOI: 10.1007/s00436-013-3701-z

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Riverine tsetse (*Glossina*) as *Glossina palpalis gambiensis* Vanderplank 1949 and *Glossina tachinoides* Westwood 1850 are the main vectors for African animal trypanosomoses in Burkina Faso. Vector control has been proven efficient in disease containment, but its success is endangered by the reinvasion of tsetse from neighbouring areas. Thus, identifying relic populations can enhance the success rate of vector control efforts. This is currently carried out through microsatellite analysis which is time-consuming and costly. Recently, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry-based analysis has become a routine method in microbial species identification. Owing to the rapidity and cost-effectiveness, this approach has been extended towards species identification of higher organisms such as tsetse. Following the recent experiences in distinguishing two genotypes of *Prototheca* spp., it is of interest to explore the validity of mass spectrometry for tsetse population differentiation. As a preliminary test, we submitted male and female *G. palpalis gambiensis* and *G. tachinoides* from Sideradougou and Folonzo, Burkina Faso (distance 60 km) to matrix-assisted laser desorption/ionisation analysis. The wing samples were utilized for protein extraction, and mass spectra in a broad mass to charge ratio (2 000-20 000 kDa) were obtained. Specific peaks appeared to represent species, sex and location. Then, a peak list was extracted, containing the peaks in mass-to-charge ratio by revealing their intensities as well. These lists were used to compute a spectral dendrogram and a principal component analysis which displayed the differences among the samples from the two different regions. The results indicate that this technique can be extended with additional tsetse species, ideally with supporting genomic data, to later assist in designing rational vector control strategies.

16797. **Hoppenheit, A., Murugaiyan, J., Bauer, B., Steuber, S., Clausen, P. H. & Roesler, U., 2013.** Identification of tsetse (G*lossina* spp.) using matrix-assisted laser desorption/ionisation time of flight mass spectrometry. *PLoS Neglected Tropical Diseases*, **7** (7): e2305.

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Glossina (G.) spp. (Diptera: Glossinidae), known as tsetse flies, are vectors of African trypanosomes that cause sleeping sickness in humans and nagana in domestic livestock. Knowledge of tsetse distribution and accurate species identification help identify potential vector intervention sites. Morphological species identification of tsetse is challenging and sometimes not accurate. The matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI TOF MS) technique, already standardised for microbial identification, could become a standard method for tsetse fly diagnostics. Therefore, a unique spectra reference database was created for five lab-reared species of riverine-, savannah- and forest-type tsetse flies and incorporated with the commercial Biotyper 3.0 database. The standard formic acid/acetonitrile extraction of male and female whole insects and their body parts (head, thorax, abdomen, wings and legs) was used to obtain the flies' proteins. The computed composite correlation index and cluster analysis revealed the suitability of any tsetse body part for a rapid taxonomical identification. Phyloproteomic analysis revealed that the peak patterns of *G. brevipalpis* differed greatly from the other tsetse. This outcome was comparable to previous theories that they might be considered as a sister group to other tsetse spp. Freshly extracted samples were found to be matched at the species level. However, sex differentiation proved to be less reliable. Similarly processed samples of the common house fly *Musca*

domestica (Diptera: Muscidae; strain: Lei) did not yield any match with the tsetse reference database. The inclusion of additional strains of morphologically defined wild caught flies of known origin and the availability of large-scale mass spectrometry data could facilitate rapid tsetse species identification in the future.

16798. **Wamwiri, F. N., Alam, U., Thande, P. C., Aksoy, E., Ngure, R. M., Aksoy, S., Ouma, J. O. & Murilla, G. A., 2013.** *Wolbachia*, *Sodalis* and trypanosome co-infections in natural populations of *Glossina austeni* and *Glossina pallidipes*. *Parasites & Vectors*, **6** (1): 232.

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Tsetse flies harbour at least three bacterial symbionts: *Wigglesworthia glossinidia*, *Wolbachia pipientis* and *Sodalis glossinidius*. *Wigglesworthia* and *Sodalis* reside in the gut in close association with trypanosomes and may influence establishment and development of midgut parasite infections. *Wolbachia* has been shown to induce reproductive effects in infected tsetse. This study was conducted to determine the prevalence of these endosymbionts in natural populations of *G. austeni* and *G. pallidipes* and to assess the degree of concurrent infections with trypanosomes. Fly samples analysed originated from Kenyan coastal forests (trapped in 2009-2011) while South African *G. austeni* were collected in 2008. The age structure was estimated by standard methods. *G. austeni* (n = 298) and *G. pallidipes* (n = 302) were analysed for infection with *Wolbachia* and *Sodalis* using PCR. Trypanosome infection was determined either by microscopic examination of dissected organs or by PCR amplification. Overall, we observed that *G. pallidipes* females had a longer lifespan (70 d) than *G. austeni* (54 d) in natural populations. *Wolbachia* infections were present in all *G. austeni* flies analysed, while in contrast, this symbiont was absent from *G. pallidipes*. The density of *Wolbachia* infections in the Kenyan *G. austeni* population was higher than that observed in South African flies. The infection prevalence of *Sodalis* ranged from 3.7 percent in *G. austeni* to about 16 percent in *G. pallidipes*. Microscopic examination of midguts revealed an overall trypanosome infection prevalence of 6 percent (n = 235) and 5 percent (n = 552), while evaluation with ITS1 primers indicated a prevalence of about 13 percent (n = 296) and 10 percent (n = 302) in *G. austeni* and *G. pallidipes*, respectively. The majority of infections (46 percent) were with *T. congolense*. Co-infection with all three organisms was observed at 1 percent and 3.3 percent in *G. austeni* and *G. pallidipes*, respectively. Eleven out of the thirteen (85 percent) co-infected flies harboured *T. congolense* and *T. simiae* parasites. While the association between trypanosomes and *Sodalis* infection was statistically significant in *G. pallidipes* (p = 0.0127), the number of co-infected flies was too few for a definite conclusion. In conclusion, the tsetse populations analysed differed in the prevalence of symbionts, despite being sympatric and therefore exposed to identical environmental factors. The density of infections with *Wolbachia* also differed between *G. austeni* populations. There were too few natural co-infections detected with the *Sodalis* and trypanosomes to suggest extensive inter-relations between these infections in natural populations. We discuss these findings in the context of potential symbiont-mediated control interventions to reduce parasite infections and/or fly populations.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also **36:** 16781, 16786**]**

16799. **Barclay, H. J. & Vreysen, M. J., 2013.** To the Editor. Which kinds of models are useful and which are not. *Journal of Economic Entomology*, **106** (5): 1939.

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Note from Editor: For response from Peck and Bouyer see ref. 16803in this Volume of TTI.

Peck and Bouyer (2012) have written an essay to apprise tsetse modellers regarding which kinds of models are useful and which are not. It seems that they do not like much of what has gone before in the insect modelling world. An overview of tsetse modelling is welcome and they make some good points. However, there are enough inconsistencies in their presentation that a response is warranted. Peck and Bouyer single out two approaches to tsetse modelling and label both as inadequate. These two are those of the Hargrove, Vale, and Torr group and the tsetse model published by us (Barclay and Vreysen, 2011). Hargrove *et al.* (2011) have pointed out various problems with our article; we have since addressed all the concerns of Hargrove *et al.* and have updated the model with a more realistic dispersal component and time step. An article has recently been published (Barclay and Vreysen, 2013) based on these changes that addresses the effects of dispersal on the ease of control of tsetse.

Peck and Bouyer cite many publications of Hargrove and his associates and note (quite rightly) that they represent some of the best modelling of tsetse yet done. However, they later single out their approach as being inadequate because it does not consider spatial effects. In fact, Hargrove and his group have used both diffusion equations and probability distributions in assessing the effects of spatial distribution on tsetse population dynamics, and hence control. Peck and Bouyer refer to efforts at modelling spatial effects using diffusion equations as deserving special attention. In fact, diffusion equations are useful in some circumstances (modelling heat transfer along a metal rod, Brownian motion, large-scale movement of animals, etc.), but have severe limitations when used in an ecological context. They assume that all particles (in this case, insects) move randomly and independently. Some species may approximate this, but tsetse flies spend a significant amount of time resting, either sleeping off a large blood meal or waiting for a larva to finish development and be born; they also appear to exhibit some fidelity to a home range. In addition, diffusion equations in their simplest form have only one parameter and so only one factor can be modelled by them, although the addition of net movement (drift) allows another parameter to be used. Further, this one parameter has units of distance squared per unit time, and this is almost un-interpretable in an ecological context. Peck and Bouyer then go on to decry the use of simplistic models that are biologically unrealistic, which seems contradictory after they have referred to the diffusion models as deserving special attention.

Peck and Bouyer claim that we have argued for the use of the sterile insect technique (SIT) as being necessary or highly desirable in tsetse control. Although we do both believe that SIT is a very valuable tool for pest control and it should not be hastily dismissed from the arsenal of control methods for tsetse, our model is designed to allow the user to test the

efficacy of the four methods included in the model, both singly and in combination, and to allow the user to determine which ones would be optimal in a given situation. Peck and Bouyer note that different models that give similar results lend confidence in the robustness of the result. This is indeed a valuable approach to assessing how realistic are the results of a particular model. However, Peck and Bouyer then conclude that the various approaches that have been used so far are so different that they cannot be used for assessing robustness. This appears contradictory, as great similarity of two models would be expected to yield similar results and could not be construed as an index of robustness (Peck and Bouyer 2012, p. 1482). We were criticized for not making the model code available. We intend to put the code on the Web along with the model in the near future.

16800. **Bouyer, J., Seck, M. T. & Sall, B. 2013.** Misleading guidance for decision making on tsetse eradication: response to Shaw *et al.,* 2013. *Preventive Veterinary Medicine*, **112**: 443-446.

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Note from Editor: Details of references, Table and supplementary information available in Journal. For response from Shaw *et. al*., see ref. 16805 in this Volume of TTI.

The paper entitled "Estimating the costs of tsetse control options: An example for Uganda" (Shaw *et al*., 2013) presents full cost estimates for eliminating or continuously controlling riverine tsetse species *Glossina fuscipes fuscipes* Newstead (*palpalis* group) in Uganda in order to facilitate decision-making and financial planning. Four tsetse control techniques were compared: i) artificial baits (insecticide- treated traps/targets or ITT), (ii) insecticide-treated cattle (ITC), (iii) aerial spraying using the sequential aerosol technique (SAT) and (iv) the addition of the sterile insect technique (SIT) to the insecticide-based methods (i–iii).

While the economic approach as such might be sound to generate a basis for estimating the cost of tsetse control campaigns, the assessment is based on a disputed model (Vale and Torr, 2005) that assumes that the above insecticide-based methods alone can succeed in eradicating riverine tsetse populations. The limitations of this model are both mathematical (appropriate criteria for using and publishing agent-based models were not respected) and ecological (important characteristics of riverine tsetse populations were neglected) (Peck and Bouyer, 2012). The model was apparently developed based on experimental field data of the *morsitans* (savannah) group of tsetse fly species (*Glossina morsitans morsitans* Westwood and *Glossina pallidipes* Austen) that have fundamental different ecological characteristics as compared to riverine species such as a two-dimensional distribution pattern, a much higher sensitivity to landscape fragmentation and a lower opportunistic feeding behaviour (Van den Bossche *et al.*, 2010). Moreover, no matter what is suggested by a mathematical model, the authors should have carefully studied the real ecological situation in Uganda, should have provided an assessment of the population dynamics of *G. f. fuscipes* in the target area and should have substantiated their parameters with appropriate references. A review of past

control efforts indicates there are no examples of successful operational eradication campaigns against riverine tsetse fly populations except those that included an SIT component (see summary in Table 1).

One of the main reasons for failure has often been the use of a single control tactic in a localized area, rather than integrating several control tactics in an area-wide approach. Evidence for a successful eradication effort using solely ITT could only be found against a *G. morsitans centralis* (savannah fly) population in Zambia (Bart *et al.,* 1993). No eradication has ever been achieved using only ITC in any tsetse group. This is not really surprising as after a control trial in Burkina Faso, Bauer *et al.* (1999) reported that "*Glossina tachinoides* persisted in a small habitat outside the ZAP (Zone Agro-Pastorale), with catches varying from 0.2 to 2.1 flies per trap per d, the majority of the surviving flies feeding on reptiles, as can be seen from the blood-meal analyses". The same problem was reported with *Glossina austeni*, which is probably closer to the riverine than to the savannah group considering its behaviour and ecology. After a failed major eradication campaign that was mainly based on ITC, a follow-up effort that included the SIT as part of an area-wide integrated pest management (AW-IPM) campaign succeeded to eradicate this species from Unguja Island (Vreysen *et al.,* 2000). In West Africa, several SAT trials, or even application of residual insecticides by air, resulted in important reductions of the riverine target population density – but never in eradication (van der Vloedt *et al*., 1980; Baldry *et al*., 1981).

Even assuming a 3-year treatment interval between SAT campaigns to maintain suppression as done by Shaw *et al.* (2013) appears unreasonably optimistic as evidence suggests that a suppressed tsetse population takes little more than one year to recover after an SAT effort (Turner and Brightwell, 1986). This frequency could even foster the generation of secondary problem scenarios, i.e. reduced cattle immunity against trypanosomes and thus breaking the endemic cycle which could result in important epidemic outbreaks (Desquesnes *et al.,* 2009; Van Den Bossche and Delespaux, 2011). The use of ITT at 7 km^{-2} failed to eradicate *G. f. fuscipes* even when used in a 32 km^2 isolated section of an island (Okoth *et al.*, 1991), which is almost double the target density used in the Shaw *et al*. (2013) paper to calculate eradication costs. Moreover, the authors suggest that an "improved target design has the potential to increase field cost-effectiveness (as measured by tsetse killed per m^2 of cloth) by a factor of 10". It must be noted, however, that the price of the cloth (around Euro 3 for a 1 m^2 target) is not the sole cost factor to be considered as the deployment cost (labour, vehicle, fuel, etc.) represents an additional Euro 5–7 per unit. Since a smaller target kills a smaller number of flies than a target of a larger size, a higher density of these smaller targets per unit of surface area is required to obtain the same tsetse mortality rate, which obviously will result in higher deployment costs (see additional file, section B for more details).

The treatment costs of ITC as calculated by Shaw *et al.* (2013) are based on the SOS campaign against *G. f. fuscipes* in Uganda where 9-22 heads of cattle/km^2 were treated every 3 months. Although the prevalence of *Trypanosoma brucei* s.l. was reduced initially, the tsetse suppression efforts failed to further reduce disease transmission; on the contrary, the prevalence increased again to the pre- control level within nine months (Morton, 2010; Selby, 2010). The intensity of treatment and its frequency were thus inadequate to have any meaningful level of tsetse suppression. A simple model (see supplementary file, section C) integrating the feeding behaviour of *G. f. fuscipes* in Uganda (Waiswa *et al.,* 2006) and the knock down rates associated to restricted application of insecticides against riverine species (Bouyer *et al.*, 2007) shows that given the treatment frequencies used in the SOS programme, the estimated daily mortality rate imposed to this species was indeed too low to induce any suppression, not even speaking of eradication.

In conclusion, the above examples clearly indicate that Shaw *et al.* (2013) have based their economic analysis on various assumptions originating from a dodgy model and that are contrary to what has been experienced in the field against riverine tsetse in general and *G. f. fuscipes* in particular. This paper is thus misleading and provides wrong advice to governments and tsetse control operators.

16801**. Hargrove, J., 2013.** An example from the world of tsetse flies. *Mathematical Biosciences & Engineering*, **10** (3): 691-704.

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In biomathematics, communication between mathematicians and biologists is crucial. This matter is illustrated using studies aimed at estimating mortality rates of tsetse flies (*Glossina* spp.). Examples are provided of apparently sound pieces of mathematics which, when applied to real data, provide obviously erroneous results. More serious objections arise when mathematical models make no attempt to address the real world in such a way that they can be tested. Unless models account for the known biology of the problem under investigation, and are challenged with data, the existence and nature of imperfections in the models will likely not be detected.

16802. **Medlock, J., Atkins, K. E., Thomas, D. N., Aksoy, S. & Galvani, A. P., 2013.** Evaluating paratransgenesis as a potential control strategy for African trypanosomiasis. *PLoS Neglected Tropical Diseases*, **7** (8): e2374.

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Genetic-modification strategies are currently being developed to reduce the transmission of vector-borne diseases, including African trypanosomiasis. For tsetse, the vector of African trypanosomiasis, a paratransgenic strategy is being considered. This approach involves modification of the commensal symbiotic bacteria *Sodalis* to express trypanosome resistance-conferring products. Modified *Sodalis* can then be driven into the tsetse population by cytoplasmic incompatibility (CI) from *Wolbachia* bacteria. To evaluate the effectiveness of this paratransgenic strategy in controlling African trypanosomiasis, we developed a three-species mathematical model of trypanosomiasis transmission among tsetse, humans, and animal reservoir hosts. Using empirical estimates of CI parameters, we found that paratransgenic tsetse have the potential to eliminate trypanosomiasis, provided that any extra mortality caused by *Wolbachia* colonization is low, that the paratransgene is effective at protecting against trypanosome transmission, and that the target tsetse species comprises a large majority of the tsetse population in the release location.

16803. **Peck, S. L. & Bouyer, J., 2013.** Reply. *Journal of Economic Entomology*, **106** (5): 1940.

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We appreciate the opportunity to respond to Barclay and Vreysen's concerns (see ref. 16799) about our article. We are confused about their claim that "P&B refer to efforts at modelling spatial effects using diffusion equations as deserving special attention," as no such claim is made in our article. The focus of the article is on simulation models and the importance of capturing ecological reality. They then argue for the importance of including ecological context above that which simple models such as diffusion models can provide, but that is actually the entire focus of our article. Their closing statement, "This appears contradictory, as great similarity of two models would be expected to yield similar results and could not be construed as an index of robustness" is cryptic because we do not know what two models they are referring to, and that matters for an assessment of robustness. We are pleased they have built another model and look forward to more thoroughly assessing it, with hopes that they have taken our suggestions for presenting an ecological simulation model seriously. The new dispersal models used by the authors include dispersal toward clumps or a percentage of non-dispersing flies at each time step, which actually better mimics reality. They, however, will need more field data to be parameterized, which is generally a limitation to their use.

16804. **Pooda, S. H., Mouline, K., De Meeus, T., Bengaly, Z. & Solano, P., 2013.** Decrease in survival and fecundity of *Glossina palpalis gambiensis* Vanderplank 1949 (Diptera: Glossinidae) fed on cattle treated with single doses of Ivermectin. *Parasites & Vectors*, **6**: 165.

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Human and animal trypanosomes are major problems for the socio-economic growth of developing countries like Burkina Faso. Ivermectin is currently used to treat humans in mass drug administration programmes in Africa, and is also commonly used for veterinary purposes. In this study, we tested the effect of Ivermectin injected into cattle on the survival and fecundity of *Glossina palpalis gambiensis*, the main vector of human and animal trypanosomes in West Africa. Three cows (local Zebu x Baoule crossbreds) were used, and received either no Ivermectin (for the control), or Ivermectin at the therapeutic dose (0.2 mg/kg) and at 10 times the therapeutic dose (2 mg/kg) respectively. *G. palpalis gambiensis* were fed on the cattle for their first blood meal, and then either on cattle or on membrane for subsequent meals. Our results showed that survival of *Glossina palpalis gambiensis* was significantly decreased when they were fed on cattle treated with Ivermectin. This decrease in survival ranged from 21 percent to 83.7 percent for the therapeutic dose (0.2 mg/kg), up to 8 d after treatment. The effects of a dose of 2 mg/kg were greater with a 78.3 percent to 93.9 percent decrease in survival, until 14 d after injection. The therapeutic dose of Ivermectin also decreased fecundity and delayed the first larviposition, but there was no significant effect on hatching rate. It is concluded that Ivermectin injected into cattle may constitute an additional potential tool for the control of *Glossina palpalis gambiensis* and possibly other vector species. Further studies will be needed to assess its effect on trypanosome transmission, and to define more precisely the adequate dose to be used for control purposes.

16805. **Shaw, A. P. M., Torr, S. J., Waiswa, C., Cecchi, G., Wint, G. R. W., Mattioli, R. C. & Robinson, T. P. 2013.** *Preventive Veterinary Medicine*, 112: 447-449. Reply to the letter to the editor by Bouyer *et al.*, 2013.

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Note from Editor: Reference details available in Journal.

Who is really misleading decision-makers about cost-effective approaches to tackling tsetse flies? Bouyer *et al*. (2013) claim we have given misleading advice in our paper (Shaw *et al*., 2013) on the costs of controlling or eliminating riverine or savannah species of tsetse using various techniques, singly or in combination. We suggested that the most cost-effective option is often tsetse control, rather than elimination. In contrast, Bouyer *et al.* (2013) focus on the importance of eliminating tsetse from whole belts of contiguous tsetse infestation, and claim that for riverine species the only means of achieving this is to rely on the sterile insect technique (SIT) to deliver the final blow to populations suppressed by other methods.

The focus adopted by Bouyer *et al.* (2013) accords with their involvement in an SIT-based elimination programme in Senegal (Vreysen *et al*., 2013). Given that SIT deals particularly poorly with the problem of tsetse invasion (Vale and Torr, 2005) it is not surprising that they link the use of SIT to the aim of widespread elimination of tsetse up to the natural barriers to invasion – the so-called area-wide policy. Nor is it surprising that they claim that SIT is essential for final elimination since the use of this complex, costly and protracted technique makes sense only if cheaper and simpler techniques cannot perform the task unaided by SIT. To maintain their stance, they ignore evidence detrimental to their cause and advance only weak arguments, which we challenge below.

First, Bouyer *et al.* (2013) criticise the model (Vale and Torr, 2005; Torr and Vale, 2011) that we used to assess conservatively the time for which various elimination techniques need to be applied. The criticisms levelled against this model and, implicitly against the model of Hargrove (2003), distract attention from the pertinent point that no alternative model has shown anything materially different. The only model that seemed to attempt this (Barclay and Vreysen, 2011a) considered technical efficacy alone, not costs, and was flawed in its structure and parameters, as outlined by Hargrove *et al*. (2011) and subsequently acknowledged by the model's authors (Barclay and Vreysen, 2011b, 2013).

Second, Bouyer *et al.* (2013) present a review table listing field operations purporting to prove that only campaigns involving SIT have ever eliminated riverine tsetse. That table is highly selective in failing to mention insecticide-based campaigns that have eliminated riverine flies without SIT, as in northern Nigeria (Davies, 1971; Spielberger *et al*., 1977; Jordan, 1986). It is particularly surprising that they did not mention the use of low technology methods to eliminate riverine tsetse from Principe Island off the west African coast (Maldonado, 1910; Jordan, 1986), since that work forms the natural counterpoint to the much

advertised SIT-assisted operations on Unguja Island, Zanzibar (Vreysen *et al.*, 2000). Moreover, their table is misleading and confused in that of the 14 separate operations listed, only six were aimed at elimination and three of these dealt with savannah tsetse. A project, such as that of Laveissière and Couret (1981), which ran for only two months, can hardly be judged as a failed elimination attempt when elimination was clearly not its objective. In general, any elimination operation that does not address a fully isolated population, or which does not maintain indefinitely an artificial barrier to invasion, runs a severe risk of eventual failure. To be objective, Bouyer *et al.* (2013) should also have commented in their table on those interventions listed that have faced this problem, including SIT-based schemes (Cuisance *et al*., 1984; Takken *et al*., 1986 as referred by Bauer *et al.*, 1988; Oluwafemi, 2009).

Third, Bouyer *et al.* (2013) claim that we have taken no account of the importance of local variations in tsetse ecology in the design and costing of operations. However, our paper aimed at a general comparison of costs for which it was appropriate to consider a theoretical, square-shaped intervention area of 10 000 km^2, homogenously infested by a single fly species. Our costings were inflated to allow for the commonly accepted need to investigate how optimal control policy in specific situations is affected by local ecology. Bouyer *et al.* (2013) appear to believe that the distribution of riverine tsetse is always going to make insecticide-based operations more problematical than with savannah tsetse, to the point that such operations can never succeed with riverine flies. In fact, the more that riverine tsetse are confined to small isolated pockets the more readily and economically they can be tackled in comparison with highly mobile tsetse spread across vast areas of savannah.

