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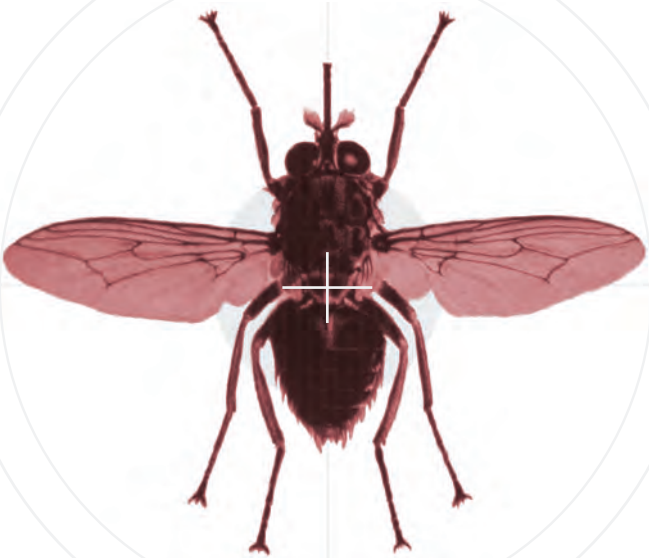

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TSETSE AND TRYPANOSOMOSIS INFORMATION

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TSETSE AND TRYPANOSOMOSIS INFORMATION

The Tsetse and Trypanosomosis Information periodical has been established to disseminate current information on all aspects of tsetse and trypanosomosis research and control to institutions and individuals involved in the problems of African trypanosomosis. This service forms an integral part of the Programme Against African Trypanosomosis (PAAT) and is jointly sponsored by the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA), the Inter-African Bureau for Animal Resources of the African Union (AU-IBAR), the World Health Organization (WHO), the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) and the Institut de recherche pour le développement (IRD).

The half-yearly periodical is prepared for publication, in both English and French editions, by the Food and Agriculture Organization of the United Nations. Each annual volume consists of two parts. Subscription is free for all recipients engaged in trypanosomosis research and control, and requests for enrolment may be sent to: Ms Maria Grazia Solari, AGAH, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy (fax +39 06 5705 5749; e-mail MariaGrazia.Solari@fao.org).

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

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

Distribution dates and copy deadlines

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

ABBREVIATIONS USED IN *TTI*

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

Organizations

ANDE	Agence Nationale de Développement de l'Elevage
AU	African Union
AU/STRC	African Union/Scientific, Technical and Research Commission
BICOT	Biological Control of Tsetse by the Sterile Insect Technique
CEBV	Communauté Economique du Bétail et de la Viande
CEMV	Centre Universitaire de Formation en Entomologie Médicale et Vétérinaire
CGIAR	Consultative Group on International Agricultural Research
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CIRAD-EMVT	Département d'Elevage et de Médecine Vétérinaire des Pays Tropicaux du CIRAD
CIRDES	Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide
CNERV	Centre National d'Elevage et de Recherches Vétérinaires
CNRS	Centre National de Recherche Scientifique
CREAT	Centre de Recherche et d'Elevage, Avétonou, Togo
CRSSA	Centre de Recherches du Service de Santé des Armées Emile Pardé
CTVM	Centre for Tropical Veterinary Medicine
DFID	Department for International Development (UK)
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

CONTENTS

SECTION A – NEWS

OBITUARY: PROFESSOR ALBERT ADEOYE ILEMOBADE

This obituary was kindly provided to TTI by Professor Peter Holmes, University of Glasgow, UK, and former Chair of the FAO/WHO/IAEA/AU-IBAR Programme Against African Trypanosomiasis (PAAT).

The tragic death has recently been announced of Professor Albert Adeoye Ilemobade. Prof. Ilemobade was one of Africa's leading veterinary parasitologists and current chairman of PAAT.

Albert Ilemobade was born on 12th April 1936. He received his primary and secondary education in Ondo State. He obtained the joint DVM degree from the Universities of Ibadan and Ahmadu Bello in 1969 and was one of the first of Nigerians to be employed as a lecturer in the Faculty of Veterinary Medicine at Ahmadu Bello University, Zaria. He was subsequently awarded a Masters degree at the Oklahoma State University and a PhD by Ahmadu Bello University.