Fourth, the more recent advances in bait methods of attacking tsetse seem particularly objectionable to Bouyer (2013). These new systems are so simple and economical that they make more credible the idea of living with locally reduced populations of the flies, instead of opting for widespread elimination. The use of insecticide-treated cattle (ITC) in the stamp out sleeping sickness (SOS) project in Uganda is singled out for special criticism. In reality, SOS has shown that ITC offers one of the most economical, effective and feasible means of dealing with riverine tsetse and sleeping sickness (Selby *et al*., 2007; Butcher, 2009; Selby, 2010; Hargrove *et al.,* 2012). And it is a reduction in disease incidence, rather than in tsetse numbers, which should be the primary goal of all operations against tsetse. The obvious point made by Bouyer *et al.* (2013), that ITC cannot be used where cattle do not occur, has long been recognised, both in theory (Torr and Vale, 2011) and in practice (Torr *et al.*, 2005) – the corrective expedient being to supplement ITC by placing insecticide-treated targets in parts of the operational area where cattle do not go, or occur in densities too low for ITC to be effective. Moreover, contrary to the view of Bouyer *et al.* (2013), the demonstration of the efficacy of tiny targets for use against riverine tsetse (Lindh *et al.*, 2009) is encouraging, especially since such targets are ideally suited to deal with tsetse in small pockets. Bouyer *et al.* (2013) have failed to appreciate that our costings for traps or targets make full provision for overall operational costs, in the same way that we consider the overall costs of other techniques.

Fifth, in promoting the use of SIT, Bouyer *et al*. (2013) fail to mention that our costing for that technique puts it in the most favourable light. Thus our estimates for the costs of SIT relate to 10 000 km^2, so that economies of scale could be realised, leading to costs within the range cited in Feldmann (2004). However, the fact that Bouyer *et al.* (2013) suggest that SIT is particularly important in dealing with small pockets, implies that the cost per km^2 for adding SIT could well be five times greater than calculated in our paper and in line with what was recorded in Unguja (Msangi *et al*., 2000). Furthermore, we accounted for only one

species of tsetse being tackled by SIT. If, as in many places, more than one species must be attacked, the cost of SIT is likely to rise by a further 60 percent per species (personal communication, Udo Feldmann). This contrasts sharply with the fact that insecticide-based control can deal with several species at once, at little or no extra cost.

Returning to the suggestion by Bouyer *et al.* (2013) that we have provided misleading advice, our paper contains only two statements that could be interpreted as advice. First, we recommended that decision-makers should consider full costs, rather than the conventionally cited partial costs. Second, we suggested that for the time being control may be the better option than elimination in many places. We stand by that advice, recognising that it is at variance with the agenda that has dominated attempts to deal with tsetse over the past 15 years, whereby decision-makers were encouraged to set elimination as their only goal, and to rely on SIT to achieve it. That agenda has failed half a generation of Africa's poor and marginalised rural inhabitants, who have received little or no support or advice on how to apply existing, cost-effective methods of tsetse control to minimise the incidence of tsetse-borne trypanosomosis in humans and livestock.

16806. **Solano, P., Torr, S. J. & Lehane, M. J., 2013.** Is vector control needed to eliminate *gambiense* human African trypanosomiasis? *Frontiers in Cellular & Infection Microbiology*, **3**: 33.

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Human African trypanosomiasis (HAT), or sleeping sickness, is a neglected tropical parasitic disease of humans due to trypanosomes transmitted by tsetse flies (*Glossina* spp.) in sub-Saharan Africa. Comparable diseases (animal African trypanosomiasis-AAT-nagana) are present in domesticated animals and these are an important constraint to animal health and production in Africa. For HAT, there is no vaccine, no chemoprophylaxis, and treatment is still long and difficult to administer despite recent improvements. In most cases HAT is fatal if untreated. Two flagellate protozoan parasites cause HAT. *Trypanosoma brucei rhodesiense* causes the *rhodesiense* form of the disease (currently <5 percent of all cases) in eastern and southern Africa, and *T. b. gambiense* causes the *gambiense* form of the disease (currently >95 percent of all cases) in Central and West Africa. Although it is accepted that tsetse control plays a central role in combatting the *rhodesiense* form of HAT, this has not been the case for the *gambiense* form. Indeed in the strategy recommended by WHO to control sleeping sickness, active case detection and treatment has always been the first, if not the only method recommended, until very recently.

Concerning whether vector control is required in *gambiense* HAT control, we would first like to make it clear that we recognize that active case detection and treatment has proved effective in HAT control in many foci and that it is a necessary intervention if those infected are not to die. However, there are several foci where active case detection and treatment without vector control, despite saving many lives, has failed to bring HAT under effective control. A mathematical model of the basic Reproductive Number (R_0) which was developed to analyse and compare vector control and active case detection and treatment in the control of *gambiense* HAT showed that when transmission rates are high (strongly influenced by fly biting rates on humans), vector control is a requirement.

Recent advances in our understanding of the epidemiology of Gambian HAT have strengthened the case for an integrated approach. First, mature *T. b. gambiense* infection rates

in tsetse (i.e. when trypanosomes have reached the salivary glands of tsetse and can be transmitted to the next mammalian host) are usually below 1 percent even in active foci, and this is also true for *T. b. rhodesiense*. This means that reduction in tsetse densities, even those not reaching total elimination, will also reduce transmission. Re-analysis of the theoretical models suggests that transmission can be halted without the elimination of tsetse. Second, there is more and more evidence that some people can live a long time while being infected by *T. b. gambiense*. This is the case for asymptomatic carriers, but also for some seropositive people who are not detected by the parasitological techniques and who will develop the disease. These two elements strongly suggest that without vector control to break the transmission cycle, elimination of *T. b. gambiense* cannot be achieved even under the assumption that HAT is an anthroponosis (i.e. transmission does not involve a non-human reservoir host). If non-humans are important reservoir hosts, then, as with Rhodesian HAT, elimination of Gambian HAT can only be achieved through a combination of medical activities and vector control. In addition to the technical arguments, tsetse control may make active case detection and treatment more efficient and affordable. Active case detection and treatment rarely covers more than 80 percent of the community treated. And it is recognized that the people who are not screened are the ones who are the most exposed (farmers, fishermen, hunters, people who work in plantations). As a result, transmission of *T. b. gambiense* will still continue after a medical intervention unless vector control is also carried out. In the absence of a vaccine or prophylactic drugs, vector control offers the only means of protecting people from infection while also reducing transmission of trypanosomes from the residual population of HAT-infected people. We suggest therefore that adding a vector control component will increase the sustainability of medical interventions.

Is vector control affordable and achievable? Restricted application of insecticide to cattle has proved cost effective and successful, and this technique currently provides good control of *rhodesiense* form HAT in Uganda and so has proved affordable and achievable. However, this only works where cattle densities are high enough, which is not the case for most areas with *gambiense* HAT. Where cattle densities are not high enough, insecticide treated cloth targets or cloth traps are often used and have been used successfully and currently tsetse control operations conducted in Gambian HAT foci use this type of technology. In all situations, the vector control phase should be implemented after a first phase of baseline data collection that will help to precisely define the identity, density, and spatial distribution of the targeted tsetse species.

Until recently, tsetse control operations have been said to be unaffordable; however recent work has changed this. Most *gambiense*-HAT is transmitted by “Palpalis group” tsetse, more commonly known as riverine tsetse. If we look more closely at the known distribution of HAT cases and the distribution of tsetse flies, we can see that the vast majority of current transmission is being caused by only two tsetse species, *G. fuscipes spp*. and *G. palpalis spp*. We have been concentrating on producing cheaper, target-based control technology for these species with funding from the Bill and Melinda Gates Foundation. A major discovery is that very small targets are highly effective for these two species. This completely changes the prospects for use of tsetse control in campaigns against *gambiense* form HAT. Commercial companies can provide long-lasting versions of these insecticide impregnated targets at ~US$1 each. Reducing target size will not only reduce material costs but also promises significant savings in operational costs. We believe that the tiny targets now available will increase deployment rates considerably and the savings associated with this increased productivity will be significant. Once deployment rates reach >4 targets per person per d we

estimate the costs fall to between US$50 to US$100 per km^2 per year which makes vector control a more feasible proposition for those involved in HAT control.

Concerning who should do the control, this can be effectively undertaken at a range of levels from regional activities involving several countries to the local village level. A possible scenario could be that control activities should be planned, organized, and implemented at the scale of the focus, by national HAT control programmes with personnel from health or vector control structures who already work in the focus. Clearly at the beginning, baseline data collection and the first vector control activities should be organized professionally by a national team who has the expertise of doing it, or who has been trained to have such expertise. Then, most of the activities (target maintenance, deployment, and tsetse densities monitoring) could be progressively transferred to NGOs or local health workers, with some supervision from the national team.

In conclusion, a change of paradigm has occurred, since it is now recognized that vector control is part of the elimination strategy of *gambiense* HAT as a complement to medical activities. Integrating medical and vector-based interventions will enable HAT-affected countries to eliminate Gambian HAT.

16807. **Yehouenou, A. P. E., Aleodjrodo, P. E., Azehoun, J. P., van Straalen, N. M., van Hattum, B., Swart, K. & van Gestel, C. A., 2014.** Pesticide residues in sediments and aquatic species in Lake Nokoue and Cotonou Lagoon in the Republic of Benin. *Environmental Monitoring & Assessment*, **186** (1): 77-86.

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Lake Nokoue and Cotonou Lagoon are the most important and most productive continental freshwaters in Benin, with an estimated fish production of over 2 tonnes per ha in Lake Nokoue. Organochlorine pesticides are used in agriculture and to repel tsetse flies, malaria mosquitoes and other diseases. Sediment, fish, shrimp and oyster species were collected in Lake Nokoue and Cotonou Lagoon for pesticide residues analysis. The main pesticides identified in sediment were pp'-DDT and its metabolites pp-DDE and pp'-DDD, with residue levels between the detection limit and 24.4 µg/kg dry weight. Fish species commonly consumed such as *Elops lacerta, Podamasys jubelini, Gobbienellus occidentalis, Ethmalosa fimbriata, Mugil cephalus* and *Hemichromis fasciatus* were contaminated with residues of seven to nine pesticides, including pp-DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT, alpha-endosulfan, aldrin, dieldrin and gamma-hexachlorocyclohexane. The levels ranged from detection limit to 289 ng/g lipid. The same pesticides were also detected in other aquatic species such as shrimp and oysters. A summed risk assessment, comparing pesticide intake levels through fish consumption with tolerable daily intake levels proposed by the World Health Organization, showed in all cases a low risk for human health.

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

[See also **36:** 16798, 16800, 16801, 16805, 16806, 16833, 16843**]**

16808. **Baldacchino, F., Muenworn, V., Desquesnes, M., Desoli, F., Charoenviriyaphap, T. & Duvallet, G., 2013.** Transmission of pathogens by *Stomoxys* flies (Diptera, Muscidae): a review. *Parasite*, **20**: 26.

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Stomoxys flies are mechanical vectors of pathogens present in the blood and skin of their animal hosts, especially livestock, but occasionally humans. In livestock, their direct effects are disturbance, skin lesions, reduction of food intake, stress, blood loss, and a global immunosuppressive effect. They also induce the gathering of animals for mutual protection; meanwhile they favour development of pathogens in the hosts and their transmission. Their indirect effect is the mechanical transmission of pathogens. In case of interrupted feeding, *Stomoxys* can re-start their blood meal on another host. When injecting saliva prior to blood-sucking, they can inoculate some infected blood remaining on their mouthparts. Beside this immediate transmission, it was observed that *Stomoxys* may keep some blood in their crop, which offers a friendly environment for pathogens that could be regurgitated during the next blood meal; thus a delayed transmission by *Stomoxys* seems possible. Such a mechanism has a considerable epidemiological impact since it allows inter-herd transmission of pathogens. Equine infectious anaemia, African swine fever, West Nile, and Rift Valley viruses are known to be transmitted by *Stomoxys*, while others are suspected. Rickettsia (*Anaplasma, Coxiella*), other bacteria and parasites (*Trypanosoma* spp., *Besnoitia* spp.) are also transmitted by *Stomoxy*s. Finally, *Stomoxys* was also found to act as an intermediate host of the helminth *Habronema microstoma* and may be involved in the transmission of some *Onchocerca* and *Dirofilaria* species. Being cosmopolite, *Stomoxys calcitrans* might have a worldwide and greater impact than previously thought on animal and human pathogen transmission.

16809. **Bouyer, J., Kone, N. & Bengaly, Z., 2013.** Dynamics of tsetse natural infection rates in the Mouhoun river, Burkina Faso, in relation with environmental factors. *Frontiers in Cellular & Infection Microbiology*, **3**: 47.

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In Burkina Faso, the cyclical vectors of African animal trypanosomoses (AAT) are riverine tsetse species, namely *Glossina palpalis gambiensis* Vanderplank (*G. p. g.*) and *Glossina tachinoides* Westwood (*G. t.*) (Diptera: Glossinidae). Experimental work demonstrated that environmental stress can increase the sensitivity of tsetse to trypanosome infection. Seasonal variations of tsetse infection rates were monitored monthly over 17 months (May 2006-September 2007) at two sites (Douroula and Kadomba). In total, 1 423

flies were dissected and the infection of the proboscis, middle intestine and salivary glands was noted. All the positive organs were analysed using monospecific polymerase chain reaction (PCR) primers. To investigate the role of different environmental factors, fly infection rates were analysed using generalized linear mixed binomial models using the species, sex, and monthly averages of the maximum, minimum and mean daily temperatures, rainfalls, land surface temperature day (LSTd) and night (LSTn) as fixed effects, and the trap position as a random effect. The overall infection rate was 10 percent from which the predominant species was *T. congolense* (7.6 percent of the flies), followed by *T. vivax* (2.2 percent of the flies). The best model (lowest AICc) for the global infection rates was the one with the maximum daily temperature only as fixed effect ($p < 0.001$). For *T. congolense*, the best model was the one with the tsetse species, sex, maximum daily temperature and rainfalls as fixed effects, where the maximum daily temperature was the main effect ($p < 0.001$). The number of *T. vivax* infections was too low to allow the models to converge. The maturation rate of *T. congolense* was very high (94 percent), and *G. t.* harboured a higher maturation rate ($p = 0.03$). The results are discussed in view of former laboratory studies showing that temperature stress can increase the susceptibility of tsetse to trypanosomes, as well as the possibility to improve AAT risk mapping using satellite images.

16810. **Dama, E., Cornelie, S., Camara, M., Somda, M. B., Poinsignon, A., Ilboudo, H., Elanga Ndille, E., Jamonneau, V., Solano, P., Remoue, F., Bengaly, Z., Belem, A. M. & Bucheton, B., 2013.** *In silico* identification of a candidate synthetic peptide (Tsgf118-43) to monitor human exposure to tsetse flies in West Africa. *PLoS Neglected Tropical Diseases*, **7** (9): e2455.

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The analysis of humoral responses directed against the saliva of blood-sucking arthropods was shown to provide epidemiological biomarkers of human exposure to vector-borne diseases. However, the use of whole saliva as antigen presents several limitations such as problems of mass production, reproducibility and specificity. The aim of this study was to design a specific biomarker of exposure to tsetse flies based on the *in silico* analysis of three *Glossina* salivary proteins (Ada, Ag5 and Tsgf1) previously shown to be specifically recognized by plasma from exposed individuals. Synthetic peptides were designed by combining several linear epitope prediction methods and Blast analysis. The most specific peptides were then tested by indirect ELISA on a bank of 160 plasma samples from tsetse infested areas and tsetse free areas. Anti-Tsgf118-43 specific IgG levels were low in all three control populations (from rural Africa, urban Africa and Europe) and were significantly higher ($p < 0.0001$) in the two populations exposed to tsetse flies (Guinean HAT foci, and South West Burkina Faso). A positive correlation was also found between anti-Tsgf118-43 IgG levels and the risk of being infected by *Trypanosoma brucei gambiense* in the sleeping sickness foci of Guinea. The Tsgf118-43 peptide is a suitable and promising candidate to develop a standardized immunoassay allowing large scale monitoring of human exposure to tsetse flies in West Africa. This could provide a new surveillance indicator for tsetse control interventions by HAT control programmes.

16811. **Desquesnes, M., Dargantes, A., Lai, D. H., Lun, Z. R., Holzmuller, P. & Jittapalapong, S., 2013.** *Trypanosoma evansi* and surra: a review and perspectives on

transmission, epidemiology and control, impact, and zoonotic aspects. *Biomed Research International*, **2013**: 1-20.

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This paper reviews the transmission modes of *Trypanosoma evansi.* Its worldwide distribution is attributed to mechanical transmission. While the role of tabanids is clear, we raise questions on the relative role of *Haematobia* spp. and the possible role of *Stomoxys* spp. in delayed transmission. A review of the available trypanocidal drugs and their efficacy in various host species is useful for understanding how they interact in disease epidemiology, which is complex. Although there are similarities with other mechanically transmitted trypanosomes, *T. evansi* has a more complex epidemiology due to the diversity of its hosts and vectors. The impact of clinical and subclinical disease is difficult to establish. A model was developed for buffaloes in the Philippines, which could be transferred to other places and livestock systems. Since *Trypanosoma evansi* was reported in humans, further research is required to investigate its zoonotic potential. Surra remains a potentially emerging disease that is a threat to Australia, Spain, and France. A number of questions about the disease have yet to be resolved. This brief review of the basic knowledge of *T. evansi* suggests that there is renewed interest in the parasite, which is spreading and has a major economic impact.

16812. **Dyer, N. A., Rose, C., Ejeh, N. O. & Acosta-Serrano, A., 2013.** Flying tryps: survival and maturation of trypanosomes in tsetse flies. *Trends in Parasitology*, **29** (4): 188-196.

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Survival in and colonization of the tsetse fly midgut are essential steps in the transmission of many species of African trypanosomes. In the fly, bloodstream trypanosomes transform into the procyclic stage within the gut lumen and later migrate to the ectoperitrophic space, where they multiply, establishing an infection. Progression of the parasite infection in the fly depends on factors inherent to the biology of trypanosomes, tsetse, and the blood meal. Flies usually eradicate infection early on with both pre-existing and inducible factors. Parasites, in contrast, respond to these stimuli by undergoing developmental changes, allowing a few to both survive and migrate within the tsetse. Here we discuss parasite and fly factors determining trypanosome colonization of the tsetse, focusing mainly on the midgut.

16813. **Echodu, R., Sistrom, M., Hyseni, C., Enyaru, J., Okedi, L., Aksoy, S. & Caccone, A., 2013.** Genetically distinct *Glossina fuscipes fuscipes* populations in the Lake Kyoga region of Uganda and its relevance for human African trypanosomiasis. *Biomed Research International*, **2013**: 614721.

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Tsetse flies (*Glossina* spp.) are the sole vectors of *Trypanosoma brucei* - the agent of human (HAT) and animal (AAT) trypanosomiases. *Glossina fuscipes fuscipes* (*G. f. f.*) is the main vector species in Uganda - the only country where the two forms of HAT disease (*rhodesiense* and *gambiense*) occur, with *gambiense* limited to the northwest. *G. f. f.* populations cluster in three genetically distinct groups in northern, southern and western Uganda, respectively, with a contact zone present in central Uganda. Understanding the dynamics of this contact zone is epidemiologically important as the merger of the two diseases is a major health concern. We used mitochondrial and microsatellite DNA data from *G. f. f.* samples in the contact zone to understand its spatial extent and temporal stability. We show that this zone is relatively narrow, extending through central Uganda along major rivers with south to north introgression but displaying no sex-biased dispersal. Lack of obvious vicariant barriers suggests that either environmental conditions or reciprocal competitive exclusion could explain the patterns of genetic differentiation observed. Lack of admixture between northern and southern populations may prevent the sympatry of the two forms of HAT disease, although continued control efforts are needed to prevent the recolonization of tsetse-free regions by neighbouring populations.

16814. **Eyford, B. A., Ahmad, R., Enyaru, J. C., Carr, S. A. & Pearson, T. W., 2013.** Identification of trypanosome proteins in plasma from African sleeping sickness patients infected with *T. b. rhodesiense*. *PLoS One*, **8** (8): e71463.

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Control of human African sleeping sickness, caused by subspecies of the protozoan parasite *Trypanosoma brucei*, is based on preventing transmission by elimination of the tsetse vector and by active diagnostic screening and treatment of infected patients. To identify trypanosome proteins that have potential as biomarkers for detection and monitoring of African sleeping sickness, we have used a “deep-mining" proteomics approach to identify trypanosome proteins in human plasma. Abundant human plasma proteins were removed by immunodepletion. Depleted plasma samples were then digested to peptides with trypsin, fractionated by basic reversed phase and each fraction analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). This sample processing and analytical method enabled identification of low levels of trypanosome proteins in pooled plasma from late stage sleeping sickness patients infected with *Trypanosoma brucei rhodesiense*. A total of 254 trypanosome proteins were confidently identified. Many of the parasite proteins identified were of unknown function, although metabolic enzymes, chaperones, proteases and ubiquitin-related/acting proteins were found. This approach to the identification of conserved, soluble trypanosome proteins in human plasma offers a possible route to improved disease diagnosis and monitoring, since these molecules are potential biomarkers for the development of a new generation of antigen-detection assays. The combined immuno-depletion/mass spectrometric approach can be applied to a variety of infectious diseases for unbiased biomarker identification.

16815. **Haines, L. R., 2013.** Examining the tsetse teneral phenomenon and permissiveness to trypanosome infection. *Frontiers in Cellular & Infection Microbiology*, **3**: 84.

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Tsetse flies are the most important vectors of African trypanosomiasis but, surprisingly, are highly refractory to trypanosome parasite infection. In populations of wild caught flies, it is rare to find mature salivarian and mouthpart parasite infection rates exceeding 1 percent and 15 percent, respectively. This inherent refractoriness persists throughout the lifespan of the fly, although extreme starvation and suboptimal environmental conditions can cause a reversion to the susceptible phenotype. The teneral phenomenon is a phenotype unique to newly emerged, previously unfed tsetse, and is evidenced by a profound susceptibility to trypanosome infection. This susceptibility persists for only a few d post-emergence and decreases with fly age and blood meal acquisition. Researchers investigating trypanosome-tsetse interactions routinely exploit this phenomenon by using young, unfed (teneral) flies to naturally boost trypanosome establishment and maturation rates. A suite of factors may contribute, at least in part, to this unusual parasite permissive phenotype. These include the physical maturity of midgut barriers, the activation of immunoresponsive tissues and their effector molecules, and the role of the microflora within the midgut of the newly emerged fly. However, at present, the molecular mechanisms that underpin the teneral phenomenon still remain unknown. This review provides a historical overview of the teneral phenomenon and examines immune-related factors that influence, and may help us better understand, this unusual phenotype.

16816. **Kohagne Tongue, L., Mavoungou, J., Fako Hendji, G., Pamba, R. & Mbatchi, B., 2013.** Is there a suburban sleeping sickness in Libreville? *African Health Sciences*, **13** (2): 266-269.

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The transmission of sleeping sickness occurs primarily in rural areas, and exposed populations are those living from rural activities such as agriculture, fishing, animal husbandry or hunting. However, urban and suburban foci are more and more reported in *T. b. gambiense* areas. In Libreville town, sleeping sickness cases are regularly diagnosed. In order to investigate the establishment of a transmission cycle of that disease, we have carried out an entomological survey in two quarters in the vicinity of the town. Vavoua traps were set out in all suitable biotopes for tsetse flies during four d and examined twice daily. Flies were collected, identified and dissected. Two species of *Glossina* were caught: *G. palpalis palpalis* (90.58 percent) and *G. caliginea* (9.42 percent). A total infection rate of 9.37 percent was observed after dissection of all non-teneral flies captured. These results suggest the establishment of a trypanosomiasis transmission cycle in the area. No salivary gland was found to be infected. Given that infected persons are regularly detected, we can think about the existence of a suburban sleeping sickness focus in Libreville. More analysis is needed concerning the identification of human trypanosomes and the origin of *Glossina* blood meals that may confirm the existence of that focus.

16817.**Majekodunmi, A. O., Fajinmi, A., Dongkum, C., Picozzi, K., Macleod, E., Thrusfield, M. V., AP, M. S. & Welburn, S. C., 2013.** Social factors affecting seasonal variation in bovine trypanosomosis on the Jos Plateau, Nigeria. *Parasites & Vectors*, **6** (1): 293.

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Animal African trypanosomosis (AAT) is a widespread disease of livestock in Nigeria and presents a major constraint to rural economic development. The Jos Plateau was considered free from tsetse flies and the trypanosomes they transmit due to its high altitude and this trypanosomiasis free status attracted large numbers of cattle-keeping pastoralists to the area. The Jos Plateau now plays a major role in the national cattle industry in Nigeria, accommodating approximately 7 percent of the national herd, supporting 300 000 pastoralists and over one million cattle. During the past two decades tsetse flies have invaded the Jos Plateau and animal trypanosomosis has become a significant problem for livestock keepers. Here we investigate the epidemiology of trypanosomosis as a re-emerging disease on the Plateau, examining the social factors that influence prevalence and seasonal variation of bovine trypanosomosis. In 2008, a longitudinal two-stage cluster survey was undertaken on the Jos Plateau. Cattle were sampled in the dry, early wet and late wet seasons. Parasite identification was undertaken using species-specific polymerase chain reactions to determine the prevalence and distribution of bovine trypanosomosis. Participatory rural appraisal was also conducted to determine knowledge, attitudes and practices concerning animal husbandry and disease control. Significant seasonal variation between the dry season and late wet season was recorded across the Jos Plateau, consistent with expected variation in tsetse populations. However, marked seasonal variations were also observed at village level to create 3 distinct groups: Group 1 in which 50 percent of villages followed the general pattern of low prevalence in the dry season and high prevalence in the wet season; Group 2 in which 16.7 percent of villages showed no seasonal variation and Group 3 in which 33.3 percent of villages showed greater disease prevalence in the dry season than in the wet season. In conclusion, there was high seasonal variation at the village level determined by management as well as climatic factors. The growing influence of management factors on the epidemiology of trypanosomosis highlights the impact of recent changes in land use and natural resource competition on animal husbandry decisions in the extensive pastoral production system.

16818. **Majekodunmi, A. O., Fajinmi, A., Dongkum, C., Picozzi, K., Thrusfield, M. V. & Welburn, S. C., 2013.** A longitudinal survey of animal African trypanosomosis in domestic cattle on the Jos Plateau, Nigeria: prevalence, distribution and risk factors. *Parasites & Vectors*, **6** (1): 239.

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In 2008 a longitudinal two-stage cluster survey was conducted on the Jos Plateau. Cattle were sampled in the dry, early wet and late wet seasons. Parasite identification was undertaken using species-specific polymerase chain reactions to determine the prevalence and distribution of bovine trypanosomosis. Logistic regression was performed to determine risk

factors for disease. The prevalence of bovine trypanosomosis (*Trypanosoma brucei brucei, Trypanosoma congolense* savannah, *Trypanosoma vivax*) across the Jos Plateau was found to be high at 46.8 percent (39.0 percent - 54.5 percent) and significant, seasonal variation was observed between the dry season and the end of the wet season. *T. b. brucei* was observed at a prevalence of 3.2 percent (1 percent - 5.5 percent); *T. congolense* at 27.7 percent (21.8 percent - 33.6 percent) and *T. vivax* at 26.7 percent (18.2 percent - 35.3 percent). High individual variation was observed in trypanosomosis prevalence between individual villages on the Plateau, ranging from 8.8 percent to 95.6 percent. Altitude was found to be a significant risk factor for trypanosomosis whilst migration also influenced risk. In conclusion, trypanosomosis is now endemic on the Jos Plateau showing high prevalence in cattle and is influenced by seasonality, altitude and migration practices. Attempts to successfully control the disease on the Plateau will need to take into account the large variability in infection rates between villages, the influence of land use and of the husbandry and management practices of the pastoralists, all of which affect the epidemiology of the disease.

16819. **Rico, E., Rojas, F., Mony, B. M., Szoor, B., Macgregor, P. & Matthews, K. R., 2013.** Bloodstream form pre-adaptation to the tsetse fly in *Trypanosoma brucei*. *Frontiers in Cellular & Infection Microbiology*, **3**: 78.

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African trypanosomes are sustained in the bloodstream of their mammalian hosts by their extreme capacity for antigenic variation. However, for life cycle progression, trypanosomes also must generate transmission stages called stumpy forms that are pre-adapted to survive when taken up during the blood meal of the disease vector, tsetse flies. These stumpy forms are rather different to the proliferative slender forms that maintain the bloodstream parasitaemia. Firstly, they are non-proliferative and morphologically distinct, secondly, they show particular sensitivity to environmental cues that signal entry to the tsetse fly and, thirdly, they are relatively robust such that they survive the changes in temperature, pH and proteolytic environment encountered within the tsetse midgut. These characteristics require regulated changes in gene expression to pre-adapt the parasite and the use of environmental sensing mechanisms, both of which allow the rapid initiation of differentiation to tsetse midgut procyclic forms upon transmission. Interestingly, the generation of stumpy forms is also regulated and periodic in the mammalian blood, this being governed by a density-sensing mechanism whereby a parasite-derived signal drives cell cycle arrest and cellular development both to optimize transmission and to prevent uncontrolled parasite multiplication overwhelming the host. In this review we detail recent developments in our understanding of the molecular mechanisms that underpin the production of stumpy forms in the mammalian bloodstream and their signal perception pathways both in the mammalian bloodstream and upon entry into the tsetse fly. These discoveries are discussed in the context of conserved eukaryotic signalling and differentiation mechanisms. Further, their potential to act as targets for therapeutic strategies that disrupt parasite development either in the mammalian bloodstream or upon their transmission to tsetse flies is also discussed.

16820. **Rotureau, B., Ooi, C. P., Huet, D., Perrot, S. & Bastin, P., 2013.** Forward motility is essential for trypanosome infection in the tsetse fly. *Cellular Microbiology.* **E Publication ahead of print, 5 November.**

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African trypanosomes are flagellated protozoan parasites transmitted by the bite of tsetse flies and responsible for sleeping sickness in humans. Their complex development in the tsetse digestive tract requires several differentiation and migration steps that are thought to rely on trypanosome motility. We used a functional approach *in vivo* to demonstrate that motility impairment prevents trypanosomes from developing in their vector. Deletion of the outer dynein arm component DNAI1 results in strong motility defects but cells remain viable in culture. However, although these mutant trypanosomes could infect the tsetse fly midgut, they were neither able to reach the foregut nor able to differentiate into the next stage, thus failing to complete their parasite cycle. This is the first *in vivo* demonstration that trypanosome motility is essential for the accomplishment of the parasite cycle.

16821. **Rotureau, B. & Van Den Abbeele, J., 2013.** Through the dark continent: African trypanosome development in the tsetse fly. *Frontiers in Cellular & Infection Microbiology*, **3**: 53.

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African trypanosomes are unicellular flagellated parasites causing trypanosomiases in Africa, a group of severe diseases also known as sleeping sickness in humans and nagana in cattle. These parasites are almost exclusively transmitted by the bite of the tsetse fly. In this review, we describe and compare the three developmental programmes of the main trypanosome species impacting human and animal health, with focus on the most recent observations. From here, some reflections are made on research issues concerning trypanosome developmental biology in the tsetse fly that are to be addressed in the future.

16822. **Selby, R., Bardosh, K., Picozzi, K., Waiswa, C. & Welburn, S. C., 2013.** Cattle movements and trypanosomes: restocking efforts and the spread of *Trypanosoma brucei rhodesiense* sleeping sickness in post-conflict Uganda. *Parasites & Vectors*, **6** (1): 281.

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The northwards spread of acute *T. b. rhodesiense* sleeping sickness in Uganda has been linked to cattle movements associated with restocking following the end to military conflict in 2006. This study examined the number of cattle traded from *T. b. rhodesiense* endemic districts, the prevalence of the parasite in cattle being traded, and the level of trypanocidal treatment at livestock markets. Between 2008 and 2009 interviews were carried out with government veterinarians from 20 districts in Uganda, 18 restocking organizations and numerous livestock traders and veterinarians. Direct observations, a review of movement permit records (2006 to 2008) and blood sampling of cattle (n = 1 758) for detection of

parasites were also conducted at 10 livestock markets in *T. b. rhodesiense* endemic districts. Records available from 8 out of 47 identified markets showed that 39.5 percent (5 238/13 267) of the inter-district cattle trade between mid-2006 and mid-2008 involved movement from endemic areas to pathogen-free districts. PCR analysis showed a prevalence of 17.5 percent *T. brucei* s.l. (n = 307/1 758 [95 percent CI: 15.7-19.2]) and 1.5 percent *T. b. rhodesiense* (n = 26/1 758 [95 percent CI: 0.9-2.0]) from these same markets. In a two-year period, between late-2006 to late-2008, an estimated 72 321 to 86 785 cattle (57 857 by 18 restocking organizations and 10 214 - 24 679 by private traders) were imported into seven pathogen-free northern districts, including districts that were endemic for *T. b. gambiense*. Between 281 and 1 302 of these cattle were likely to have carried *T. b. rhodesiense*. While government organisations predominantly adhered to trypanocidal treatment, most non-government organizations (NGOs) and private traders did not. Inadequate market infrastructure, poor awareness, the need for payment for drug treatments, and the difficulty in enforcing a policy of treatment at point of sale contributed to non-compliance. It is concluded that with increasing private trade, preventing the spread of Rhodesian sleeping sickness in Uganda requires government support to ensure mandatory trypanocidal treatment at livestock markets, investment in market infrastructure and possible drug subsidy. Mapping the northern reaches of *T. b. rhodesiense* in livestock and preparation of risk assessments for cattle trading could mitigate future outbreaks.

16823. **Van Den Abbeele, J. & Rotureau, B., 2013.** New insights in the interactions between African trypanosomes and tsetse flies. *Frontiers in Cellular & Infection Microbiology*, **3**: 63.

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Note from Editor: Reference details available in Journal paper.

This Research Topic hosted in *Frontiers in Cellular and Infection Microbiology* focuses on the state-of-the-art on some key aspects of the fascinating biological interplay between African trypanosomes and the tsetse fly. To prepare for life cycle progression, *T. brucei* parasites present in the bloodstream of an infected mammalian host have to generate stumpy forms that are pre-adapted to survive in the tsetse fly once they are taken up by the fly blood feeding. The molecular mechanisms that underpin the production of these stumpy forms and their signal perception pathways upon entry into the tsetse fly are detailed within recent discoveries in the review of Vidal *et al*., 2013. In the tsetse fly, trypanosomes have to go through specific developmental programmes in order to survive and produce transmissible stage. This mini-review focuses on the major advancements in our understanding of this trypanosome development in the tsetse fly (Rotureau and Van Den Abbeele, 2013). The different types of parasite cycle and the critical stages of the three epidemiologically most relevant trypanosome species, namely *T. vivax*, *T. congolense* and *T. brucei* are compared. More emphasis is on *T. brucei* development since the parasite undergoes multiple morphological changes during the successive invasion of the tsetse alimentary tract to finally achieve the mammalian-infective stage in the salivary glands. Another review attempts to elucidate how these morphological changes are possible for a parasite that has evolved a

highly robust cell structure to survive the chemically and physically diverse environments in the fly (Ooi and Bastin, 2013). During their journey in the tsetse fly, trypanosomes are challenged by a robust innate defence system. This is reviewed by Haines, 2013 with a focus on the tissues intimately associated with host defence. Moreover, the established symbiotic associations of tsetse flies with bacterial and viral microorganisms also modulate its vector competence for trypanosomes. A first review article will provide a detailed description of the tsetse symbiotic microbiome, and describes the physiology underlying host-symbiont and symbiont-symbiont interactions that occur in this fly (Wang *et al*., 2013). The diversity of the gut bacterial flora in the tsetse fly and the possible impact of newly identified bacteria to the vector competence are also discussed in the mini-review of Geiger *et al.* (2013). In combination to the multiple trypanosome-tsetse cross-talk, environmental factors can also affect the parasite developmental barriers in the fly. Here, experimental work from Burkina Faso demonstrated that temperature stress could increase the susceptibility of tsetse to trypanosomes (Bouyer *et al.,* 2013), thus opening the possibility to improve AAT risk mapping using satellite images. Since African trypanosomes rely on tsetse flies for their transmission and dissemination, it is clear that a comprehensive knowledge on the multiple interactions between the parasite, the tsetse fly, its symbiotic flora, and the environment will allow for a better understanding of the transmission dynamics of these parasites in the natural context and could open new avenues for vector transmission control. In this respect, a change of paradigm has recently occurred since it is now recognized that vector control is an important part of the elimination strategy of *gambiense* HAT, as a complement to medical activities (Solano *et al*., 2013).

16824. **Wardrop, N. A., Fevre, E. M., Atkinson, P. M. & Welburn, S. C., 2013.** The dispersal ecology of Rhodesian sleeping sickness following its introduction to a new area. *PLoS Neglected Tropical Diseases*, **7** (10): e2485.

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Tsetse-transmitted human and animal trypanosomiases are constraints to both human and animal health in sub-Saharan Africa, and although these diseases have been known for over a century, there is little recent evidence demonstrating how the parasites circulate in natural hosts and ecosystems. The spread of Rhodesian sleeping sickness (caused by *Trypanosoma brucei rhodesiense*) within Uganda over the past 15 years has been linked to the movement of infected, untreated livestock (the predominant reservoir) from endemic areas. However, despite an understanding of the environmental dependencies of sleeping sickness, little research has focused on the environmental factors controlling transmission establishment or the spatially heterogeneous dispersal of disease following a new introduction. In the current study, an annually stratified case-control study of Rhodesian sleeping sickness cases from Serere District, Uganda was used to allow the temporal assessment of correlations between the spatial distribution of sleeping sickness and landscape factors. Significant relationships were detected between Rhodesian sleeping sickness and selected factors, including elevation and the proportion of land which was "seasonally flooding grassland" or "woodlands and dense savannah." Temporal trends in these relationships were detected, illustrating the dispersal of Rhodesian sleeping sickness into more “suitable” areas over time, with diminishing dependence on the point of introduction in concurrence with an increasing dependence on environmental and landscape factors. These results provide a novel insight

into the ecology of Rhodesian sleeping sickness dispersal and may contribute towards the implementation of evidence-based control measures to prevent its further spread.

16825. **Weiss, B. L., Wang, J., Maltz, M. A., Wu, Y. & Aksoy, S., 2013.** Trypanosome infection establishment in the tsetse fly gut is influenced by microbiome-regulated host immune barriers. *PLoS Pathogens*, **9** (4): e1003318.

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Tsetse flies (*Glossina* spp.) vector pathogenic African trypanosomes, which cause sleeping sickness in humans and nagana in domesticated animals. Additionally, tsetse harbour three maternally transmitted endosymbiotic bacteria that modulate their host's physiology. Tsetse are highly resistant to infection with trypanosomes, and this phenotype depends on multiple physiological factors at the time of challenge. These factors include host age, density of maternally-derived trypanolytic effector molecules present in the gut, and symbiont status during development. In this study, we investigated the molecular mechanisms that result in tsetse's resistance to trypanosomes. We found that following parasite challenge, young susceptible tsetse present a highly attenuated immune response. In contrast, mature refractory flies express higher levels of genes associated with humoral (attacin and pgrp-lb) and epithelial (inducible nitric oxide synthase and dual oxidase) immunity. Additionally, we discovered that tsetse must harbour its endogenous microbiome during intrauterine larval development in order to present a parasite refractory phenotype during adulthood. Interestingly, mature aposymbiotic flies (Gmm (Apo) present a strong immune response earlier in the infection process than do wild type flies that harbour symbiotic bacteria throughout their entire lifecycle. However, this early response fails to confer significant resistance to trypanosomes. Gmm (Apo) adults present a structurally compromised peritrophic matrix (PM), which lines the fly midgut and serves as a physical barrier that separates luminal contents from immune responsive epithelial cells. We propose that the early immune response we observe in Gmm (Apo) flies following parasite challenge results from the premature exposure of gut epithelia to parasite-derived immunogens in the absence of a robust PM. Thus, tsetse's PM appears to regulate the timing of host immune induction following parasite challenge. Our results document a novel finding, which is the existence of a positive correlation between tsetse's larval microbiome and the integrity of the emerging adult PM gut immune barrier.

5. HUMAN TRYPANOSOMOSIS

(a) SURVEILLANCE

[See also **36**: 16776, 16777, 16782, 16788, 16814, 16816, 16824.]

16826. **Abdulla, M. H., Bakhiet, M., Lejon, V., Andersson, J., McKerrow, J., Al-Obeed, O. & Harris, R. A., 2013.** TLTF in cerebrospinal fluid for detection and staging of *T. b. gambiense* infection. *PLoS One*, **8** (11): e79281.

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Trypanosome-derived lymphocyte triggering factor (TLTF) is a molecule released by African trypanosomes that interacts with the host immune system, resulting in increased levels of IFN-gamma production. TLTF and anti-TLTF antibodies were assessed in sera and cerebrospinal fluid (CSF) from patients infected with *Trypanosoma brucei gambiense* (*T. b. gambiense*) in an attempt to identify alternative markers for diagnosis and stage determination of human African trypanosomiasis or sleeping sickness. Seventy-four serum and sixty-one CSF samples from patients with parasitologically confirmed infection and known disease stage along with 13 sera and CSF from uninfected controls were tested. In serum the levels of anti-TLTF antibodies were unrelated to the disease stage. In contrast, levels of anti-TLTF antibodies in CSF were higher in intermediate/late stages than in early stage disease patients. Specificity of the detected antibodies was assessed by inhibition of TLTF bioactivity as represented by its ability to induce IFN-gamma production. Additionally, TLTF was detected in CSF from late stage patients by Western blotting with the anti-TLTF specific monoclonal antibody MO3. These findings suggest a new possibility for disease diagnosis with focus on involvement of the CNS through detection of TLTF and anti-TLTF antibodies in the CSF.

16827. **Dobrodenkova, S., 2013.** Sleeping sickness in Buikwe South health sub-district: neuroinfection situation report. *Neuroendocrinology Letters*, **34** (Suppl. 1): 17-23.

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This paper describes the incidence of *Trypanosoma brucei rhodesiense* sleeping sickness in the last functioning treatment centre in Buikwe South HSD in Southeast Uganda, in Mukono District, over a 19-year period (1989-2008). This is a report on the treatment outcome, structure of population affected, comparison with the published data on general incidence of *T. b rhodesiense* in Uganda, and functioning of the sleeping sickness control programme. Cross-sectional sleeping sickness data from 1989 to 2008 were collected retrospectively in 2009 at Buikwe Sleeping Sickness Center to identify case counts and measures of disease magnitude per sub-county per year. Data were collected from all available records of sleeping sickness patients. Case counts from the Buikwe South sub-counties, and even some neighbouring sub-counties over 19 years (1989-2008) were collected and analysed by Microsoft Excel and EpiInfo programs. In the period from 1989 to 2008, 372 cases of sleeping sickness were diagnosed and treated. Children under 5 years were 12 (3.22 percent) - males 6, females 6; patients in the age from 6 to 15 years were 51 (13.7 percent) - males 30, females 21; and patients above 15 were 309 (83.06 percent) - males 176, females 133. In the categories 5-15 years and above 15 years there was a significant gender difference closely connected to the professional exposure. The oldest patient was 80 years old, the youngest was 3 months old. The average age of the patients was 30.8 years. From all 372 patients with trypanosomiasis 30 had died - 10 females and 20 males, which means an 8 percent case fatality. The case fatality rate in the late stage of the disease was 14 percent. From this group 6 patients (20 percent) had negative BS. The average interval between the diagnosis and death was 14.4 d. in 10 patients, the exact date of death being not recorded. The average age of the patients that died was 30.6 years. In conclusion, sleeping sickness still remains a serious public health problem. Since the preventive and educational activities for the control of this neglected disease are not functioning, it can very easily re-emerge. In the

future, it is also essential to support research into less toxic drugs, interventions related to parasite transmission through cattle movements and potential changes in vector-human exposure in central Ugandan districts as well as strengthening of the necessary, relevant surveillance systems.

16828. **Gillet, P., Mumba Ngoyi, D., Lukuka, A., Kande, V., Atua, B., van Griensven, J., Muyembe, J. J., Jacobs, J. & Lejon, V., 2013.** False positivity of non-targeted infections in malaria rapid diagnostic tests: the case of human African trypanosomiasis. *PLoS Neglected Tropical Diseases*, **7** (4): e2180.

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In endemic settings, diagnosis of malaria increasingly relies on the use of rapid diagnostic tests (RDTs). False positivity of such RDTs is poorly documented, although it is especially relevant in those infections that resemble malaria, such as human African trypanosomiasis (HAT). We therefore examined the specificity of malaria RDT products among patients infected with *Trypanosoma brucei gambiense*. Blood samples of 117 HAT patients and 117 matched non-HAT controls were prospectively collected in the Democratic Republic of the Congo. Reference malaria diagnosis was based on real-time PCR. Ten commonly used malaria RDT products were assessed including three two-band and seven three-band products, targeting HRP-2, Pf-pLDH and/or pan-pLDH antigens. Rheumatoid factor was determined in PCR negative subjects. Specificity of the 10 malaria RDT products varied between 79.5 percent and 100 percent in HAT-negative controls and between 11.3 percent and 98.8 percent in HAT patients. For seven RDT products, specificity was significantly lower in HAT patients compared with controls. False positive reactions in HAT tests were mainly observed for pan-pLDH test lines (specificities between 13.8 percent and 97.5 percent), but also occurred frequently for the HRP-2 test line (specificities between 67.9 percent and 98.8 percent). The Pf-pLDH test line was not affected by false-positive lines in HAT patients (specificities between 97.5 percent and 100 percent). False positivity was not associated to rheumatoid factor, detected in 7.6 percent of controls and 1.2 percent of HAT patients. In conclusion, the specificity of some malaria RDT products in HAT tests was surprisingly low, and constitutes a risk for misdiagnosis of a fatal but treatable infection. Our results show the importance of assessing RDT specificity in non-targeted infections when evaluating diagnostic tests.

16829. **Hardwick, R. J., Menard, A., Sironi, M., Milet, J., Garcia, A., Sese, C., Yang, F., Fu, B., Courtin, D. & Hollox, E. J., 2013.** Haptoglobin (HP) and haptoglobin-related protein (HPR) copy number variation, natural selection, and trypanosomiasis. *Human Genetics*. **E Publication ahead of print, 5 September**.

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Haptoglobin, coded by the HP gene, is a plasma protein that acts as a scavenger for free haeme, and haptoglobin-related protein (coded by the HPR gene) forms part of the trypanolytic factor TLF-1, together with apolipoprotein L1 (ApoL1). We analyse the polymorphic small intragenic duplication of the HP gene, with alleles Hp1 and Hp2, in 52

populations, and find no evidence for natural selection either from extended haplotype analysis or from correlation with pathogen rich matrices. Using fibre-FISH, the paralogue ratio test, and array-CGH data, we also confirm that the HPR gene is copy number variable, with duplication of the whole HPR gene at polymorphic frequencies in west and central Africa, up to an allele frequency of 15 percent. The geographical distribution of the HPR duplication allele overlaps the region where the pathogen causing chronic human African trypanosomiasis, *Trypanosoma brucei gambiense*, is endemic. The HPR duplication has occurred on one SNP haplotype, but there is no strong evidence of extended homozygosity, a characteristic of recent natural selection. The HPR duplication shows a slight, insignificantly reduced transmission to human African trypanosomiasis-affected children of unaffected parents in the Democratic Republic of Congo. However, taken together with alleles of APOL1, there is an overall significant under-transmission of putative protective alleles to human African trypanosomiasis-affected children.

16830. **Mitashi, P., Hasker, E., Ngoyi, D. M., Pyana, P. P., Lejon, V., Van der Veken, W., Lutumba, P., Buscher, P., Boelaert, M. & Deborggraeve, S., 2013.** Diagnostic accuracy of Loopamp *Trypanosoma brucei* detection kit for diagnosis of human African trypanosomiasis in clinical samples. *PLoS Neglected Tropical Diseases*, **7** (10): e2504.