At Ahmadu Bello University he rose through the ranks, becoming a Professor of Parasitology and Entomology in October 1980. He served in different academic and administrative capacities at that university before his translation in 1983 to the newly established Federal University of Technology, at Akure – first as Head of Department of Animal Production and Health, and subsequently as Dean of the School of Agriculture and Agricultural Technology. He was to become the first Deputy Vice-Chancellor of the University in 1987 and in November 1988, he was appointed Vice-Chancellor, a position he held until January 1996, after serving two terms.

In his academic career, apart from teaching undergraduate students and guiding numerous graduate students, Professor Ilemobade carried out extensive veterinary research which earned him travel grants and fellowships to the USA and Europe. For his work on blood parasitic diseases of animals he became widely recognised as a leading veterinary scientist both nationally and internationally. He served as an Expert Consultant in blood parasitic diseases of livestock to the Federal Government of Nigeria, FAO, IAEA, WHO and AU-IBAR. He served as President of the ISCTRC. He was a member of the board of the Programme Against African Trypanosomiasis (PAAT) from its inception in 1997 and was chairman from 2002. He was also a member of the Policy and Mobilization Committee of the Pan African Tsetse and Trypanosomiasis Campaign (PATTEC) of the African Union.

In addition to these international responsibilities, Professor Ilemobade was highly influential in many social and religious activities in Nigerian society. Until his recent death, Professor Ilemobade was the President and Chief Executive of Upline Resources Foundation, a non-profit, registered NGO based in Akure. Professor Ilemobade also served on numerous councils and foundations including the Governing Councils of Adekunle Ajasin University, Akungba Akoko; the Federal Polytechnic, Ado-Ekiti; and the Nigerian Institute of Trypanosomiasis Research, Kaduna.

Professor Ilemobade was the recipient of many honours and awards including Foundation Fellow of the College of Veterinary Surgeons of Nigeria and the Pfizer Animal Health award in recognition of outstanding contributions to the advancement of knowledge in Veterinary Medicine.

Professor Ilemobade enjoyed good health and a strong family life, being happily married to Christiana Olakitan (née Akinrodoye), and together they were blessed with four children: Ayokanmi, Adesola, Adeseni, and Tolulade and eight grand-children.

Albert Ilemobade was wonderfully warm-hearted and supportive to his extensive family and to his many colleagues within Nigeria and beyond. He provided outstanding leadership in all his activities with a fine mixture of humour, strategic guidance and inclusiveness. His very large circle of family, friends and colleagues all mourn his passing.

FROM WHO

WHO NETWORK FOR HAT ELIMINATION. HUMAN AFRICAN TRYPANOSOMIASIS: UPDATE OF THE METHODOLOGICAL FRAMEWORK FOR CLINICAL TRIALS. REPORT OF THE FIRST MEETING ON THE DEVELOPMENT OF NEW TOOLS SUBGROUP. GENEVA, 24 SEPTEMBER 2014

Researchers involved in clinical trials for the evaluation of new treatment modalities for human African trypanosomiasis (HAT), also known as sleeping sickness, face a number of challenges that are rarely, if ever, encountered in this combination in other diseases. Many of these challenges are related to the fact that both the disease and the populations it affects are neglected and that, prior to 2004, there was no background of generally accepted – and ubiquitously feasible – diagnostic and treatment standards for the planning and conduct of clinical evaluation of new treatment modalities for a disease.

In 2004, the World Health Organization (WHO) organized an expert consultation to establish a methodological framework for clinical trials on HAT in order to facilitate collaboration among research actors and comparison of the data obtained by different groups (WHO 2007). The agreed common criteria were applied from that point by the different researchers, which created a new harmony and a collaborative environment.

During the following decade, thanks to renewed research efforts, new diagnostic tools and new knowledge on assessing treatment outcomes became available (WHO 2013), which may allow improvement of the clinical trial methodology. In addition, the number of HAT cases reported annually to WHO has fallen to fewer than 7 000, and the disease has been targeted for elimination. These new facts justify an update of some of the criteria adopted in 2004.

This meeting was framed by the WHO Network for HAT Elimination and it convened specifically the sub-group “Development of new tools”, with the following objectives:

- To review and discuss how the new knowledge made available since 2004 could impact the implementation of clinical trials; and
- To update the consensus framework for the planning, conduct and analysis of clinical trials in the future in a way that would promote the acquisition of data that can be readily compared and used in meta-analysis.