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Molecular methods have great potential for sensitive parasite detection in the diagnosis of human African trypanosomiasis (HAT), but the requirements in terms of laboratory infrastructure limit their use to reference centres. A recently developed assay detects the Trypanozoon repetitive insertion mobile element (RIME) DNA under isothermal amplification conditions and has been transformed into a ready-to-use kit format, the Loopamp *Trypanosoma brucei*. In this study, we have evaluated the diagnostic performance of the Loopamp *Trypanosoma brucei* assay (hereafter called LAMP) in confirmed *T. b. gambiense* HAT patients, HAT suspects and healthy endemic controls from the Democratic Republic of the Congo (DRC). 142 *T. b. gambiense* HAT patients, 111 healthy endemic controls and 97 HAT suspects with unconfirmed status were included in this retrospective evaluation. Reference standard tests were parasite detection in blood, lymph or cerebrospinal fluid. Archived DNA from blood of all study participants was analysed in duplicate with LAMP. Sensitivity of LAMP in parasitologically confirmed cases was 87.3 percent (95 percent CI 80.9-91.8 percent) in the first run and 93.0 percent (95 percent CI 87.5-96.1 percent) in the second run. Specificity in healthy controls was 92.8 percent (95 percent CI 86.4-96.3 percent) in the first run and 96.4 percent (95 percent CI 91.1-98.6 percent) in the second run. Reproducibility was excellent with a kappa value of 0.81. In this laboratory-based study, the Loopamp *Trypanosoma brucei* detection kit showed good diagnostic accuracy and excellent reproducibility. Further studies are needed to assess the feasibility of its routine use for diagnosis of HAT under field conditions.

16831. **Mukadi, P., Gillet, P., Lukuka, A., Atua, B., Sheshe, N., Kanza, A., Mayunda, J. B., Mongita, B., Senga, R., Ngoyi, J., Muyembe, J. J., Jacobs, J. & Lejon, V.,**

2013. External quality assessment of Giemsa-stained blood film microscopy for the diagnosis of malaria and sleeping sickness in the Democratic Republic of the Congo. *Bulletin of the World Health Organization*, **91** (6): 441-448.

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This publication reports the findings of a second external quality assessment of Giemsa-stained blood film microscopy in the Democratic Republic of the Congo, performed one year after the first. A panel of four slides was delivered to diagnostic laboratories in all provinces of the country. The slides contained: (i) *Plasmodium falciparum* gametocytes; (ii) *P. falciparum* trophozoites (reference density: 113 530 per µL); (iii) *Trypanosoma brucei* subspecies; and (iv) no parasites. Of 356 laboratories contacted, 277 (77.8 percent) responded. Overall, 35.0 percent of the laboratories reported all four slides correctly but 14.1 percent reported correct results for 1 or 0 slides. Major errors included not diagnosing trypanosomiasis (50.4 percent), not recognizing *P. falciparum* gametocytes (17.5 percent) and diagnosing malaria from the slide with no parasites (19.0 percent). The frequency of serious errors in assessing parasite density and in reporting false-positive results was lower than in the previous external quality assessment: 17.2 percent and 52.3 percent, respectively, ($p < 0.001$) for parasite density and 19.0 percent and 33.3 percent, respectively, ($p < 0.001$) for false-positive results. Laboratories that participated in the previous quality assessment performed better than first-time participants and laboratories in provinces with a high number of sleeping sickness cases recognized trypanosomes more frequently (57.0 percent versus 31.2 percent, $p < 0.001$). Malaria rapid diagnostic tests were used by 44.3 percent of laboratories, almost double the proportion observed in the previous quality assessment. It is concluded that the overall quality of blood film microscopy was poor but was improved by participation in external quality assessments. The failure to recognize trypanosomes in a country where sleeping sickness is endemic is a concern.

16832. **Mwanakasale, V., Songolo, P. & Daka, V., 2013.** Challenges in the control of human African trypanosomiasis in the Mpika district of Zambia. *BMC Research Notes*, **6**: 180.

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Human African trypanosomiasis is one of the Neglected Tropical Diseases that is targeted for elimination by the World Health Organization. Strong health delivery systems in endemic countries are required for a control programme to eliminate this disease. In Zambia, human African trypanosomiasis is lowly endemic in the northeastern part of the country. We conducted a cross-sectional survey of health institutions in the Mpika district in Northern Province of Zambia from 9th to 23rd November 2011. The aim of this study was to assess the current health delivery system in the management of human African trypanosomiasis cases in Mpika district, Northern Province of Zambia. Ten health institutions were covered in the survey. Two structured questionnaires targeting health workers were used to collect the data on general knowledge on HAT and state of health care facilities in relation to HAT management from the surveyed health institution. Only 46 percent of the 28 respondents scored more than 50 percent from the questionnaire on general knowledge about human African trypanosomiasis disease. None of the respondents knew how to differentiate the two

clinical stages of human African trypanosomiasis disease. There were only three medical doctors to attend to all cases and other diseases at the only diagnostic and treatment hospital in Mpika district. The supply of anti-trypanosomal drugs to the only treatment centre was erratic. Only one refresher course on human African trypanosomiasis case diagnosis and management for health staff in the district had been organized by the Ministry of Health in conjunction with the World Health Organization in 2009. The referral system for suspected cases from Rural Health Centres (RHCs) to the diagnostic/treatment centre was inefficient. In conclusion, a number of challenges were identified and need to be addressed if human African trypanosomiasis is to be eliminated in a lowly endemic country such as Zambia. These include shortage of trained health workers, inadequate diagnostic and treatment centres, lack of more sensitive laboratory diagnostic techniques, shortage of trypanocides among others; these are discussed in detail here.

16833. **Rouamba, J., Bruneau, J. C., Sory, I., Kagbadouno, M., Coulibaly, B., Jamonneau, V., Solano, P., Rayaisse, J. B., Camara, M. & Courtin, F., 2013.** Settlements, landscapes, and risks of sleeping sickness at the mouth of the Rio Pongo in Guinea-Conakry. *Médecine et Santé Tropicales*, **23** (2): 225.

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Seeking to understand how humans, by the settlements they create (among other means), influence the operation of the pathogen system of sleeping sickness, the authors performed a diachronic analysis of the landscape and settlement dynamics by comparing topographic maps from 1957, a satellite image from 2004, and geo-referenced censuses from 2009 and 2001. It appears that the extreme mobility of the population between the continent and the islands is the principal cause for the continuation of this disease at the mouth of the Rio Pongo.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **36**: 16766, 16775, 16787]

16834. **Koko, J., Ategbo, S. J., Gahouma, D., Engohan-Aloghe, E. & Moussavou, A., 2013.** Human African trypanosomiasis: report of three cases. *Archives of Pediatrics*, **20** (8): 871-873.

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Prolonged fever is an important cause of morbidity in pediatric practice, especially in tropical areas. It is above all a problem of aetiological diagnosis given the vast number of aetiologies. In sub-Saharan Africa, practitioners more often focus on bacterial infections and malaria at the expense of other infectious diseases such as human African trypanosomiasis (HAT), most often leading to over-use of antibiotics and antimalarials. A dramatic resurgence of HAT, also called sleeping sickness, has been reported during the last few decades in large areas of Central Africa. Furthermore, with the development of air transport, cases of children infected during a trip to Africa can be exported outside endemic areas, making diagnosis even more difficult. This parasitic infection causes a protracted, often initially unrecognized, illness

with episodes of fever, headache, and malaise, accompanied by progressive lymphadenopathy, before the development of a progressive meningoencephalitis. These three case reports aim to remind practitioners of clinical and biological signs suggestive of HAT diagnosis in children living in endemic areas or having stayed there during the months prior to visiting the doctor. The prognosis is largely dependent on the precocity of diagnosis and therapeutic support.

16835. **Pale, C. A. & Vigna, L., 2013.** Images in clinical medicine. African trypanosomiasis in Argentina. *New England Journal of Medicine*, **369** (8): 763.

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A 65-year-old man was admitted to the hospital with a 4-d history of fever, asthenia, myalgia, and arthralgia. The physical examination revealed two violaceous lesions measuring 3 cm in diameter with shallow ulceration in the lower portions of both legs. He reported having been bitten by a tsetse fly during an annual hunting trip in Zambia and had returned to Argentina 10 d before his admission. Microscopical examination of blood and fluid from one of the lesions revealed *Trypanosoma brucei rhodesiense (T. b. rhodesiense),* which causes human African trypanosomiasis (also called sleeping sickness). Tanzania, Malawi, Zambia, and Zimbabwe are typical countries of origin for cases of *T. b. rhodesiense* human African trypanosomiasis, which may be an acute, life-threatening disease characterized by fever (39.4 to 40.6 °C), parasitaemia, and chancre. Because of difficulties in obtaining suramin, we initially administered pentamidine and switched to suramin 48 h later. Subsequently, the patient's condition worsened, and he was admitted to the intensive care unit with the acute respiratory distress syndrome, along with hepatic and renal failure. Mechanical ventilation and haemodialysis were initiated. Disseminated intravascular coagulation developed, and the patient died 3 d later from ventricular arrhythmia.

16836. **Pickrell, W. O., Sudarshi, D., Eligar, V., Brown, M. & Walter, L. J., 2013.** Tripped up by an unusual diagnosis? *Journal of Neurology, Neurosurgery and Psychiatry,* **84**: (11) e2 doi:10.1136/jnnp-2013-306573.41.

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A 62-year-old man presented with a 3-month history of increasing daytime somnolence, unsteadiness, falls, tremor and amnesia. For the last 3 years he had been immunosuppressed with prednisolone and azathioprine due to suspected Sjögren's disease with positive anti–Ro antibodies and raised ESR. The azathioprine was stopped 4 months prior to admission as there was some doubt of the diagnosis of Sjögren's. He had a pituitary macroadenoma and had recently been started on the dopamine agonist Carbergoline. He was born in Sierra Leone but had lived in the UK for the last 41 years, his last visit to Africa being 29 years ago. On examination he was orientated; profoundly bradyphrenic; Parkinsonian and had slowed horizontal saccades. There was mild right-sided facial weakness but no limb weakness or signs of ataxia or neuropathy. He had a normocytic anaemia and thrombocytopaenia. Voltage–gated–potassium channel antibody (VGKC Ab) and n-methyl-d-aspartate receptor

antibody (NMDAR Ab) titres were markedly elevated. MRI brain showed grey and white matter abnormalities including bilateral signal change in the substantia nigra. There were radiolucent lesions in the skull. Cerebrospinal fluid (CSF) analysis showed 250 lymphocytes/mm^3, protein of 0.57 g/L and a glucose of 4.7 mmol/L. Three months after admission he was stuporose and having frequent generalized tonic-clonic seizures. At this time results from the bone marrow, undertaken because of pancytopaenia, became available and revealed trypanosomes, pathognomic for human African trypanosomiasis (CSF PCR later confirmed the species as *Trypanosoma brucei gambiense*). The patient was then transferred for urgent treatment and management at the Hospital for Tropical Diseases. He received nifurtimox and eflornithine combination treatment for the trypanosomiasis and two courses of plasma exchange for persistently elevated VGKC Ab titres. He was discharged home 3 months later, with resolution of the Parkinsonism and significant cognitive and functional improvement. West African trypanosomiasis ("African sleeping sickness") is caused by the protozoa *Trypanosoma brucei gambiense*. Neurological symptoms, characterized by increasing somnolence and cognitive disturbance are manifest during stage II of the disease when the parasite invades the central nervous system. Stage II disease is generally fatal if left untreated. We believe that this case demonstrates the longest reported latent period between trypanosome infection and clinical presentation with important implications for the understanding of trypanosome immunology. Trypanosomiasis can cause hypergammaglobulinaemia which could explain the raised levels of a number of autoantibodies in the years preceeding the presentation. The very raised VGKC Ab levels in this case (1 780) are interesting and do raise the possibility that they were contributing to the clinical presentation despite the fact that they may have been produced as a consequence of the trypanosomiasis.

16837**. Uzureau, P., Uzureau, S., Lecordier, L., Fontaine, F., Tebabi, P., Homble, F., Grelard, A., Zhendre, V., Nolan, D. P., Lins, L., Crowet, J. M., Pays, A., Felu, C., Poelvoorde, P., Vanhollebeke, B., Moestrup, S. K., Lyngso, J., Pedersen, J. S., Mottram, J. C., Dufourc, E. J., Perez-Morga, D. & Pays, E., 2013.** Mechanism of *Trypanosoma brucei gambiense* resistance to human serum. *Nature*, **501** (7467): 430-434.

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The African parasite *Trypanosoma brucei gambiense* accounts for 97 percent of human sleeping sickness cases. *T. b. gambiense* resists the specific human innate immunity acting against several other tsetse fly-transmitted trypanosome species such as *T. b. brucei*, the causative agent of nagana disease in cattle. Human immunity to some African trypanosomes is due to two serum complexes designated trypanolytic factors (TLF-1 and -2), which both contain haptoglobin-related protein (HPR) and apolipoprotein LI (APOL1). Whereas HPR association with haemoglobin (Hb) allows TLF-1 binding and uptake via the trypanosome receptor TbHpHbR, TLF-2 enters trypanosomes independently of TbHpHbR. APOL1 kills trypanosomes after insertion into endosomal/lysosomal membranes. Here we report that *T. b. gambiense* resists TLFs via a hydrophobic beta-sheet of the *T. b. gambiense*-specific glycoprotein (TgsGP), which prevents APOL1 toxicity and induces stiffening of membranes upon interaction with lipids. Two additional features contribute to resistance to TLFs: reduction of sensitivity to APOL1 requiring cysteine protease activity, and TbHpHbR

inactivation due to a L210S substitution. According to such a multifactorial defence mechanism, transgenic expression of *T. b. brucei* TbHpHbR in *T. b. gambiense* did not cause parasite lysis in normal human serum. However, these transgenic parasites were killed in hypohaptoglobinaemic serum, after high TLF-1 uptake in the absence of haptoglobin (Hp) that competes for Hb and receptor binding. TbHpHbR inactivation preventing high APOL1 loading in hypohaptoglobinaemic serum may have evolved because of the overlapping endemic area of *T. b. gambiense* infection and malaria, the main cause of haemolysis-induced hypohaptoglobinaemia in western and central Africa.

(c) TREATMENT

[See also **36**: 16769, 16770, 16784]

16838. **Babokhov, P., Sanyaolu, A. O., Oyibo, W. A., Fagbenro-Beyioku, A. F. & Iriemenam, N. C., 2013.** A current analysis of chemotherapy strategies for the treatment of human African trypanosomiasis. *Pathogens & Global Health*, **107** (5): 242-252.

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Despite the recent advances in drug research, finding a safe, effective, and easy to use chemotherapy for human African trypanosomiasis (HAT) remains a challenging task. The four current anti-trypanosomiasis drugs have major disadvantages that limit more widespread use of these drugs in the endemic regions of sub-Saharan Africa. Pentamidine and suramin are limited by their effectiveness against the first stage of *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, respectively. In addition, melarsoprol and eflornithine (two second stage drugs) each have disadvantages of their own. The former is toxic and has increasing treatment failures while the latter is expensive, laborious to administer, and lacks efficacy against *T. b. rhodesiense*. Furthermore, melarsoprol's toxicity and decreasing efficacy are glaring problems and phasing out the drug as a frontline treatment against *T. b. gambiense* is now possible with the emergence of competent, safe combination chemotherapies such as nifurtimox-eflornithine combination treatment (NECT). The future of eflornithine, on the other hand, is more promising. The drug is useful in the context of combination chemotherapy and potential orally administered analogues. Due to the limits of monotherapies, greater emphasis should be placed on the research and development of combination chemotherapies based on the successful clinical tests with NECT and its current use as a frontline anti-trypanosomiasis treatment. This review discusses the current and future chemotherapy strategies for the treatment of HAT.

16839. **Lutje, V., Seixas, J. & Kennedy, A., 2013.** Chemotherapy for second-stage human African trypanosomiasis. *Cochrane Database of Systemic Reviews*, **6**: CD006201.

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Human African trypanosomiasis, or sleeping sickness, is a painful and protracted disease affecting people in the poorest parts of Africa and is fatal without treatment. Few drugs are currently available for second-stage sleeping sickness, with considerable adverse

events and variable efficacy. To evaluate the effectiveness and safety of drugs for treating second-stage human African trypanosomiasis, we searched the Cochrane Infectious Diseases Group Specialized Register (January 2013), CENTRAL (The Cochrane Library Issue 12 2012), MEDLINE (1966 to January 2013), EMBASE (1974 to January 2013), LILACS (1982 to January 2013), BIOSIS (1926-January 2013), mRCT (January 2013) and reference lists. We contacted researchers working in the field and organizations. Randomized and quasi-randomized controlled trials including adults and children with second-stage HAT, treated with anti-trypanosomal drugs were used as selection criteria while two authors (VL and AK) extracted data and assessed methodological quality; a third author (JS) acted as an arbitrator. Included trials only reported dichotomous outcomes, and we present these as risk ratio (RR) with 95 percent confidence intervals (CI). Nine trials with 2 577 participants, all with *Trypanosoma brucei gambiense* HAT, were included. Seven trials tested currently available drugs: melarsoprol, eflornithine, nifurtimox, alone or in combination; one trial tested pentamidine, and one trial assessed the addition of prednisolone to melarsoprol. The frequency of death and number of adverse events were similar between patients treated with fixed 10-d or 26-d regimens of melarsoprol. Melarsoprol monotherapy gave fewer relapses than pentamidine or nifurtimox, but resulted in more adverse events. Later trials evaluated nifurtimox combined with eflornithine (NECT), showing this gave few relapses and was well tolerated. It also has practical advantages in reducing the frequency and number of eflornithine slow infusions to twice a day, thus easing the burden on health personnel and patients. In conclusion, choice of therapy for second stage *Gambiense* HAT will continue to be determined by what is locally available, but eflornithine and NECT are likely to replace melarsoprol, with careful parasite resistance monitoring. We need research on reducing adverse effects of currently used drugs, testing different regimens, and experimental and clinical studies of new compounds, effective for both stages of the disease.

16840. **Nzoumbou-Boko, R., Dethoua, M., Gabriel, F., Buguet, A., Cespuglio, R., Courtois, P., Daulouede, S., Bouteille, B., Ngampo, S., Mpandzou, G., Semballa, S. & Vincendeau, P., 2013.** Serum arginase, a biomarker of treatment efficacy in human African trypanosomiasis. *Journal of Clinical Microbiology*, **51** (7): 2379-2381.

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Arginase serum levels were increased in human African trypanosomiasis patients and returned to control values after treatment. Arginase hydrolyzes l-arginine to l-ornithine, which is essential for parasite growth. Moreover, l-arginine depletion impairs immune functions. Arginase may be considered as a biomarker for treatment efficacy.

6. ANIMAL TRYPANOSOMOSIS

(a) SURVEY AND DISTRIBUTION

[See also **36**: 16767, 16781, 16811, 16817, 16818, 16822]

16841. **Elhaig, M. M., Youssef, A. I. & El-Gayar, A. K., 2013.** Molecular and parasitological detection of *Trypanosoma evansi* in camels in Ismailia, Egypt. *Veterinary Parasitology*, **198** (1-2): 214-218.

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Trypanosoma evansi (*T. evansi*) infection is an endemic disease of camels and other domestic animals in Egypt. This study aimed to determine the prevalence of clinical and sub-clinical *T. evansi* infection among camels in Ismailia, Egypt, as well as to survey their owners for *T. evansi* infection. The diagnostic sensitivity of three different PCR assays for detection of *T. evansi* in blood samples was evaluated. Blood samples were collected from 100 camels and 20 of their owners in the Ismailia governorate. Results revealed that the percentage of infected camels with *T. evansi* varied with the detection method, ranging from 10 percent to 46 percent by PCR compared with 12 percent by microscopic examination of stained blood smears. Targeting the highly repeated sequence of mini-chromosome satellite DNA (TBR1/2 primer set) was more often seen in the PCR method (46 percent positive) compared with targeting ITS 1 (16 percent positive) or RoTat 1.2 VSG (10 percent positive) sequences. A partial sequence of RoTat 1.2 VSG gene was identical to the *T. evansi* sequences reported from India and Kenya, but varied similarity was seen when aligned with Egyptian *T. evansi* sequences. None of the camel owners was positive for *T. evansi* by microscopic examination of stained blood smears or PCR assays. PCR assay based on TBR sets is useful in the diagnosis and control of disease and reducing economic losses.

16842. **Elshafie, E. I., Sani, R. A., Hassan, L., Sharma, R., Bashir, A. & Abubakar, I. A., 2013.** Active infection and morphometric study of *Trypanosoma evansi* among horses in Peninsular Malaysia. *Tropical Biomedicine*, **30** (3): 444-450.

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Apart from occasional reports of clinical disease affecting horses, there is no information about *Trypanosoma evansi* in horses in Peninsular Malaysia. Thus, a cross-sectional study was conducted in eight states in Peninsular Malaysia to determine the active presence of *T. evansi* in horses. A total of 527 blood samples were obtained and examined by the haematocrit centrifugation technique (HCT), Giemsa-stained thin blood smear (GSS), morphometric measurements, polymerase chain reaction (PCR) and cloning of PCR products. The results showed an overall parasitological prevalence of 0.57 percent (3/527, CI: 1.6-0.19 percent) with both HCT and GSS. Morphometric study revealed the mean total length of the trypanosomes including the free flagellum was 27.94 +/- 2.63 µm. PCR successfully amplified a trypanosome specific 257 bp product in 1.14 percent of samples (6/527, CI: 2.4-0.52 percent) and was confirmed by nucleotide sequences. The mean packed cell volume (PCV) for the positive cases detected by HCT was lower (23 percent +/- 7.00) compared with the positive cases detected by PCR alone in the state of Terengganu (35 percent +/- 4.73). In conclusion, this study showed that *T. evansi* infection occurred at low frequency in horses in Peninsular Malaysia, and anaemia coincided with parasitaemic animals. PCR is considered as a sensitive diagnostic tool when parasitaemia is undetectable. The slightly lengthier parasite and anaemia may indicate a virulent strain of *T. evansi* circulating throughout the country. Thus, it is highly recommended to shed more light on the host-parasite relationship for a better epidemiological understanding.

16843 **Pumhom, P., Pognon, D., Yangtara, S., Thaprathorn, N., Milocco, C., Douangboupha, B., Herder, S., Chaval, Y., Morand, S., Jittapalapong, S. & Desquesnes, M., 2013.** Molecular prevalence of *Trypanosoma* spp. in wild rodents of Southeast Asia: influence of human settlement habitat. *Epidemiology & Infection,* **2013**: 1-10.

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This study investigated the molecular prevalence of *Trypanosoma lewisi* and *T. evansi* in wild rodents from Cambodia, Lao PDR and Thailand. Between 2008 and 2012, rodents (and shrews) were trapped at nine locations and 616 of these were tested using three sets of primers: TRYP1 (amplifying ITS1 of ribosomal DNA of all trypanosomes), TBR (amplifying satellite genomic DNA of Trypanozoon parasites), and LEW1 (amplifying ITS1 of ribosomal DNA of *T. lewisi*). Based on the size of the PCR products using TRYP1, 17 percent were positive for *T. lewisi* and 1.0 percent positive for Trypanozoon. Results were confirmed by sequencing PCR products and by using more specific primers (LEW1 and TBR). The specificity of TRYP1 primers, however, failed as rodent DNA was amplified in some instances, giving unexpected product sizes. Using LEW1 primers, 13.3 percent of the samples were confirmed positive for *T. lewisi*, both by PCR and sequencing. In Thailand, *T. lewisi* was found in *Rattus tanezumi, R. exulans* and *Berylmys*; in Lao PDR in *R. tanezumi* and *R. exulans*, and in Cambodia in *R. tanezumi, R. exulans* and *R. norvegicus*. Using TBR, 1.3 percent of the samples tested positive for Trypanozoon by PCR and sequencing; *T. evansi* is the only species of the Trypanozoon subgenus possibly present in wild Asian rodents. These results confirmed its presence in rodents from Thailand (*R. tanezumi*), Lao PDR (*R. tanezumi, R. nitidus*) and Cambodia (*R. tanezumi, Niviventer fulvescens, Maxomys surifer*). Based on the information related to rodent trapping, it was found that rodent species trapped in and around human dwellings had a higher prevalence of *T. lewisi* infection. *R. tanezumi* and *R. exulans*, two synanthropic species, were mainly found infected in this habitat suggesting a role as reservoirs and thus potential sources of *T. lewisi* for human infection.

16844. **Sharma, A., Das Singla, L., Tuli, A., Kaur, P., Batth, B. K., Javed, M. & Juyal, P. D., 2013.** Molecular prevalence of *Babesia bigemina* and *Trypanosoma evansi* in dairy animals from Punjab, India, by duplex PCR: a step forward to the detection and management of concurrent latent infections. *Biomed Research International*, **2013**: 893862.

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Specific duplex polymerase chain reaction (PCR) was employed on 411 (386 cattle and 25 buffaloes) blood samples of dairy animals from 9 districts of Punjab, India, for simultaneous detection of *Babesia bigemina* and *Trypanosoma evansi*. The results were compared and correlated with conventional Giemsa stained thin blood smear (GSTBS) examination and haematological alterations to determine the clinical status and pathogenicity of infections. The Bg3/Bg4 and TR3/TR4 primers were used in duplex PCR for *B. bigemina*

and *T. evansi* amplified products of 689 bp and 257 bp, respectively. The overall prevalence by duplex PCR was found to be 36.49 percent, 2.43 percent, and 3.41 percent for *T. evansi*, *B. bigemina*, and dual infections, respectively. A more significant difference was observed for dual infection status (p </= 0.005) as compared with *T. evansi* (p </= 0.05) and *B. bigemina* (p </= 0.01) among various districts under study. A very low prevalence of *T. evansi* (0.73 percent) and *B. bigemina* (0.48 percent) was recorded by GSTBS. The highly sensitive, specific, and cost-effective duplex PCR was able to detect latent *T. evansi* and *B. bigemina* infections in cattle and buffaloes. Haematological evaluation revealed marked pathology in the *B. bigemina* infected and in the dual infected groups in contrast to that infected with *T. evansi* alone.

16845. **Sivakumar, T., Lan, D. T., Long, P. T., Yoshinari, T., Tattiyapong, M., Guswanto, A., Okubo, K., Igarashi, I., Inoue, N., Xuan, X. & Yokoyama, N., 2013.** PCR detection and genetic diversity of bovine hemoprotozoan parasites in Vietnam. *Journal of Veterinary Medical Science*, **75** (11): 1455-1462.

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Haemoprotozoan infections often cause serious production losses in livestock. In the present study, we conducted a PCR-based survey of *Babesia bovis, Babesia bigemina, Theileria annulata*, *Theileria orientalis*, *Trypanosoma evansi* and *Trypanosoma theileri*, using 423 DNA samples extracted from blood samples of cattle (n = 202), water buffaloes (n = 43), sheep (n = 51) and goats (n = 127) bred in the Hue and Hanoi provinces of Vietnam. With the exception of *T. annulata* and *T. evansi*, all other parasite species (*B. bovis, B. bigemina*, *T. orientalis* and *T. theileri*) were detected in the cattle populations, with *B. bovis* being the most common among them. Additionally, four water buffaloes and a single goat were infected with *B. bovis* and *B. bigemina*, respectively. The Hue province had more haemoprotozoan-positive animals than those from the Hanoi region. In the phylogenetic analyses, *B. bovis*-MSA-2b, *B. bigemina*-AMA-1 and *T. theileri*-CATL gene sequences were dispersed across four, one and three different clades in the respective phylograms. This is the first study in which the presence of *Babesia, Theileria* and *Trypanosoma* parasites was simultaneously investigated by PCR in Vietnam. The findings suggest that haemoprotozoan parasites, some of which are genetically diverse, continue to be a threat to the livestock industry in this country.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **36**: 16787, 16811]

16846. **Desquesnes, M., Holzmuller, P., Lai, D. H., Dargantes, A., Lun, Z. R. & Jittaplapong, S., 2013.** *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *Biomedical Research International*, **2013**: 194176.

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Trypanosoma evansi, the agent of "surra," is a salivarian trypanosome, originating from Africa. It is thought to derive from *Trypanosoma brucei* by deletion of the maxicircle kinetoplastic DNA (genetic material required for cyclical development in tsetse flies). It is mostly mechanically transmitted by tabanids and stomoxes, initially to camels, in sub-Saharan Africa. The disease spread from North Africa towards the Middle East, Turkey, India, up to 53 degrees North in Russia, across all South-East Asia, down to Indonesia and the Philippines, and it was also introduced by the conquistadores into Latin America. It can affect a very large range of domestic and wild hosts including camelids, equines, cattle, buffaloes, sheep, goats, pigs, dogs and other carnivores, deer, gazelles, and elephants. It found a new large range of wild and domestic hosts in Latin America, including reservoirs (capybaras) and biological vectors (vampire bats). Surra is a major disease in camels, equines, and dogs, in which it can often be fatal in the absence of treatment, and exhibits nonspecific clinical signs (anaemia, loss of weight, abortion, and death), which are variable from one host and one place to another; however, its immunosuppressive effects interfering with intercurrent diseases or vaccination campaigns might be its most significant and questionable aspect.

16847. **Seyoum, Z., Terefe, G. & Ashenafi, H., 2013.** Farmers' perception of impacts of bovine trypanosomosis and tsetse fly in selected districts in Baro-Akobo and Gojeb river basins, Southwestern Ethiopia. *BMC Veterinary Research*, **9** (1): 214.

Unit of Paraclinical Studies, Faculty of Veterinary Medicine, University of Gondar, P.O. Box:196, Gondar, Ethiopia; Department of Pathology and Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University, Debre Zeit, Ethiopia. [zewdus@yahoo.com].

Trypanosomosis, via causing anaemia, emaciation, production loss and death, is arguably the most important constraint to livestock development in sub-Saharan countries, including Ethiopia and its impact in Baro-Akobo and Gojeb river basins (endemic areas for tsetse flies) is unknown. This study was carried out from November 2011 to April 2012 to assess farmers' perception of the presence, impact, management and the need of intervention programmes of bovine trypanosomosis and tsetse fly in selected districts located in the Baro-Akobo and Gojeb river basins, Southwestern Ethiopia. A standardized questionnaire survey was employed to collect the relevant information from the farmers. The result of this study showed that 94.1 percent of the respondents considered bovine trypanosomosis as an economically important cattle disease which accounted for 64.6 percent of the total annual deaths in the year 2011/2012. Estimated mean annual financial loss via mortality due to trypanosomosis was reported to be 3 501 Ethiopian Birr (US$200)/household. The reported trypanosomosis suggestive signs were consistent with published reports and farmers strongly associated the occurrence of the disease with biting flies (particularly tsetse fly). Respondents also explained the seasonality of the disease and its vectors, i.e. May and June are peak risk months of the year. Chemotherapy was reported the major method to combating the problem, mean frequency of treatment being 5.7 times per animal per year. Because of the economic burden of the disease, farmers expressed their strong interest and support for the establishment of an intervention programme in their area. The study revealed that livestock keepers are familiar with bovine trypanosomosis and its vectors as well as its impacts. Thus, trypanosomosis and tsetse control strategies should be integrated with the local communities' participation to minimize the impacts of the disease and its vectors in the area.

16848. **Madeira, M. F., Almeida, A. B., Barros, J. H., Oliveira, T. S., Sousa, V. R., Alves, A. S., Miranda, L. F., Schubach, A. O. & Marzochi, M. C., 2013.** *Trypanosoma caninum,* a new parasite described in dogs in Brazil: aspects of natural infection. *Journal of Parasitology.* **E Publication ahead of print, 13 December.**

Fundacao Oswaldo Cruz, Research, Fundacao Oswaldo Cruz. Av. Brazil, 4365 - Manguinhos, Rio de Janeiro - CEP: 21040-360, Brazil.
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Trypanosoma caninum constitutes the most recent trypanosomatid species infecting dogs in Brazil. Due to the limited data available about this parasite, this study aimed to determine the clinical and laboratory findings from 14 naturally infected dogs. The dogs were diagnosed during a cross-sectional survey in Cuiaba (Mato Grosso, Brazil) and followed up at intervals of 3, 6 and 12 months in order to evaluate the clinical evolution, and to investigate the parasite and/or DNA in different biological samples (intact skin, cutaneous scar, blood, bone marrow and lymph node aspirate) by parasitological (culture and smear exam) and molecular (DNA-based) methods. Specific anti-*T. caninum* and anti-*Leishmania* antibody production was also evaluated. Ten of 14 dogs infected by *T. caninum* showed a good general state at the time of diagnosis, and this status did not vary during the follow-up. Anti-*T. caninum* and anti-*Leishmania* IgG antibodies were detected by IFAT in 10 and 2 animals, respectively. Concomitant infection by *Leishmania chagasi* was confirmed in 2 dogs, evidence for the overlap of endemic areas in Cuiaba. Our results indicate that *T. caninum* infection can be asymptomatic with low humoral immune response. Furthermore, despite examination of several biological samples, *T. caninum* (parasite or DNA) was found only in the intact skin in all animals.

(c) TRYPANOTOLERANCE

16849. **Silbermayr, K., Li, F., Soudre, A., Muller, S. & Solkner, J., 2013.** A novel qPCR assay for the detection of African animal trypanosomosis in trypanotolerant and trypanosusceptible cattle breeds. *PLoS Neglected Tropical Diseases*, **7** (8): e2345.

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This study was conducted to (i) determine the prevalence of animal African trypanosomosis (AAT) in tsetse-challenged areas, (ii) compare conventional with qPCR detection systems, and (iii) evaluate the host genetic background and biology as risk factors. AAT prevalence studies are often confronted with low levels of parasitaemia. Hence, we designed a novel qPCR assay using primers and species specific probes amplifying the internal transcribed spacer 1 (ITS1) gene. Thereby all three AAT species could be detected simultaneously. 368 individuals from three cattle types (Baoule, Zebu and hybrids) originating from 72 farms in Burkina Faso were analysed. Farmers were interviewed and morphometric measurements of the cattle taken. A chi-squared test and a logistic regression model were calculated to detect associations with infection. In our study, the overall rate of prevalence detected with the novel qPCR assay was 11.14 percent. Compared with conventional PCR, we identified a concordance of 91.30 percent. We tested 41 animals

positive for trypanosome DNA, and five animals showed multiple infections. Zebus were twice as often infected (21.74 percent) compared with Baoule (9.70 percent) and hybrids (9.57 percent). *Trypanosoma vivax* was the dominant species (9.24 percent), followed by *T. congolense* (2.44 percent) and *T. brucei* (0.82 percent). The chi-squared tests linking the infection events to the breeds (Zebu vs. Baoule and Zebu vs. hybrids) were on the border of significance. No significant association with other tested parameters could be detected. To conclude, we introduced a novel qPCR technique for the fast, sensitive and simultaneous detection of the three AAT species. Our results suggest that associations with breed and infection exist since Zebu cattle are more likely to be infected compared with Baoule and hybrids. Indigenous taurine cattle breeds, like the Baoule, therefore provide a unique and valuable genetic resource.

(d) TREATMENT

[See also 36: 16781, 16804, 16822].

16850. **Ranjithkumar, M., Saravanan, B. C., Yadav, S. C., Kumar, R., Singh, R. & Dey, S., 2013.** Neurological trypanosomiasis in quinapyramine sulphate-treated horses: a breach of the blood-brain barrier? *Tropical Animal Health & Production*. **E Publication ahead of print, 6 November.**

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Trypanosoma evansi infection typically produces a wasting disease, but it can also develop into a neurological or meningoencephalitis form in equids. Trypanosomosis in horses was treated with quinapyramine sulphate, and all the 14 infected animals recovered clinically. After clinical recovery, four animals developed a neurological form of the disease at various intervals. Two of these animals treated with diminazene aceturate recovered temporarily. Repeated attempts failed to find the parasite in the blood or the cerebrospinal fluid (CSF), but all of the animals were positive in an enzyme-linked immunosorbent assay. The calculation of the antibody index (AI) in the serum and the CSF and polymerase chain reaction (PCR) analysis of the CSF and brain tissue were carried out to confirm the neuro-infection. We found PCR and AI analyses of the CSF to be useful tools in the diagnosis of the neurological form of trypanosomosis when the organism cannot be found in the blood or CSF. The increased albumin quotient is indicative of barrier leakage due to neuroinflammation. The biochemical changes in the CSF due to nervous system trypanosomosis include increases in the albumin quotient, total protein, and urea nitrogen. This seems to be the first report on relapse of the nervous form of trypanosomosis in equids even after quinapyramine treatment in endemic areas.

7. EXPERIMENTAL TRYPANOSOMOSIS

(a) DIAGNOSTICS

[See also **36**: 16772]

16851. **McLatchie, A. P., Burrell-Saward, H., Myburgh, E., Lewis, M. D., Ward, T. H.,**

Mottram, J. C., Croft, S. L., Kelly, J. M. & Taylor, M. C., 2013. Highly sensitive i*n vivo* imaging of *Trypanosoma brucei* expressing "red-shifted" luciferase. *PLoS Neglected Tropical Diseases*, **7** (11): e2571.

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Human African trypanosomiasis is caused by infection with parasites of the *Trypanosoma brucei* species complex, and threatens over 70 million people in sub-Saharan Africa. Development of new drugs is hampered by the limitations of current rodent models, particularly for stage II infections, which occur once parasites have accessed the CNS. Bioluminescence imaging of pathogens expressing firefly luciferase (emission maximum 562 nm) has been adopted in a number of *in vivo* models of disease to monitor dissemination, drug treatment and the role of immune responses. However, lack of sensitivity in detecting deep tissue bioluminescence at wavelengths below 600 nm has restricted the widespread use of *in vivo* imaging to investigate infections with *T. brucei* and other trypanosomatids. Here, we report a system that allows the detection of fewer than 100 bioluminescent *T. brucei* parasites in a murine model. As a reporter, we used a codon-optimised red-shifted *Photinus pyralis* luciferase (PpyRE9H) with a peak emission of 617 nm. Maximal expression was obtained following targeted integration of the gene, flanked by an upstream 5'-variant surface glycoprotein untranslated region (UTR) and a downstream 3'-tubulin UTR, into a *T. brucei* ribosomal DNA locus. Expression was stable in the absence of selective drug for at least 3 months and was not associated with detectable phenotypic changes. Parasite dissemination and drug efficacy could be monitored in real time, and brain infections were readily detectable. The level of sensitivity *in vivo* was significantly greater than achievable with a yellow firefly luciferase reporter. The optimised bioluminescent reporter line described here will significantly enhance the application of *in vivo* imaging to study stage II African trypanosomiasis in murine models. The greatly increased sensitivity provides a new framework for investigating host-parasite relationships, particularly in the context of CNS infections. It should be ideally suited to drug evaluation programmes.

16852. **Pillay, D., Izotte, J., Fikru, R., Buscher, P., Mucache, H., Neves, L., Boulange, A., Seck, M. T., Bouyer, J., Napier, G. B., Chevtzoff, C., Coustou, V. & Baltz, T., 2013.** *Trypanosoma vivax* GM6 antigen: a candidate antigen for diagnosis of animal African trypanosomosis in cattle. *PLoS One*, **8** (10): e78565.

Microbiologie Fondamentale et Pathogénicité, UMR 5234, Centre National de la Recherche Scientifique (CNRS), Université Bordeaux Segalen, Bordeaux, France. [davita.pillay@u-bordeaux2.fr].

Diagnosis of animal African trypanosomosis is vital to controlling this severe disease which hampers development across 10 million km^2 of Africa endemic to tsetse flies. Diagnosis at the point of treatment is currently dependent on parasite detection which is unreliable, and on clinical signs, which are common to several other prevalent bovine diseases. The repeat sequence of the GM6 antigen of *Trypanosoma vivax* (TvGM6), a flagellar-associated protein, was analysed from several isolates of *T. vivax* and found to be almost identical despite the fact that *T. vivax* is known to have high genetic variation. The TvGM6 repeat was recombinantly expressed in *E. coli* and purified. An indirect ELISA for

bovine sera based on this antigen was developed. The TvGM6 indirect ELISA had a sensitivity of 91.4 percent (95 percent CI: 91.3 to 91.6) in the period following 10 d post experimental infection with *T. vivax*, which decreased tenfold to 9.1 percent (95 percent CI: 7.3 to 10.9) one month post treatment. With field sera from cattle infected with *T. vivax* from two locations in East and West Africa, 91.5 percent (95 percent CI: 83.2 to 99.5) sensitivity and 91.3 percent (95 percent CI: 78.9 to 93.1) specificity was obtained for the TvGM6 ELISA using the whole trypanosome lysate ELISA as a reference. For heterologous *T. congolense* field infections, the TvGM6 ELISA had a sensitivity of 85.1 percent (95 percent CI: 76.8 to 94.4). This study is the first to analyse the GM6 antigen of *T. vivax* and the first to test the GM6 antigen on a large collection of sera from experimentally and naturally infected cattle. This study demonstrates that the TvGM6 is an excellent candidate antigen for the development of a point-of-treatment test for diagnosis of *T. vivax*, and to a lesser extent *T. congolense* animal African trypanosomosis in cattle.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **36**: 16857, 16881, 16901, 16943, 16909]

16853. **Bal, M. S., Singla, L. D., Kumar, H., Vasudev, A., Gupta, K. & Juyal, P. D., 2012.** Pathological studies on experimental *Trypanosoma evansi* infection in Swiss albino mice. *Journal of Parasitic Diseases*, **36** (2): 260-264.

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The pathology of *Trypanosoma evansi* infection was studied in Swiss albino mice using a cattle isolate of the parasite. Sixteen Swiss albino mice were used in the experiment and were divided into two groups viz. infected group (I) and uninfected healthy control group (II) comprising 12 and four mice, respectively. The mice from group I were infected with 1 x 10^5 purified trypanosomes. Systematic necropsy examination of the infected mice as well as of healthy controls was performed and pathological changes were recorded. The different tissue samples were collected in 10 percent neutral saline buffered with formaline and used to study the histopathological changes. Gross post-mortem examination revealed enlargement of spleen and petechial haemorrhages in liver in the terminal stages of disease. Tissue sections revealed the presence of numerous trypanosomes in blood vessels of the liver, spleen, brain and kidneys. Microscopically, the liver revealed lesions varying from vacuolar degeneration and coagulative necrosis along with congestion and haemorrhages. Spleens showed extensive haemorrhages in the red pulp area, haemosiderosis and aggregation of histiocytes resulting in multinuclear giant cell formation. Lungs revealed oedema, congestion and mild inflammatory changes. Brains revealed mild degenerative changes along with congestion of meningeal blood vessels. Kidneys showed tubular degeneration, congestion and cellular infiltration. Hearts revealed mild degenerative changes along with interstitial oedema. All changes were consistent with trypanosome infection and were confirmed by the presence of trypanosomes in most of the tissue sections examined.

16854. **Balogun, E. O., Balogun, J. B., Yusuf, S., Inuwa, H. M., Ndams, I. S., Sheridan, P., Inaoka, D. K., Shiba, T., Harada, S., Kita, K., Esievo, K. A. & Nok, A. J., 2014.** Anaemia amelioration by lactose infusion during trypanosomosis could be

associated with erythrocyte membrane de-galactosylation. *Veterinary Parasitology,* **199***:*259-263.

Department of Biochemistry, Ahmadu Bello University, Zaria 2222, Nigeria; Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan; Department of Applied Biology, Graduate School of Science and Technology, Kyoto Institute of Technology, Sakyo-ku, Kyoto 606-8585, Japan. [balogun1@m.u-tokyo.ac.jp].

African trypanosomosis is a potentially fatal disease that is caused by extracellular parasitic protists known as African trypanosomes. These parasites inhabit the blood stream of their mammalian hosts and produce a number of pathological features, amongst which is anaemia. Aetiology of the anaemia has been partly attributed to an autoimmunity-like mediated erythrophagocytosis of de-sialylated red blood cells (dsRBCs) by macrophages. Lactose infusion to infected animals has proven effective in delaying progression of the anaemia. However, the mechanism of this anaemia prevention is yet to be well characterized. Here, the hypothesis of a likely induced further modification of the dsRBCs was investigated. RBC membrane galactose (RBC m-GAL) and packed cell volume (PCV) were measured during the course of experimental trypanosomosis in mice infected with *Trypanosoma congolense* (stb 212). Intriguingly, while the membrane galactose on the RBCs of infected and lactose-treated mice (group D) decreased as a function of parasitaemia, that of the lactose-untreated infected group (group C) remained relatively constant, as was the case for the uninfected lactose-treated control (group B) animals. At the peak of infection, the respective cumulative percent decreases in PCV and membrane galactose were 30 percent and 185 percent for group D, and 84 percent and 13 percent for group C. From the observed inverse relationship between RBCs membrane galactose and PCV, it is logical to rationalize that the delay in anaemia progression during trypanosomosis produced by lactose might have resulted from an induction of galactose depletion from dsRBCs, thereby preventing their recognition by the macrophages.

16855. **Basso, B., Moretti, E. & Fretes, R., 2014.** Vaccination with *Trypanosoma rangeli* induces resistance of guinea pigs to virulent *Trypanosoma cruzi. Veterinary Immunology & Immunopathology*, **157** (1-2): 119-123.

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Chagas disease, endemic in Latin America, is spread in natural environments through animal reservoirs, including marsupials, mice and guinea pigs. Farms breeding guinea pigs for food are located in some Latin American countries with consequent risk of digestive infection. The aim of this work was to study the effect of vaccination with *Trypanosoma rangeli* in guinea pigs challenged with *Trypanosoma cruzi*. Animals were vaccinated with fixated epimastigotes of *T. rangeli,* emulsified with saponin. Controls received only PBS. Before being challenged with *T. cruzi*, parasitaemia, survival rates and histological studies were performed. The vaccinated guinea pigs revealed significantly lower parasitaemias than controls ($p<0.0001$-0.01) and a discrete lymphomonocytic infiltrate in cardiac and skeletal muscles was present. In the chronic phase, the histological appearance was normal. In

contrast, the control group revealed amastigote nests and typical histopathological alterations compatible with chagasic myocarditis, endocarditis and pericarditis. These results, together with previous work in our laboratory, show that *T. rangeli* induces immunoprotection in three species of animals: mice, guinea pigs and dogs. The development of vaccines for use in animals, like domestic dogs and guinea pigs in captivity, opens up new opportunities for preventive tools, and could reduce the risk of infection with *T. cruzi* in the community.

16856. **Capewell, P., Clucas, C., DeJesus, E., Kieft, R., Hajduk, S., Veitch, N., Steketee, P. C., Cooper, A., Weir, W. & MacLeod, A., 2013.** The TgsGP gene is essential for resistance to human serum in *Trypanosoma brucei gambiense*. *PLoS Pathogens*, **9** (10): e1003686.

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Trypanosoma brucei gambiense causes 97 percent of all cases of African sleeping sickness, a fatal disease of sub-Saharan Africa. Most species of trypanosome, such as *T. b. brucei*, are unable to infect humans due to the trypanolytic serum protein apolipoprotein-L1 (APOL1) delivered via two trypanosome lytic factors (TLF-1 and TLF-2). Understanding how *T. b. gambiense* overcomes these factors and infects humans is of major importance in the fight against this disease. Previous work indicated that a failure to take up TLF-1 in *T. b. gambiense* contributes to resistance to TLF-1, although another mechanism is required to overcome TLF-2. Here, we have examined a *T. b. gambiense* specific gene, TgsGP, which had previously been suggested, but not shown, to be involved in serum resistance. We show that TgsGP is essential for resistance to lysis as deletion of TgsGP in *T. b. gambiense* renders the parasites sensitive to human serum and recombinant APOL1. Deletion of TgsGP in *T. b. gambiense* modified to take up TLF-1 showed sensitivity to TLF-1, APOL1 and human serum. Reintroducing TgsGP into knockout parasite lines restored resistance. We conclude that TgsGP is essential for human serum resistance in *T. b. gambiense*.

16857. **Dalla Rosa, L., Da Silva, A. S., Ruchel, J. B., Gressler, L. T., Oliveira, C. B., Franca, R. T., Lopes, S. T., Leal, D. B. & Monteiro, S. G., 2013.** Influence of treatment with 3'-deoxyadenosine associated deoxycoformycin on haematological parameters and activity of adenosine deaminase in infected mice with *Trypanosoma evansi*. *Experimental Parasitology*, **135** (2): 357-362.

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This study aimed to verify the effect of 3'-deoxyadenosine and deoxycoformycin on haematologic parameters and adenosine deaminase (ADA) activity in plasma and brain of mice infected with *Trypanosoma evansi*. Seventy animals were divided into seven groups, which were each divided into two subgroups for sampling on d 4 and d 8 post-infection (PI). The groups were composed of three uninfected groups (A-C), namely, not-treated (A), treated with 3'-deoxyadenosine (B), and treated with deoxycoformycin (C), and four groups infected with *T. evansi* (D-G), namely, not-treated (D), treated with 3'-deoxyadenosine (E), treated with deoxycoformycin (F), and treated with a combination of 3'-deoxyadenosine and deoxycoformycin (G). Haematological parameters and ADA activity were evaluated in

plasma and brain. Animals in groups B and C exhibited a reduction in the levels of plasma total protein compared group A. Animals in groups D and F showed changes in the haematological parameters. The ADA activity was significantly reduced in the animals of groups C, D, F and G. Mice in group E presented increased ADA activity in plasma. Therefore, we conclude that the treatment interferes significantly with the haematologic parameters in mice infected with *T. evansi*. On the other hand, when the ADA inhibitor was used we recorded significant decreases in the values for haematocrit, total erythrocytes, and haemoglobin concentration. Deoxycoformycin was able to inhibit the ADA activity of the parasite suggesting that it may be one of the mechanisms underpinning the efficacy of this treatment.

16858. **Elliott, E. B., McCarroll, D., Hasumi, H., Welsh, C. E., Panissidi, A. A., Jones, N. G., Rossor, C. L., Tait, A., Smith, G. L., Mottram, J. C., Morrison, L. J. & Loughrey, C. M., 2013.** *Trypanosoma brucei* cathepsin-L increases arrhythmogenic sarcoplasmic reticulum-mediated calcium release in rat cardiomyocytes. *Cardiovascular Research*, **100** (2): 325-335.

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African trypanosomiasis, caused by *Trypanosoma brucei* species, leads to both neurological and cardiac dysfunction and can be fatal if untreated. While the neurologically-related pathogenesis is well studied, the cardiac pathogenesis remains unknown. The current study exposed isolated ventricular cardiomyocytes and adult rat hearts to *T. brucei* to test whether trypanosomes can alter cardiac function independent of a systemic inflammatory/immune response. Using confocal imaging, *T. brucei* and culture media (supernatant) caused an increased frequency of arrhythmogenic spontaneous diastolic sarcoplasmic reticulum (SR)-mediated Ca^{2+} release (Ca^{2+} waves) in isolated adult rat ventricular cardiomyocytes. Studies utilising inhibitors, recombinant protein and RNAi all demonstrated that this altered SR function was due to *T. brucei* cathepsin-L (TbCatL). Separate experiments revealed that TbCatL induced a 10-15 percent increase of SERCA activity but reduced SR Ca^{2+} content, suggesting a concomitant increased SR-mediated Ca^{2+} leak. This conclusion was supported by data demonstrating that TbCatL increased Ca^{2+} wave frequency. These effects were abolished by autocamtide-2-related inhibitory peptide, highlighting a role for CaMKII in the TbCatL action on SR function. Isolated Langendorff perfused whole heart experiments confirmed that supernatant caused an increased number of arrhythmic events. These data demonstrate for the first time that African trypanosomes alter cardiac function independent of a systemic immune response, via a mechanism involving extracellular cathepsin-L-mediated changes in SR function.

16859. **Eze, J. I., Okeke, M. C., Ngene, A. A., Omeje, J. N. & Abonyi, F. O., 2013.** Effects of dietary selenium supplementation on parasitaemia, anaemia and serum proteins of *Trypanosoma brucei brucei* infected rats. *Experimental Parasitology*, **135** (2): 331-336.

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Trypanosomosis has been associated with immunosuppression, anaemia and oxidative damage while selenium possesses both immunostimulatory and antioxidative effects. This study was designed to assess the effect of dietary selenium supplementation on parasitaemia, anaemia, survival pattern and serum protein profiles of trypanosome-infected rats. Twenty five rats were divided into five groups (A-E) each of 5 animals. They were treated as follows: three groups received respectively 4, 8 or 16 ppm (ppm) of selenium in their feed throughout the experimental period and were infected with *Trypanosoma brucei brucei* on d 14 post supplementation; a further group was infected but not supplemented and one group E served as the negative control. Supplementation at 4 and 8 ppm increased the packed cell volume (PCV) and haemoglobin (Hb) concentration on d 7 of supplementation when compared with the unsupplemented groups. Following infection at 14 d post supplementation, the PCV and Hb levels of the group given 16 ppm and of the group infected and not supplemented were significantly ($p < 0.05$) lower than other groups on d 28 and 35 after supplementation. Supplementation did not lead to significant ($p > 0.05$) changes in total protein, albumin and globulin levels by day 14 after supplementation. Infection, however, caused significant ($p > 0.05$) decreases in the total protein and albumin levels from d 28. The supplementation did not significantly ($p > 0.05$) increase the pre-patent period but caused a significant reduction in parasitaemia levels and increased survival intervals. Dietary selenium supplementation may show promise in the management of African trypanosomosis as supplementation was able to reduce anaemia and parasitaemia and increase survival intervals of trypanosome infected rats.

16860. **Hall, J. P., Wang, H. & Barry, J. D., 2013.** Mosaic VSGs and the scale of *Trypanosoma brucei* antigenic variation. *PLoS Pathogens*, **9** (7): e1003502.

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A main determinant of prolonged *Trypanosoma brucei* infection and transmission and on success of the parasite is the interplay between host acquired immunity and antigenic variation of the parasite variant surface glycoprotein (VSG) coat. About 0.1 percent of trypanosome divisions produce a switch to a different VSG through differential expression of an archive of hundreds of silent VSG genes and pseudogenes, but the patterns and extent of the trypanosome diversity phenotype, particularly in chronic infection, are unclear. We applied longitudinal VSG cDNA sequencing to estimate variant richness and test whether pseudogenes contribute to antigenic variation. We show that individual growth peaks can contain at least 15 distinct variants, are estimated computationally to comprise many more, and that antigenically distinct "mosaic" VSGs arise from segmental gene conversion between donor VSG genes or pseudogenes. The potential for trypanosome antigenic variation is probably much greater than VSG archive size; mosaic VSGs are core to antigenic variation and chronic infection.

16861. **Kisalu, N. K., Langousis, G., Bentolila, L. A., Ralston, K. S. & Hill, K. L., 2014.** Mouse infection and pathogenesis by *Trypanosoma brucei* motility mutants. *Cellular Microbiol*ogy. **E Publication ahead of print, 8 January.**

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The flagellum of *Trypanosoma brucei* is an essential and multifunctional organelle that drives parasite motility and is receiving increased attention as a potential drug target. In the mammalian host, parasite motility is suspected to contribute to infection and disease pathogenesis. However, it has not been possible to test this hypothesis owing to lack of motility mutants that are viable in the bloodstream life cycle stage that infects the mammalian host. We recently identified a bloodstream form motility mutant in 427-derived *T. brucei* in which point mutations in the LC1 dynein subunit disrupt propulsive motility but do not affect viability. These mutants have an actively beating flagellum, but cannot translocate. Here we demonstrate that the LC1 point mutant fails to show enhanced cell motility upon increasing viscosity of the surrounding medium, which is a hallmark of wild type *T. brucei*, thus indicating that motility of the mutant is fundamentally altered compared with wild type cells. We next used the LC1 point mutant to assess the influence of trypanosome motility on infection in mice. We surprisingly found that disrupting parasite motility has no discernible effect on *T. brucei* bloodstream infection. Infection time-course, maximum parasitaemia, number of waves of parasitaemia, clinical features and disease outcome are indistinguishable between motility mutant and control parasites. Our studies provide an important step toward understanding the contribution of parasite motility to infection and a foundation for future investigations of *T. brucei* interaction with the mammalian host.

16862. **Kobo, P. I., Ayo, J. O., Aluwong, T., Zezi, A. U., Maikai, V. & Ambali, S. F., 2013.** Flavonoid mixture ameliorates increase in erythrocyte osmotic fragility and malondialdehyde concentration induced by *Trypanosoma brucei brucei* infection in Wistar rats. *Research in Veterinary Science,* **96**: 139-142.

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The experiment was performed with the aim of investigating the effect of a flavonoid mixture, Daflon® 500 mg (DF) on the erythrocyte fragility and lipoperoxidative changes, induced by *Trypanosoma brucei brucei* infection in Wistar rats. Fifty adult male rats randomly divided into five groups of 10 animals each were used. Rats in the control group were administered (1mL/kg) distilled water only, while the other groups were infected with *T. brucei* and treated with Daflon® 500 mg and/or diminazene aceturate. At the end of 5 weeks, EDTA-blood samples and serum samples were collected from the rats, and were used to determine erythrocyte osmotic fragility (EOF) and serum malondialdehyde (MDA) concentration. The results showed that EOF and MDA concentration significantly ($p < 0.05$) increased in the infected untreated group when compared with the treatment groups. Treatment with Daflon® 500 mg and diminazene aceturate significantly ($p < 0.05$) reduced trypanosome-induced increases in EOF and lipoperoxidative changes, suggesting possible antioxidant properties of Daflon® 500mg and its therapeutic value in trypanosomosis.

16863. **Nyawira Maranga, D., Kagira, J. M., Kinyanjui, C. K., Muturi Karanja, S., Wangari Maina, N. & Ngotho, M., 2013.** IL-6 is upregulated in late-stage disease in monkeys experimentally infected with *Trypanosoma brucei rhodesiense*. *Clinical & Developmental Immunology*, **2013**: 320509.

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The management of human African trypanosomiasis (HAT) is constrained by lack of simple-to-use diagnostic, staging, and treatment tools. The search for novel biomarkers is, therefore, essential in the fight against HAT. The current study aimed at investigating the potential of IL-6 as an adjunct parameter for HAT stage determination in vervet monkey model. Four adult vervet monkeys (*Chlorocebus aethiops*) were experimentally infected with *Trypanosoma brucei rhodesiense* and treated subcuratively at 28 d after infection (dpi) to induce late stage disease. Three non-infected monkeys formed the control group. Cerebrospinal fluid (CSF) and blood samples were obtained at weekly intervals and assessed for various biological parameters. A typical HAT-like infection was observed. The late stage was characterized by significant ($p < 0.05$) elevation of CSF IL-6, white blood cell count, and total protein starting 35 dpi with peak levels of these parameters coinciding with relapse parasitaemia. Brain immunohistochemical staining revealed an increase in brain glial fibrillary acidic protein expression indicative of reactive astrogliosis in infected animals which were euthanized in late-stage disease. The elevation of IL-6 in CSF which accompanied other HAT biomarkers indicates the onset of parasite neuroinvasion and shows potential for use as an adjunct late-stage disease biomarker in the Rhodesian sleeping sickness.

16864. **Onyilagha, C., Okwor, I., Kuriakose, S., Singh, R. & Uzonna, J., 2013.** Low dose intradermal infection with *Trypanosoma congolense* leads to expansion of regulatory T cells and enhanced susceptibility to reinfection. *Infection & Immunity*. **E Publication ahead of print, 16 December.**

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BALB/c mice are highly susceptible to experimental intraperitoneal *T. congolense* infection. However, a recent report showed that these mice are relatively resistant to primary intradermal low dose infection. Paradoxically, repeated low dose intradermal infections predispose mice to enhanced susceptibility to an otherwise non-infectious dose challenge. Here, we explored the mechanisms responsible for this low dose-induced susceptibility to subsequent low dose challenge infection. We found that akin to intraperitoneal infection, low dose intradermal infection led to production of IL-10, IL-6, IL-12, TNF-alpha, TGF-beta and IFN-gamma by spleen and draining lymph node cells. Interestingly, despite the absence of parasitaemia, low dose intradermal infection led to expansion of $CD4^+CD25^+Foxp3^+$ cells (Tregs) in both the spleens and lymph nodes draining the infection site. Depletion of Tregs by anti-CD25 mAb treatment during primary or before challenge infection following repeated low dose infection completely abolished the low dose-induced enhanced susceptibility. In addition, Tregs depletion was associated with dramatic reduction in serum levels of TGF-beta and IL-10. Collectively, these findings show that low dose intradermal infection leads to rapid expansion of Tregs and these cells mediate enhanced susceptibility to subsequent infection.

16865. **Wolkmer, P., Paim, F. C., CB, D. A. S., Gai, B. M., Carvalho, F. B., AC, D. A. S., MM, D. A. R., AS, D. A. S., Pereira, P. R., Lopes, S. T., Nogueira, C. W., Rubin, M. A., Monteiro, S. G. & Mazzanti, C. M., 2013.** *Trypanosoma evansi* infection impairs memory, increases anxiety behaviour and alters neurochemical parameters in rats. *Parasitology*, **140** (11): 1432-1441.

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The aim of this study was to investigate neurochemical and enzymatic changes in rats infected with *Trypanosoma evansi*, and their interference in the cognitive parameters. Behavioural assessment (assessment of cognitive performance), evaluation of cerebral L-[^{3}H]glutamate uptake, acetylcholinesterase (AChE) activity and Ca^{2+} and Na^{+}, K^{+}-ATPase activity were evaluated at 5 and 30 d post infection (dpi). This study demonstrates a cognitive impairment in rats infected with *T. evansi*. At 5 dpi memory deficit was demonstrated by an inhibitory avoidance test. With the chronicity of the disease (30 dpi) animals showed anxiety symptoms. It is possible the inhibition of cerebral Na^{+}, K^{+}-ATPase activity, AChE and synaptosomal glutamate uptake is involved in cognitive impairment in infected rats by *T. evansi*. The understanding of the cerebral host-parasite relationship may shed some light on the cryptic symptoms of animals and possibly human infection where patients often present with other central nervous system (CNS) disorders.

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[See also **36**: 16774, 16857, 16881 16897, 16913, 16937, 16943]

16866. **Adelodun, V. O., Elusiyan, C. A., Olorunmola, F. O., Adewoyin, F. B., Omisore, N. O., Adepiti, A. O., Agbedahunsi, J. M. & Adewunmi, C. O., 2013.** Evaluation of anti-*Trypanosomal* and anti-inflammatory activities of selected Nigerian medicinal plants in mice. *African Journal of Traditional, Complementary & Alternative Medicines*, **10** (6): 469-476.

Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

The extracts of nine selected Nigerian medicinal plants were investigated on *Trypanosoma brucei brucei* infected mice. The anti-inflammatory properties of the hexane fraction of the most promising *Uvaria chamae* extract was assessed by acute oedema of the mice paw model while the modulatory effect of the extract on delayed-type hypersensitivity (DTH) response on *in vivo* leucocyte mobilization was evaluated. “Dose-probing acute toxicity tests” established an oral and intraperitoneal LD_{50} for *T. ivorensis* stem bark as >1 600 < 5 000 mg/kg and 100 mg/kg respectively, while the oral LD_{50} of *Uvaria chamae* was >5 000 mg/kg. Extracts of *Khaya senegalensis, Harungana madagascariensis, Terminalia ivorensis, Curcuma longa, Ocimum gratissimum* and *Alcornea cordifolia* showed weak anti-trypanosomal effect and did not exhibit significant clearance in parasitaemia at the test dose administered compared with the positive control (Diminal®). However, the leaf extract of *U. chamae* and its hexane fraction demonstrated a significant response ($p < 0.01$). The fraction at 1 000 mg/kg inhibited oedema by 107 percent. *Uvaria chamae* demonstrated both

antitrypanosomal and anti-inflammatory properties by increasing the survival time of infected mice due to reduction in parasitaemia caused by *T. brucei brucei*.

16867. **Alibu, V. P., Daunes, S. & D'Silva, C., 2013.** N-benzyloxycarbonyl-S-(2,4-dinitrophenyl) glutathione dibutyl diester is inhibitory to melarsoprol resistant cell lines overexpressing the *T. brucei* MRPA transporter. *Bioorganic & Medicinal Chemistry Letters*, **23** (15): 4351-4353.

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16868. **Ayyari, M., Salehi, P., Ebrahimi, S. N., Zimmermann, S., Portmann, L., Krauth-Siegel, R. L., Kaiser, M., Brun, R., Rezadoost, H., Rezazadeh, S. & Hamburger, M., 2014.** Antitrypanosomal isothiocyanate and thiocarbamate glycosides from *Moringa peregrina*. *Planta Medica,* **80** (01): 86-89

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O-Methyl (1), O-ethyl (2), and O-butyl (3) 4-[(alpha-L-rhamnosyloxy) benzyl] thiocarbamate (E), along with 4-(alpha-L-rhamnosyloxy) benzyl isothiocyanate (4) were isolated from the aerial parts of *Moringa peregrina*. The compounds were tested for *in vitro* activity against *Trypanosoma brucei rhodesiense* and cytotoxicity in rat skeletal myoblasts (L6 cells). The most potent compound was 4 with an IC_{50} of 0.10 µM against *T. b. rhodesiense* and a selectivity index of 73, while the thiocarbamate glycosides 1, 2, and 3 showed only moderate activity. Intraperitoneal administration of 50 mg/kg b.w./d of 4 in the *T. b. rhodesiense* STIB 900 acute mouse model revealed significant *in vivo* toxicity. Administration of 10 mg/kg b.w./d resulted in a 95 percent reduction of parasitaemia on d 7 post-infection, but did not cure the animals. Because of its high *in vitro* activity and its ability to irreversibly inhibit trypanothione reductase, an attractive parasite-specific target enzyme, 4-[(alpha-L-rhamnosyloxy) benzyl] isothiocyanate (4), can be considered as a lead structure for the development and characterization of novel antitrypanosomal drugs.

16869. **Baumann, A., Pfeifer, T., Melles, D. & Karst, U., 2013.** Investigation of the biotransformation of melarsoprol by electrochemistry coupled to complementary LC/ESI-MS and LC/ICP-MS analysis. *Analytical & Bioanalytical Chemistry*, **405** (15): 5249-5258.

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Melarsoprol is the only currently available drug for treatment of the late stage of African trypanosomiasis (sleeping sickness). Unfortunately, the arsenic-containing drug causes serious side effects, for which the mechanisms have not been elucidated so far. This investigation describes the study of the melarsoprol biotransformation processes by electrochemical (EC) techniques. Based on EC, potential oxidation reactions of melarsoprol are examined. Moreover, the reactivity of melarsoprol, its metabolite melarsen oxide, and their oxidation products toward the tripeptide glutathione and the proteins haemoglobin and

human serum albumin are evaluated. The combination of different analytical techniques allows the identification as well as the quantification of the biotransformation products. The combination of liquid chromatography (LC) and electrospray ionization mass spectrometry (ESI-MS) is applied for identification and structure elucidation, which enables the determination of exact masses and fragmentation patterns. For the selective detection of arsenic containing metabolites, LC coupled with inductively coupled plasma mass spectrometry is utilized. Based on the data obtained, the oxidative biotransformation of melarsoprol can be predicted, revealing novel species which have been suspected, but not identified up to now. The results of the protein studies prove that melarsen oxide, the active derivative of melarsoprol, strongly binds to human haemoglobin and forms different adducts via the free cysteinyl groups of the haemoglobin alpha- and beta-chain.

16870. **Branquinha, M. H., Marinho, F. A., Sangenito, L. S., Oliveira, S. S., Goncalves, K. C., Ennes-Vidal, V., d'Avila-Levy, C. M. & Santos, A. L., 2013.** Calpains: potential targets for alternative chemotherapeutic intervention against human pathogenic trypanosomatids. *Current Medicinal Chemistry*, **20** (25): 3174-3185.

Laboratorio de Investigacao de Peptidases, Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Goes-IMPG, Centro de Ciencias da Saude-CCS, Bloco Esubsolo, Sala 05, Universidade Federal do Rio de Janeiro-UFRJ, Rio de Janeiro, Brazil. [mbranquinha@micro.ufrj.br].

16871. **Buchanan-Kilbey, G., Djumpah, J., Papadopoulou, M. V., Bloomer, W., Hu, L., Wilkinson, S. R. & Ashworth, R., 2013.** Evaluating the developmental toxicity of trypanocidal nitroaromatic compounds on zebrafish. *Acta Tropica*, **128** (3): 701-705.

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Current therapies against African and American trypanosomiases are problematic and with no immediate prospect of a vaccine there is an urgent need for cheap, more effective treatments. To aid the drug discovery pipeline, we report a novel *in vivo* screening approach using zebrafish (*Danio rerio*) embryos as a means of rapidly assessing a compound's developmental toxicity. This technique, amenable to high-throughput screening, was validated using several trypanocidal nitroaromatic prodrugs including nifurtimox and benznidazole.

16872. **Derbyshire, E. R. & Clardy, J., 2013.** Closing in on a new treatment for sleeping sickness. *Elife*, **2**: e01042.

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, USA. [emily_derbyshire@hms.harvard.edu].

A chemoproteomics approach was employed to identify a kinase that could be used as a druggable target in efforts to develop new treatments for African sleeping sickness.

16873. **Dethoua, M., Nzoumbou-Boko, R., Truc, P., Daulouede, S., Courtois, P., Bucheton, B., Cuny, G., Semballa, S. & Vincendeau, P., 2013.** Evaluation of

trypanocidal drugs used for human African trypanosomosis against *Trypanosoma lewisi*. *Parasite*, **20**: 39.

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Trypanosomes from animals are potential pathogens for humans. Several human cases infected by *Trypanosoma lewisi*, a parasite of rats, have been reported. The number of these infections is possibly underestimated. Some infections were self-cured, others required treatment with drugs used in human African trypanosomosis. An *in vitro* evaluation of these drugs and fexinidazole, a new oral drug candidate, were performed against *T. lewisi* in comparison with *T. brucei gambiense*. All have comparable activities against the two parasites. Suramin was not effective. *In vivo*, drugs were tested in rats immunosuppressed by cyclophosphamide. The best efficacy was obtained for fexinidazole, and pentamidine (15 mg/kg): rats were cured in 7 d and 10 d respectively. Rats receiving nifurtimox-eflornithine combination therapy (NECT) or pentamidine (4 mg/kg) were cured after 28 d, while melarsoprol was weakly active. The identification of efficient drugs with reduced toxicity will help in the management of new cases of atypical trypanosomosis.

16874. **Dunny, E., Doherty, W., Evans, P., Malthouse, J. P., Nolan, D. & Knox, A. J., 2013.** Vinyl sulphone-based peptidomimetics as anti-trypanosomal agents: design, synthesis, biological and computational evaluation. *Journal of Medicinal Chemistry*, **56** (17): 6638-6650.

Centre for Synthesis and Chemical Biology, School of Chemistry and Chemical Biology, University College Dublin, Dublin 4, Ireland. [J.Paul.G.Malthouse@ucd.ie].

16875. **Ehmke, V., Winkler, E., Banner, D. W., Haap, W., Schweizer, W. B., Rottmann, M., Kaiser, M., Freymond, C., Schirmeister, T. & Diederich, F., 2013.** Optimization of triazine nitriles as rhodesain inhibitors: structure-activity relationships, bioisosteric imidazopyridine nitriles, and X-ray crystal structure analysis with human cathepsin L. *ChemMedChem*, **8** (6): 967-975.

Laboratorium fur Organische Chemie, ETH Zurich, Zurich, Switzerland. [schirmei@uni-mainz.de].

16876. **Kpoviessi, S., Bero, J., Agbani, P., Gbaguidi, F., Kpadonou-Kpoviessi, B., Sinsin, B., Accrombessi, G., Frederich, M., Moudachirou, M. & Quetin-Leclercq, J., 2013.** Chemical composition, cytotoxicity and *in vitro* antitrypanosomal and antiplasmodial activity of the essential oils of four *Cymbopogon* species from Benin. *Journal of Ethnopharmacology,* **151**: 652-659.

Laboratory of Physics and Synthesis Organic Chemistry (LaCOPS), University of Abomey-Calavi (UAC), Faculty of Sciences and Technics (FAST), BP: 4521 Cotonou, Benin. [joelle.leclercq@uclouvain.be].

Cymbopogon species are largely used in folk medicine for the treatment of many diseases some of which relate to parasitic diseases as fevers and headaches. As part of our

research on antiparasitic essential oils from Beninese plants, we decided to evaluate the *in vitro* antiplasmodial and antitrypanosomal activities of essential oils of four *Cymbopogon* species used in traditional medicine as well as their cytotoxicity. The essential oils of *Cymbopogon citratus* (I), *Cymbopogon giganteus* (II), *Cymbopogon nardus* (III) and *Cymbopogon schoenantus* (IV) from Benin obtained by hydrodistillation were analysed by GC/MS and GC/FID and were tested *in vitro* against *Trypanosoma brucei brucei* and *Plasmodium falciparum* respectively for antitrypanosomal and antiplasmodial activities. Cytotoxicity was evaluated *in vitro* against Chinese hamster ovary (CHO) cells and the human non-cancer fibroblast cell line (WI38) through an MTT assay to evaluate the selectivity. All tested oils showed a strong antitrypanosomal activity with a good selectivity. Sample II was the most active against *Trypanosoma brucei brucei* and could be considered as a good candidate. It was less active against *Plasmodium falciparum*. Samples II, III and IV had low or no cytotoxicity, but the essential oil of *Cymbopogon citratus* (I), was toxic against CHO cells and moderately toxic against WI38 cells and needs further toxicological studies. Sample I (29 compounds) was characterized by the presence as main constituents of geranial, neral, beta-pinene and cis-geraniol; sample II (53 compounds) by trans-p-mentha-1(7),8-dien-2-ol, trans-carveol, trans-p-mentha-2,8-dienol, cis-p-mentha-2,8-dienol, cis-p-mentha-1(7),8-dien-2-ol, limonene, cis-carveol and cis-carvone; sample III (28 compounds) by beta-citronellal, nerol, beta-citronellol, elemol and limonene, and sample IV (41 compounds) by piperitone, (+)-2-carene, limonene, elemol and beta-eudesmol. Our study shows that essential oils of Cymbopogon genus can be a good source of antitrypanosomal agents. This is the first report on the activity of these essential oils against *Trypanosoma brucei brucei, Plasmodium falciparum* and analysis of their cytotoxicity.

16877. **Lam, C. F., Pearce, A. N., Tan, S. H., Kaiser, M. & Copp, B. R., 2013.** Discovery and evaluation of thiazinoquinones as anti-protozoal agents. *Marine Drugs*, **11** (9): 3472-3499.

School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand. [b.copp@auckland.ac.nz].

16878. **Loureiro, I., Faria, J., Clayton, C., Ribeiro, S. M., Roy, N., Santarem, N., Tavares, J. & Cordeiro-da-Silva, A., 2013.** Knockdown of asparagine synthetase A renders *Trypanosoma brucei* auxotrophic to asparagine. *PLoS Neglected Tropical Diseases*, **7** (12): e2578.

Parasite Disease Group, Instituto de Biologia Molecular e Celular da Universidade do Porto, Porto, Portugal. [jtavares@ibmc.up.pt].

16879. **Lu, J., Vodnala, S. K., Gustavsson, A. L., Gustafsson, T. N., Sjoberg, B., Johansson, H. A., Kumar, S., Tjernberg, A., Engman, L., Rottenberg, M. E. & Holmgren, A., 2013.** Ebsulphur is a benzisothiazolone cytocidal inhibitor targeting the trypanothione reductase of *Trypanosoma brucei*. *Journal of Biological Chemistry*, **288** (38): 27456-27468.

Dept. of Microbiology, Tumor and Cell Biology, Karolinska Institutet, SE-17177 Stockholm, Sweden. [Martin.Rottenberg@ki.se].

16880. **Mokoka, T. A., Xolani, P. K., Zimmermann, S., Hata, Y., Adams, M., Kaiser, M., Moodley, N., Maharaj, V., Koorbanally, N. A., Hamburger, M., Brun, R. & Fouche, G., 2013.** Antiprotozoal screening of 60 South African plants, and the identification of the antitrypanosomal germacranolides schkuhrin I and II. *Planta Medica*, **79** (14): 1380-1384.

Natural Product Chemistry, Biosciences, Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa.

Two hundred and seven extracts were prepared from sixty plants from South Africa and screened for *in vitro* activity against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, and *Plasmodium falciparum*. For the 21 extracts which inhibited the growth of one or more parasites by more than 95 percent at 10 µg/mL, the IC_{50} values against all four protozoal parasites and cytotoxic IC_{50} values against L6 myoblasts were determined. Amongst the most notable results are the activities of *Psoralea pinnata* (IC_{50} of 0.15 µg/mL), *Schkuhria pinnata* (2.04 µg/mL), and *Vernonia mespilifolia* (1.01 µg/mL) against *Trypanosoma brucei rhodesiense*. HPLC-based activity profiling was used to identify the active constituents in the extracts, and the germacranolide sesquiterpene lactones schkuhrin I and II from *S. pinnata*, and cynaropicrin from *V. mespilifolia* were identified, with IC_{50} values of 0.9, 1.5, and 0.23 µM, respectively.

16881. **Myburgh, E., Coles, J. A., Ritchie, R., Kennedy, P. G., McLatchie, A. P., Rodgers, J., Taylor, M. C., Barrett, M. P., Brewer, J. M. & Mottram, J. C., 2013.** *In vivo* imaging of trypanosome-brain interactions and development of a rapid screening test for drugs against CNS stage trypanosomiasis. *PLoS Neglected Tropical Diseases*, **7** (8): e2384.

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New drugs to treat the second-stage HAT disease are urgently needed, yet testing of novel drug candidates is a slow process because the established animal model relies on detecting parasitemia in the blood as late as 180 d after treatment. To expedite compound screening, we have modified the GVR35 strain of *Trypanosoma brucei brucei* to express luciferase, and have monitored parasite distribution in infected mice following treatment with trypanocidal compounds using serial, non-invasive, bioluminescence imaging. Parasites were detected in the brains of infected mice following treatment with diminazene, a drug which cures stage 1 but not stage 2 disease. Intravital multi-photon microscopy revealed that trypanosomes enter the brain meninges as early as d 5 post-infection but can be killed by diminazene, whereas those that cross the blood-brain barrier and enter the parenchyma by d 21 survived treatment and later caused bloodstream recrudescence. In contrast, all bioluminescent parasites were permanently eliminated by treatment with melarsoprol and DB829, compounds known to cure stage 2 disease. We show that this use of imaging reduces by two thirds the time taken to assess drug efficacy and provides a dual-modal imaging platform for monitoring trypanosome infection in different areas of the brain.

16882. **Patrick, D. A., Bakunov, S. A., Bakunova, S. M., Wenzler, T., Brun, R. & Tidwell, R. R., 2014.** Antiprotozoal activity of dicationic 3,5-diphenylisoxazoles, their prodrugs and aza-analogues. *Bioorganic & Medicinal Chemistry,* **1**: 559-576.

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Fifty novel prodrugs and aza-analogues of 3,5-bis (4-amidinophenyl) isoxazole and its derivatives were prepared. Eighteen of the 24 aza-analogues exhibited IC_{50} values below 25nM against *Trypanosoma brucei rhodesiense* or *Plasmodium falciparum*. Six compounds had antitrypanosomal IC_{50} values below 10nM. Twelve analogues showed similar antiplasmodial activities, including three with sub-nanomolar potencies. Forty-four diamidines (including 16 aza-analogues) and the 26 prodrugs were evaluated for efficacy in mice infected with *T. b. rhodesiense* STIB900. Six diamidines cured 4/4 mice at daily 5mg/kg intraperitoneal doses for 4 d, giving results far superior to pentamidine and furamidine. One prodrug attained 3/4 cures at daily 25mg/kg oral doses for 4 d.

16883. **Pizarro, J. C., Hills, T., Senisterra, G., Wernimont, A. K., Mackenzie, C., Norcross, N. R., Ferguson, M. A., Wyatt, P. G., Gilbert, I. H. & Hui, R., 2013.** Exploring the *Trypanosoma brucei* Hsp83 potential as a target for structure guided drug design. *PLoS Neglected Tropical Diseases*, **7** (10): e2492.

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Human African trypanosomiasis is a neglected parasitic disease that is fatal if untreated. The current drugs available to eliminate the causative agent *Trypanosoma brucei* have multiple liabilities, including toxicity, increasing problems due to treatment failure and limited efficacy. There are two approaches to discover novel antimicrobial drugs - whole-cell screening and target-based discovery. In the latter case, there is a need to identify and validate novel drug targets in *Trypanosoma* parasites. The heat shock proteins (Hsp), while best known as cancer targets with a number of drug candidates in clinical development, are a family of emerging targets for infectious diseases. In this paper, we report the exploration of *T. brucei* Hsp83 - a homologue of human Hsp90 - as a drug target using multiple biophysical and biochemical techniques. Our approach included the characterization of the chemical sensitivity of the parasitic chaperone against a library of known Hsp90 inhibitors by means of differential scanning fluorimetry (DSF). Several compounds identified by this screening procedure were further studied using isothermal titration calorimetry (ITC) and X-ray crystallography, as well as tested in parasite growth inhibition assays. These experiments led us to the identification of a benzamide derivative compound capable of interacting with TbHsp83 more strongly than with its human homologues and structural rationalization of this selectivity. The results highlight the opportunities created by subtle structural differences to develop new series of compounds to selectively target the *Trypanosoma brucei* chaperone and effectively kill the sleeping sickness parasite.

16884. **Raber, G., Raber, T., Raml, R., Murko, M., Magnes, C. & Francesconi, K. A., 2013.** Determination of the trypanocidal drug melarsoprol and its conversion products in biological fluids with HPLC-ICPMS/ESMS. *Talanta*, **116**: 876-881.

Institute of Chemistry-Analytical Chemistry, University of Graz, Universitaetsplatz 1, 8010 Graz, Austria. [georg.raber@uni-graz.at].

16885. **Roussaki, M., Hall, B., Lima, S. C., da Silva, A. C., Wilkinson, S. & Detsi, A., 2013.** Synthesis and anti-parasitic activity of a novel quinolinone-chalcone series. *Bioorganic & Medicinal Chemistry Letters*, **23** (23): 6436-6441.

Laboratory of Organic Chemistry, School of Chemical Engineering, National Technical University of Athens, Zografou Campus, 15780 Athens, Greece. [adetsi@chemeng.ntua.gr].

16886. **Schmidt, T. J., Da Costa, F. B., Lopes, N. P., Kaiser, M. & Brun, R., 2013.** *In silico* prediction and experimental evaluation of furanoheliangolide sesquiterpene lactones as potent agents against *Trypanosoma brucei rhodesiense*. *Antimicrobial Agents & Chemotherapy*. **E Publication ahead of print, 28 October.**

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16887. **Thuita, J. K., Wolf, K. K., Murilla, G. A., Liu, Q., Mutuku, J. N., Chen, Y., Bridges, A. S., Mdachi, R. E., Ismail, M. A., Ching, S., Boykin, D. W., Hall, J. E., Tidwell, R. R., Paine, M. F., Brun, R. & Wang, M. Z., 2013.** Safety, pharmacokinetic, and efficacy studies of oral DB868 in a first stage vervet monkey model of human African trypanosomiasis. *PLoS Neglected Tropical Diseases*, **7** (6): e2230.

Trypanosomiasis Research Centre, Kenya Agricultural Research Institute, Kikuyu, Kenya. [michael.wang@ku.edu].

There are no oral drugs for human African trypanosomiasis (HAT, sleeping sickness). A successful oral drug would have the potential to reduce or eliminate the need for patient hospitalization, thus reducing healthcare costs of HAT. The development of oral medications is a key objective of the Consortium for Parasitic Drug Development (CPDD). In this study, we investigated the safety, pharmacokinetics, and efficacy of a new orally administered CPDD diamidine prodrug, 2,5-bis [5-(N-methoxyamidino)-2-pyridyl] furan (DB868; CPD-007-10), in the vervet monkey model of first stage HAT. DB868 was well tolerated at a dose up to 30 mg/kg/d for 10 d, a cumulative dose of 300 mg/kg. Mean plasma levels of biomarkers indicative of liver injury (alanine aminotransferase, aspartate aminotransferase) were not significantly altered by drug administration. In addition, no kidney-mediated alterations in creatinine and urea concentrations were detected. Pharmacokinetic analysis of plasma confirmed that DB868 was orally available and was converted to the active compound DB829 in both uninfected and infected monkeys. Treatment of infected monkeys with DB868 began 7 d post-infection. In the infected monkeys, DB829 attained a median C_{max} (dosing regimen) that was 12-fold (3 mg/kg/d for 7 d), 15-fold (10 mg/kg/d for 7 d), and 31-fold (20

mg/kg/d for 5 d) greater than the IC_{50} (14 nmol/L) against *T. b. rhodesiense* STIB900. DB868 cured all infected monkeys, even at the lowest dose tested. In conclusion, oral DB868 cured monkeys with first stage HAT at a cumulative dose 14-fold lower than the maximum tolerated dose and should be considered a lead preclinical candidate in efforts to develop a safe, short course (5-7 d), oral regimen for first stage HAT.

16888. **Van Reet, N., Pyana, P., Roge, S., Claes, F. & Buscher, P., 2013.** Luminescent multiplex viability assay for *Trypanosoma brucei gambiense*. *Parasites & Vectors*, **6**: 207.

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New compounds for the treatment of human African trypanosomiasis (HAT) are urgently required. *Trypanosoma brucei* (*T. b.*) *gambiense* is the leading cause of HAT, yet *T. b. gambiense* is often not the prime target organism in drug discovery. This may be attributed to the difficulties in handling this subspecies and the lack of an efficient viability assay to monitor drug efficacy. In this study, a *T. b. gambiense* strain, recently isolated in the D.R. Congo, was made bioluminescent by transfection with *Renilla luciferase* (RLuc) without altering its *in vitro* and *in vivo* growth characteristics. A luminescent multiplex viability assay (LMVA), based on measurement of the *Renilla luciferase* activity and the ATP content of the cells within the same experiment, was investigated as an alternative to the standard fluorimetric resazurin viability assay for drug sensitivity testing of *T. b. gambiense*. In a 96-well format, the RLuc transfected strain showed a detection limit of 2×10^4 cells mL^{-1} for the *Renilla luciferase* measurement and 5×10^3 cells mL^{-1} for the ATP measurement. Both assays of the LMVA showed linearity up to 10^6 cells mL^{-1} and correlated well with the cell density during exponential growth of the long slender bloodstream forms. The LMVA was compared with the fluorimetric resazurin viability assay for drug sensitivity testing of pentamidine, eflornithine, nifurtimox and melarsoprol with both the wild type and the RLuc transfected population. For each drug, the IC_{50} value of the RLuc population was similar to that of the wild type when determined with either the fluorimetric resazurin method or the LMVA. For eflornithine, nifurtimox and melarsoprol we found no difference between the IC_{50} values in both viability assays. In contrast, the IC_{50} value of pentamidine was higher when determined with the fluorimetric resazurin method than in both assays of the LMVA. In conclusion, LMVA has some advantages for viability measurement of *T. b. gambiense*: it requires less incubation time for viability detection than the fluorimetric resazurin assay and in LMVA, two sensitive and independent viability assays are performed in the same experiment.

16889**. Vlachakis, D., Pavlopoulou, A., Roubelakis, M. G., Feidakis, C., Anagnou, N. P. & Kossida, S., 2013.** 3D molecular modelling and evolutionary study of the *Trypanosoma brucei* DNA topoisomerase IB, as a new emerging pharmacological target. *Genomics*. **E Publication ahead of print, 5 December.**

Bioinformatics & Medical Informatics Team, Biomedical Research Foundation, Academy of Athens, Soranou Efessiou 4, Athens 11527, Greece. [skossida@bioacademy.gr].

16890. **Vodnala, S. K., Lundback, T., Yeheskieli, E., Sjoberg, B., Gustavsson, A. L., Svensson, R., Olivera, G. C., Eze, A. A., de Koning, H. P., Hammarstrom, L. G. & Rottenberg, M. E., 2013.** Structure-activity relationships of synthetic cordycepin analogues as experimental therapeutics for African trypanosomiasis. *Journal of Medicinal Chemistry,* **56** (24): 9861-9873.

Department of Microbiology, Tumor and Cell Biology and Division of Translational Medicine and Chemical Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden. [Martin.Rottenberg@ki.se].

16891. **Wenzler, T., Yang, S., Braissant, O., Boykin, D. W., Brun, R. & Wang, M. Z., 2013.** Pharmacokinetics, *Trypanosoma brucei gambiense* efficacy, and time of drug action of DB829, a preclinical candidate for treatment of second-stage human African trypanosomiasis. *Antimicrobial Agents & Chemotherapy*, **57** (11): 5330-5343.

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Human African trypanosomiasis (HAT, also called sleeping sickness), a neglected tropical disease endemic to sub-Saharan Africa, is caused by the parasites *Trypanosoma brucei gambiense* and *T. brucei rhodesiense*. Current drugs against this disease have significant limitations, including toxicity, increasing resistance, and/or a complicated parenteral treatment regimen. DB829 is a novel aza-diamidine that demonstrated excellent efficacy in mice infected with *T. b. rhodesiense* or *T. b. brucei* parasites. The current study examined the pharmacokinetics, *in vitro* and *in vivo* activity against *T. b. gambiense*, and time of drug action of DB829 in comparison to pentamidine. DB829 showed outstanding *in vivo* efficacy in mice infected with parasites of *T. b. gambiense* strains, despite having higher *in vitro* 50 percent inhibitory concentrations (IC_{50}s) than against *T. b. rhodesiense* strain STIB900. A single dose of DB829 administered intraperitoneally (5 mg/kg b.w.) cured all mice infected with different *T. b. gambiense* strains. No cross-resistance was observed between DB829 and pentamidine in *T. b. gambiense* strains isolated from melarsoprol-refractory patients. Compared with pentamidine, DB829 showed a greater systemic exposure when administered intraperitoneally, partially contributing to its improved efficacy. Isothermal microcalorimetry and *in vivo* time-to-kill studies revealed that DB829 is a slower-acting trypanocidal compound than pentamidine. A single dose of DB829 (20 mg/kg) administered intraperitoneally clears parasites from mouse blood within 2 to 5 d. In summary, DB829 is a promising preclinical candidate for the treatment of first- and second-stage HAT caused by both *Trypanosoma brucei* subspecies.

16892. **Woodring, J. L., Bland, N. D., Ochiana, S. O., Campbell, R. K. & Pollastri, M. P., 2013.** Synthesis and assessment of catechol diether compounds as inhibitors of trypanosomal phosphodiesterase B1 (TbrPDEB1). *Bioorganic & Medicinal Chemistry Letters*, **23** (21): 5971-5974.

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

16893. **Wring, S., Gaukel, E., Nare, B., Jacobs, R., Beaudet, B., Bowling, T., Mercer, L., Bacchi, C., Yarlett, N., Randolph, R., Parham, R., Rewerts, C., Platner, J. & Don, R., 2014.** Pharmacokinetics and pharmacodynamics utilizing unbound target tissue exposure as part of a disposition-based rationale for lead optimization of benzoxaboroles in the treatment of stage 2 human African trypanosomiasis. *Parasitology,* **141** (Special Issue 1): 14-118.

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This review presents a progression strategy for the discovery of new anti-parasitic drugs that uses *in vitro* susceptibility, time-kill and reversibility measures to define the therapeutically relevant exposure required in target tissues of animal infection models. The strategy is exemplified by the discovery of SCYX-7158 as a potential oral treatment for stage 2 (CNS) human African trypanosomiasis (HAT). A critique of current treatments for stage 2 HAT is included to provide context for the challenges of achieving target tissue disposition and the need for establishing pharmacokinetic-pharmacodynamic (PK-PD) measures early in the discovery paradigm. The strategy comprises 3 stages. Initially, compounds demonstrating promising *in vitro* activity and selectivity for the target organism over mammalian cells are advanced to *in vitro* metabolic stability, barrier permeability and tissue binding assays to establish that they will likely achieve and maintain therapeutic concentrations during *in-vivo* efficacy studies. Secondly, *in vitro* time-kill and reversibility kinetics are employed to correlate exposure (based on unbound concentrations) with *in vitro* activity, and to identify pharmacodynamic measures that would best predict efficacy. Lastly, this information is used to design dosing regimens for pivotal pharmacokinetic-pharmacodyamic studies in animal infection models.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY, CHARACTERISATION OF ISOLATES

[See also **36**: 16897]

16894. **Acosta, I. D., da Costa, A. P., Nunes, P. H., Gondim, M. F., Gatti, A., Rossi Jr, J. L., Gennari, S. M. & Marcili, A., 2013.** Morphological and molecular characterization and phylogenetic relationships of a new species of trypanosome in *Tapirus terrestris* (lowland tapir), *Trypanosoma teixeirae* sp. nov., from the Atlantic rainforest of southeastern Brazil. *Parasites & Vectors*, **6** (1): 349.

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The Lowland tapir (*Tapirus terrestris*) is the largest Brazilian mammal and despite

being distributed in various Brazilian biomes, it is seriously endangered in the Atlantic rainforest. These hosts were never evaluated for the presence of parasites. Lowland tapirs were captured in the Brazilian southeastern Atlantic rainforest, Espirito Santo state. Trypanosomes were isolated by haemoculture, and the molecular phylogeny based on small subunit rDNA (SSU rDNA) and glycosomal-3-phosphate dehydrogenase (gGAPDH) gene sequences and the ultrastructural features seen via light microscopy and scanning and transmission electron microscopy are described. Phylogenetic trees using combined SSU rDNA and gGAPDH data sets clustered the trypanosomes of Lowland tapirs, which were highly divergent from other trypanosome species. The phylogenetic position and morphological discontinuities, mainly in epimastigote culture forms, made it possible to classify the trypanosomes from Lowland tapirs as a separate species. The isolated trypanosomes from *Tapirus terrestris* are a new species, *Trypanosoma teixeirae* sp. n., and were positioned in a new a clade, named *T. teixeirae* clade.

16895. **Duffy, C. W., Maclean, L., Sweeney, L., Cooper, A., Turner, C. M., Tait, A., Sternberg, J., Morrison, L. J. & Macleod, A., 2013.** Population genetics of *Trypanosoma brucei rhodesiense*: clonality and diversity within and between foci. *PLoS Neglected Tropical Diseases*, **7** (11): e2526.

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African trypanosomes are unusual among pathogenic protozoa in that they can undergo their complete morphological life cycle in the tsetse fly vector with mating as a non-obligatory part of this development. *Trypanosoma brucei rhodesiense*, which infects humans and livestock in East and Southern Africa, has classically been described as a host-range variant of the non-human infective *Trypanosoma brucei* that occurs as stable clonal lineages. We have examined *T. b. rhodesiense* populations from East (Uganda) and Southern (Malawi) Africa using a panel of microsatellite markers, incorporating both spatial and temporal analyses. Our data demonstrate that Ugandan *T. b. rhodesiense* existed as clonal populations, with a small number of highly related genotypes and substantial linkage disequilibrium between pairs of loci. However, these populations were not stable as the dominant genotypes changed and the genetic diversity also reduced over time. Thus these populations do not conform to one of the criteria for strict clonality, namely stability of predominant genotypes over time, and our results show that, in a period in the mid-1990s, the previously predominant genotypes were not detected but were replaced by a novel clonal population with limited genetic relationship to the original population present between 1970 and 1990. In contrast, the Malawi *T. b. rhodesiense* population demonstrated significantly greater diversity and evidence for frequent genetic exchange. Therefore, the population genetics of *T. b. rhodesiense* is more complex than previously described. This has important implications for the spread of the single copy *T. b. rhodesiense* gene that allows human infectivity, and therefore the epidemiology of the human disease, as well as suggesting that these parasites represent an important organism to study the influence of optional recombination upon population genetic dynamics.

16896. **Goodhead, I., Capewell, P., Bailey, J. W., Beament, T., Chance, M., Kay, S., Forrester, S., MacLeod, A., Taylor, M., Noyes, H. & Hall, N., 2013.** Whole-

genome sequencing of *Trypanosoma brucei* reveals introgression between subspecies that is associated with virulence. *MBio*, **4:** 4.

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Human African trypanosomiasis is caused by two subspecies of *Trypanosoma brucei*. *Trypanosoma brucei rhodesiense* is found in East Africa and frequently causes acute disease, while *Trypanosoma brucei gambiense* is found in West Africa and is associated with chronic disease. Samples taken from a single focus of a Ugandan outbreak of *T. b. rhodesiense* in the 1980s were associated with either chronic or acute disease. We sequenced the whole genomes of two of these isolates, which showed that they are genetically distinct from each other. Analysis of single nucleotide polymorphism markers in a panel of 31 Ugandan isolates plus 32 controls revealed a mixture of East African and West African haplotypes, and some of these haplotypes were associated with the different virulence phenotypes. It has been shown recently that *T. b. brucei* and *T. b. rhodesiense* populations undergo genetic exchange in natural populations. Our analysis showed that these strains from the Ugandan epidemic were intermediate between the reference genome sequences of *T. b. gambiense* and *T. b. brucei* and contained haplotypes that were present in both subspecies. This suggests that the human-infective subspecies of *T. brucei* are not genetically isolated, and our data are consistent with genomic introgression between East African and West African *T. b. brucei* subspecies. This has implications for the control of the parasite, the spread of drug resistance, and understanding the variation in virulence and the emergence of human infectivity.

16897. **Graf, F. E., Ludin, P., Wenzler, T., Kaiser, M., Brun, R., Pyana, P. P., Buscher, P., de Koning, H. P., Horn, D. & Maser, P., 2013.** Aquaporin 2 mutations in *Trypanosoma brucei gambiense* field isolates correlate with decreased susceptibility to pentamidine and melarsoprol. *PLoS Neglected Tropical Diseases*, **7** (10): e2475.

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The predominant mechanism of drug resistance in African trypanosomes is decreased drug uptake due to loss-of-function mutations in the genes for the transporters that mediate drug import. The role of transporters as determinants of drug susceptibility is well documented from laboratory-selected *Trypanosoma brucei* mutants. But clinical isolates, especially of *T. b. gambiense*, are less amenable to experimental investigation since they do not readily grow in culture without prior adaptation. Here we analyse a selected panel of 16 *T. brucei* ssp. field isolates that (i) have been adapted to axenic *in vitro* cultivation and (ii) mostly stem from treatment-refractory cases. For each isolate, we quantify the sensitivity to melarsoprol, pentamidine, and diminazene, and sequence the genomic loci of the transporter genes TbAT1 and TbAQP2. The former encodes the well-characterized aminopurine permease P2 which transports several trypanocides including melarsoprol, pentamidine, and diminazene. We find that diminazene-resistant field isolates of *T. b. brucei* and *T. b. rhodesiense* carry the same set of point mutations in TbAT1 that was previously described from lab. mutants. Aquaglyceroporin 2 has only recently been identified as a second transporter involved in melarsoprol/pentamidine cross-resistance. Here we describe two different kinds of TbAQP2 mutations found in *T. b. gambiense* field isolates: simple loss of

TbAQP2, or loss of wild-type TbAQP2 allele combined with the formation of a novel type of TbAQP2/3 chimera. The identified mutant *T. b. gambiense* are 40- to 50-fold less sensitive to pentamidine and 3- to 5-times less sensitive to melarsoprol than the reference isolates. We thus demonstrate for the first time that rearrangements of the TbAQP2/TbAQP3 locus accompanied by TbAQP2 gene loss also occur in the field, and that the *T. b. gambiense* carrying such mutations correlate with a significantly reduced susceptibility to pentamidine and melarsoprol.

16898**. Kabore, J., De Meeus, T., Macleod, A., Ilboudo, H., Capewell, P., Camara, M., Gaston Belem, A. M., Bucheton, B. & Jamonneau, V., 2013.** A protocol to improve genotyping of problematic microsatellite loci of *Trypanosoma brucei gambiense* from body fluids. *Infection, Genetics & Evolution*, **20**: 171-176.

Centre International de Recherche-Développement sur l'Elevage en zones Subhumides (CIRDES), Unité de Recherches sur les Bases Biologiques de la Lutte Intégrée, 01 BP 454 Bobo-Dioulasso 01, Burkina Faso; Université Polytechnique de Bobo-Dioulasso, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso. [jacqueskabore@yahoo.fr].

Microsatellite genotyping of *Trypanosoma brucei gambiense*, the causative agent of human African trypanosomiasis or sleeping sickness, and population genetics tools, are useful for inferring population parameters such as population size and dispersal. Amplifying parasite DNA directly from body fluids (i.e. blood, lymph or cerebrospinal fluid) allows avoiding costly and tedious isolation phases. It is, however, associated with increased frequencies of amplification failures (allelic dropouts and/or null alleles) at some loci. In this paper, we present a study focused on three *T. brucei gambiense* microsatellite loci suspected to present amplification problems when amplified from body fluids sampled in Guinean sleeping sickness foci. We checked for the real nature of blank and apparent homozygous genotypes of parasite DNA directly amplified from body fluids and tested the effect of three different DNA quantities of trypanosomes. Our results show that some initially blank and homozygous genotypes happen to be actual heterozygous genotypes. In Guinea, lymph from the cervical lymph nodes, known to contain the highest concentrations of parasites, appeared to provide the best amplification results. Simply repeating the PCR may be enough to retrieve the correct genotype, but we also show that increasing initial DNA content provides better results while undertaking first amplification. We finally propose an optimal protocol for amplifying trypanosome's DNA directly from body fluids that should be adapted to local characteristics and/or constraints.

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

[See also **36**: 16773, 16841, 16842, 16843, 16844, 16845, 16849, 16856, 16863, 16872, 16878, 16883, 16892]

16899. **Achcar, F., Barrett, M. P. & Breitling, R., 2013.** Explicit consideration of topological and parameter uncertainty gives new insights into a well-established model of glycolysis. *FEBS Journal*, **280** (18): 4640-4651.

Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK. [rainer.breitling@manchester.ac.uk].

16900. **Adeyemi, O. S. & Whiteley, C. G., 2013.** Interaction of nanoparticles with arginine kinase from *Trypanosoma brucei*: kinetic and mechanistic evaluation. *International Journal of Biological Macromolecules*, **62**: 450-456.

Department of Biochemistry, Microbiology & Biotechnology Rhodes University, Grahamstown, South Africa. [C.Whiteley@ru.ac.za].

16901. **Ammar, Z., Plazolles, N., Baltz, T. & Coustou, V., 2013.** Identification of trans-sialidases as a common mediator of endothelial cell activation by African trypanosomes. *PLoS Pathogens*, **9** (10): e1003710.

French National Centre for Scientific Research (CNRS), Université Bordeaux Segalen, Microbiologie Fondamentale et Pathogénicité, UMR 5234, Bordeaux, France. [virginie.coustou@u-bordeaux2.fr].

Understanding African trypanosomiasis (AT) host-pathogen interaction is the key to an "anti-disease vaccine", a novel strategy to control HAT. Here we provide a better insight into this poorly described interaction by characterizing the activation of a panel of endothelial cells by bloodstream forms of four African trypanosome species, known to interact with host endothelium. *T. congolense*, *T. vivax*, and *T. b. gambiense* activated the endothelial NF-kappaB pathway, but interestingly, not *T. b. brucei*. The parasitic TS (trans-sialidases) mediated this NF-kappaB activation, remarkably via their lectin-like domain and induced production of pro-inflammatory molecules not only *in vitro* but also *in vivo*, suggesting a considerable impact on pathogenesis. For the first time, TS activity was identified in *T. b. gambiense* BSF which distinguishes it from the subspecies *T. b. brucei*. The corresponding TS were characterized and shown to activate endothelial cells, suggesting that TS represent a common mediator of endothelium activation among trypanosome species with divergent physiopathologies.

16902. **Ammerman, M. L., Tomasello, D. L., Faktorova, D., Kafkova, L., Hashimi, H., Lukes, J. & Read, L. K., 2013.** A core MRB1 complex component is indispensable for RNA editing in insect and human infective stages of *Trypanosoma brucei*. *PLoS One*, **8** (10): e78015.

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

16903. **Andre, J., Harrison, S., Towers, K., Qi, X., Vaughan, S., McKean, P. G. & Ginger, M. L., 2013.** The tubulin cofactor C family member TBCCD1 orchestrates cytoskeletal filament formation. *Journal of Cell Science*, **126** (23): 5350-5356.

Faculty of Health and Medicine, Division of Biomedical and Life Sciences, Lancaster University, Lancaster LA1 4YQ, UK. [m.ginger@lancaster.ac.uk].

16904. **Andre, J., Kerry, L., Qi, X., Hawkins, E., Drizyte, K., Ginger, M. L. & McKean, P. G., 2013.** An alternative model for the role of RP2 in flagellum assembly in the

African trypanosome. *Journal of Biological Chemistry*. **E Publication ahead of print, 20 November.**

Faculty of Health and Medicine, Biomedical and Life Sciences, Lancaster University, Lancaster LA1 4YQ, UK. [m.ginger@lancaster.ac.uk].

16905. **Aphasizheva, I., Maslov, D. A. & Aphasizhev, R., 2013.** Kinetoplast DNA-encoded ribosomal protein S12: a possible functional link between mitochondrial RNA editing and translation in *Trypanosoma brucei*. *RNA Biology*, **10** (11).

Department of Molecular and Cell Biology; Boston University Goldman School of Dental Medicine, Boston, MA., USA. [maslov@ucr.edu].

16906. **Bauer, S., Morris, J. C. & Morris, M. T., 2013.** Environmentally regulated glycosome protein composition in the African trypanosome. *Eukaryotic Cell*, **12** (8): 1072-1079.

Department of Genetics and Biochemistry, Clemson University, Clemson, South Carolina, USA. [mmorri3@clemson.edu].

16907**. Boynak, N. Y., Rojas, F., D'Alessio, C., Vilchez Larrea, S. C., Rodriguez, V., Ghiringhelli, P. D. & Tellez-Inon, M. T., 2013.** Identification of a Wee1-like kinase gene essential for procyclic *Trypanosoma brucei* survival. *PLoS One*, **8** (11): e79364.

Instituto de Investigaciones en Ingenieria Genetica y Biologia Molecular "Dr. Hector N. Torres", (INGEBI-CONICET), Buenos Aires, Argentina. [mtellez@dna.uba.ar].

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The surface of *Trypanosoma brucei* is covered by a dense coat of glycosylphosphatidylinositol-anchored glycoproteins. The major component is the variant surface glycoprotein (VSG) which is glycosylated by both paucimannose and oligomannose N-glycans. Surface glycans are poorly accessible and killing mediated by peptide lectin-VSG

complexes is hindered by active endocytosis. However, contrary to previous observations, here we show that high-affinity carbohydrate binding agents bind to surface glycoproteins and abrogate growth of *T. brucei* bloodstream forms. Specifically, binding of the mannose-specific *Hippeastrum* hybrid agglutinin (HHA) resulted in profound perturbations in endocytosis and parasite lysis. Prolonged exposure to HHA led to the loss of triantennary oligomannose structures in surface glycoproteins as a result of genetic rearrangements that abolished expression of the oligosaccharyltransferase TbSTT3B gene and yielded novel chimeric enzymes. Mutant parasites exhibited markedly reduced infectivity thus demonstrating the importance of specific glycosylation patterns in parasite virulence.

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Institut fur Anatomie und Zellbiologie, Giessen, 35392, Germany. [cclayton@zmbh.uni-heidelberg.de].

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Selenium (Se) is an essential trace element for several organisms and is present in proteins as selenocysteine (Sec or U), an amino acid that is chemically distinct from serine and cysteine by a single atom (Se instead of O or S, respectively). Sec is incorporated into selenoproteins at an in-frame UGA codon specified by an mRNA stem-loop structure called the selenocysteine incorporating sequence (SECIS) presented in selenoprotein mRNA and specific selenocysteine synthesis and incorporation machinery. Selenoproteins are presented in all domains but are not found in all organisms. Although several functions have been

attributed to this class, the majority of the proteins are involved in oxidative stress defence. Here, we discuss the kinetoplastid selenocysteine pathway and how selenium supplementation is able to alter the infection course of trypanosomatids in detail. These organisms possess the canonical elements required for selenoprotein production such as phosphoseryl tRNA kinase (PSTK), selenocysteine synthase (SepSecS), selenophosphase synthase (SelD or SPS), and elongation factor EFSec (SelB), whereas other important factors presented in mammal cells, such as SECIS binding protein 2 (SBP) and SecP 43, are absent. The selenoproteome of trypanosomatids is small, as is the selenoproteome of others parasites, which is in contrast to the large number of selenoproteins found in bacteria, aquatic organisms and higher eukaryotes. *Trypanosoma* and *Leishmania* are sensitive to auranofin, a potent selenoprotein inhibitor; however, the probable drug mechanism is not related to selenoproteins in kinetoplastids. Selenium supplementation decreases the parasitaemia of various trypanosome infections and reduces important parameters associated with diseases such as anaemia and parasite-induced organ damage. New experiments are necessary to determine how selenium acts, but evidence suggests that immune response modulation and increased host defence against oxidative stress contribute to control of the parasite infection.

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In order to promote infection, the blood-borne parasite *Trypanosoma brucei* releases factors that upregulate arginase expression and activity in myeloid cells. By screening a cDNA library of *T. brucei* with an antibody neutralizing the arginase-inducing activity of parasite released factors, we identified a kinesin heavy chain isoform, termed TbKHC1, as responsible for this effect. Following interaction with mouse myeloid cells, natural or recombinant TbKHC1 triggered SIGN-R1 receptor-dependent induction of IL-10 production, resulting in arginase-1 activation concomitant with reduction of nitric oxide (NO) synthase activity. This TbKHC1 activity was IL-4Ralpha-independent and did not mirror M2 activation of myeloid cells. As compared with wild-type *T. brucei*, infection by TbKHC1 KO parasites was characterized by strongly reduced parasitaemia and prolonged host survival time. By treating infected mice with ornithine or with NO synthase inhibitor, we observed that during the first wave of parasitaemia the parasite growth-promoting effect of TbKHC1-mediated arginase activation resulted more from increased polyamine production than from reduction of NO synthesis. In late stage infection, TbKHC1-mediated reduction of NO synthesis appeared to contribute to liver damage linked to shortening of host survival time. In conclusion, a kinesin heavy chain released by *T. brucei* induces IL-10 and arginase-1 through SIGN-R1 signalling in myeloid cells, which promotes early trypanosome growth and favours parasite settlement in the host. Moreover, in the late stage of infection, the inhibition of NO synthesis by TbKHC1 contributes to liver pathogenicity.

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Rhodesain, a cathepsin L-like cysteine protease of *T. brucei rhodesiense*, is considered a potential target for the treatment of human African trypanosomiasis. Recent findings have confirmed that rhodesain, a lysosomal protease, is essential for parasite survival. Rhodesain is required by *T. brucei* to cross the blood-brain barrier, degrade host immunoglobulins, and turn over variant surface coat glycoproteins of *T. brucei*, which impair effective host immune responses. In this perspective, we discuss the main classes of rhodesain inhibitors, including peptidic, peptidomimetic, and nonpeptidic structures, emphasizing those that have exhibited an optimal match between enzymatic affinity and trypanocidal profile and those for which preclinical investigations are currently in progress.

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The protozoan parasites *Trypanosoma brucei* spp. cause important human and livestock diseases in sub-Saharan Africa. In mammalian blood, two developmental forms of the parasite exist: proliferative “slender” forms and arrested “stumpy” forms that are responsible for transmission to tsetse flies. The slender to stumpy differentiation is a density-dependent response that resembles quorum sensing in microbial systems and is crucial for the parasite life cycle, ensuring both infection chronicity and disease transmission. This response is triggered by an elusive “stumpy induction factor” (SIF) whose intracellular signalling pathway is also uncharacterized. Laboratory-adapted (monomorphic) trypanosome strains respond inefficiently to SIF but can generate forms with stumpy characteristics when exposed

to cell-permeable cAMP and AMP analogues. Exploiting this, we have used a genome-wide RNA interference library screen to identify the signalling components driving stumpy formation. In separate screens, monomorphic parasites were exposed to 8-(4-chlorophenylthio)-cAMP (pCPT-cAMP) or 8-pCPT-2'-O-methyl-5'-AMP to select cells that were unresponsive to these signals and hence remained proliferative. Genome-wide ion torrent based RNAi target sequencing identified cohorts of genes implicated in each step of the signalling pathway, from purine metabolism, through signal transducers (kinases, phosphatases) to gene expression regulators. Genes at each step were independently validated in cells naturally capable of stumpy formation, confirming their role in density sensing *in vivo*. The putative RNA-binding protein, RBP7, was required for normal quorum sensing and promoted cell-cycle arrest and transmission competence when overexpressed. This study reveals that quorum sensing signalling in trypanosomes shares similarities to fundamental quiescence pathways in eukaryotic cells, its components providing targets for quorum-sensing interference-based therapeutics.

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Trypanosoma brucei drug transporters include the TbAT1/P2 aminopurine transporter and the high-affinity pentamidine transporter (HAPT1), but the genetic identity of HAPT1 is unknown. We recently reported that loss of *T. brucei* aquaglyceroporin 2 (TbAQP2) caused melarsoprol/pentamidine cross-resistance (MPXR) in these parasites and the current study aims to delineate the mechanism by which this occurs. The TbAQP2 loci of isogenic pairs of drug-susceptible and MPXR strains of *T. brucei* subspecies were sequenced. Drug susceptibility profiles of trypanosome strains were correlated with expression of mutated TbAQP2 alleles. Pentamidine transport was studied in *T. brucei* subspecies expressing TbAQP2 variants. All MPXR strains examined contained TbAQP2 deletions or rearrangements, regardless of whether the strains were originally adapted *in vitro* or *in vivo* to arsenicals or to pentamidine. The MPXR strains and AQP2 knockout strains had lost HAPT1 activity. Reintroduction of TbAQP2 in MPXR trypanosomes restored susceptibility to the drugs and reinstated HAPT1 activity, but did not change the activity of TbAT1/P2. Expression of TbAQP2 sensitized *Leishmania mexicana* promastigotes 40-fold to

pentamidine and > 1 000-fold to melaminophenyl arsenicals and induced a high-affinity pentamidine transport activity indistinguishable from HAPT1 by K_m and inhibitor profile. Grafting the TbAQP2 selectivity filter amino acid residues onto a chimeric allele of AQP2 and AQP3 partly restored susceptibility to pentamidine and an arsenical. It is concluded that TbAQP2 mediates high-affinity uptake of pentamidine and melaminophenyl arsenicals in trypanosomes and TbAQP2 encodes the previously reported HAPT1 activity. This finding establishes TbAQP2 as an important drug transporter.

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T. brucei, the causative parasite for African trypanosomiasis, faces an interesting dilemma in its life cycle. It has to successfully complete its infection cycle in the tsetse vector to be able to infect other vertebrate hosts. *T. brucei* has to undergo multiple morphological changes as it invades the alimentary canal of the tsetse to finally achieve infectivity in the salivary glands. In this review, we attempt to elucidate how these morphological changes are possible for a parasite that has evolved a highly robust cell structure to survive the chemically and physically diverse environments it finds itself in. To achieve this, we juxtaposed the experimental evidence that has been collected from *T. brucei* forms that are cultured *in vitro* with the observations that have been carried out on tsetse-infective forms *in vivo*. Although the accumulated knowledge on *T. brucei* biology is by no means trivial, several outstanding

questions remain about how the parasite mechanistically changes its morphology as it traverses the tsetse and how those changes are triggered. However, we conclude that with recent breakthroughs allowing for the replication of the tsetse-infection process of *T. brucei in vitro*, these outstanding questions can finally be addressed.

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African trypanosomes are capable of both *de novo* synthesis and salvage of pyrimidines. The last two steps in *de novo* synthesis are catalysed by UMP synthase (UMPS) - a bifunctional enzyme comprising orotate phosphoribosyl transferase (OPRT) and orotidine monophosphate decarboxylase (OMPDC). To investigate the essentiality of pyrimidine biosynthesis in *Trypanosoma brucei*, we generated a umps double knockout (DKO) line by gene replacement. The DKO was unable to grow in pyrimidine-depleted medium *in vitro*, unless supplemented with uracil, uridine, deoxyuridine or UMP. DKO parasites were completely resistant to 5-fluoroorotate and hypersensitive to 5-fluorouracil, consistent with loss of UMPS, but remained sensitive to pyrazofurin indicating that, unlike mammalian cells, the primary target of pyrazofurin is not OMPDC. The null mutant was unable to infect mice indicating that salvage of host pyrimidines is insufficient to support growth. However, following prolonged culture *in vitro*, parasites regained virulence in mice despite retaining pyrimidine auxotrophy. Unlike the wild-type, both pyrimidine auxotrophs secreted substantial quantities of orotate, significantly higher in the virulent DKO line. We propose that this may be responsible for the recovery of virulence in mice, due to host metabolism converting orotate to uridine, thereby bypassing the loss of UMPS in the parasite.

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It is well known that an amino acid can be encoded by more than one codon, called synonymous codons. The preferential use of one particular codon for coding an amino acid is referred to as codon usage bias (CUB). A quantitative analytical method, CUB and a related tool, codon adaptative index (CAI) have been applied to comparatively study whole genomes of a few pathogenic Trypanosomatid species. This quantitative attempt is of direct help in the comparison of qualitative features like mutational and translational selection. Pathogens of the *Leishmania* and *Trypanosoma* genus cause debilitating diseases and suffering in human beings and animals. Of these, whole genome sequences are available for only five species. The complete coding sequences (CDS), highly expressed, essential and low expressed genes have all been studied for their CUB signature. The codon usage bias of essential genes and highly expressed genes show distribution similar to codon usage bias of all CDSs in Trypanosomatids. Translational selection is the dominant force selecting the preferred codon, and selection due to mutation is negligible. In contrast to an earlier study done on these pathogens, it is found in this work that CUB and CAI may be used to distinguish the Trypanosomatid genomes at the sub-genus level. Further, CUB may effectively be used as a signature of the species differentiation by using principal component analysis (PCA).

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African trypanosomes cause disease in humans and livestock, generating significant health and welfare problems throughout sub-Saharan Africa. When ingested in a tsetse fly bloodmeal, trypanosomes must detect their new environment and initiate the developmental responses that ensure transmission. The best-established environmental signal is citrate/cis aconitate (CCA), this being transmitted through a protein phosphorylation cascade involving two phosphatases: one that inhibits differentiation (TbPTP1) and one that activates differentiation (TbPIP39). Other cues have been also proposed (mild acid, trypsin exposure, glucose depletion) but their physiological relevance and relationship to TbPTP1/TbPIP39 signalling is unknown. Here we demonstrate that mild acid and CCA operate through TbPIP39 phosphorylation, whereas trypsin attack of the parasite surface uses an alternative pathway that is dispensable in tsetse flies. Surprisingly, glucose depletion is not an important signal. Mechanistic analysis through biophysical methods suggests that citrate promotes differentiation by causing TbPTP1 and TbPIP39 to interact.

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Nuclear DNA replication is, arguably, the central cellular process in eukaryotes, because it drives propagation of life and intersects with many other genome reactions. Perhaps surprisingly, our understanding of nuclear DNA replication in kinetoplastids was limited until a clutch of studies emerged recently, revealing new insights into both the machinery and genome-wide coordination of the reaction. Here, we discuss how these studies suggest that the earliest acting components of the kinetoplastid nuclear DNA replication machinery - the factors that demarcate sites of the replication initiation, termed origins - are diverged from model eukaryotes. In addition, we discuss how origin usage and replication dynamics relate to the highly unusual organisation of transcription in the genome of *Trypanosoma brucei*.

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