The discussions and conclusions of the meeting were driven by the need to evaluate the efficacy of new treatment regimens, but are in some cases directly applicable or easily adaptable to the evaluation of new diagnostics. The conclusions focused on the acquisition of

data from clinical trials, since data acquired according to common criteria are a prerequisite for any meaningful comparison between the outcomes of different clinical trials. With the objective of direct comparability of published data on drug efficacy in mind, a framework for analysis and reporting of the efficacy of the treatment regimens under evaluation was also agreed upon. The group did not discuss the safety evaluation aspect of HAT clinical trials. This document concerns *gambiense* HAT (g-HAT). In the case of *rhodesiense* HAT (r-HAT), the body of clinical evidence is extremely limited and therefore the elements developed here cannot always be applied in studies of clinical products addressed to r-HAT.

The full report is available at:
http://apps.who.int/iris/bitstream/10665/173583/1/9789241508834_eng.pdf?ua=1

REPORT OF THE FIRST WHO STAKEHOLDERS MEETING ON RHODESIENSEHUMAN AFRICAN TRYPANOSOMIASIS. GENEVA, 20–22 OCTOBER 2014

An excellent summary of this meeting is given in this Volume of TTI (Abstract No. 17438) by Professor P. Holmes. The full report is available at:
http://apps.who.int/iris/bitstream/10665/181167/1/9789241508650_eng.pdf?ua=1.

CASES OF SLEEPING SICKNESS DROP TO LOWEST LEVEL IN 75 YEARS. GENEVA, 19 MAY 2015

The number of new cases of human African trypanosomiasis (also known as sleeping sickness) reported to WHO has dropped to 3 796 – the lowest level since the start of systematic global data collection 75 years ago. Collaboration with regional offices – the Regional Office for Africa and the Regional Office for the Eastern Mediterranean – has greatly contributed to reducing transmission of the disease. At the height of a resurgence of the disease in 1998, nearly 38 000 cases were reported. "*This is a historic achievement*" said Dr Jean Jannin, Coordinator, Department of Control of Neglected Tropical Diseases. "*We are on track to achieving WHO's Roadmap target of eliminating the disease as a public health problem by 2020.*"

Strengthened control and surveillance by National Sleeping Sickness Control Programmes in endemic countries over the past 15 years have progressively reduced the number of cases – falling to below 10 000 cases in 2009 and to 6 314 cases in 2013. "*Reduction in the number of cases could not have been possible without the invaluable support of mobile health units in endemic countries*" said Dr José Ramón Franco, Medical Officer. "*As cases continue to decline, we are adapting our control programme to the new epidemiological situation, improving surveillance by reinforcing the integration of passive case-finding in the peripheral health system.*"

In 2014 a coordination network for HAT was established under WHO leadership to ensure strengthened and sustained efforts to eliminate the disease. The stakeholders include national sleeping sickness control programmes, groups developing new tools to fight HAT, international and non-governmental organizations involved in control, and donors.

Collaboration

Since 2000 and 2001, WHO public-private partnerships with Sanofi (formerly Aventis Pharma) and Bayer HealthCare have enabled the creation of a WHO-led control and surveillance programme, providing support to endemic countries in their control activities and the supply

of medicines free of charge. WHO ensures the access of the donated anti-trypanosome medicines to endemic countries through this public-private partnership with Sanofi (pentamidine, melarsoprol and eflornithine) and with Bayer HealthCare (suramin and nifurtimox).

The disease

Human African trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease. It is caused by infection with protozoan parasites belonging to the genus *Trypanosoma*. They are transmitted to humans by tsetse fly (*Glossina* genus) bites which have acquired their infection from human beings or from animals harbouring the human pathogenic parasites. There are two main forms of human African trypanosomiasis: *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. Although there have been several epidemics in Africa over the last century, the disease was brought under control through successive control programmes. By the mid-1960s, less than 5 000 cases were reported in the whole continent. But after this success, surveillance was relaxed and the disease re-emerged, reaching epidemic proportions in several regions by 1970. The efforts of WHO, national control programmes, bilateral cooperation and non-governmental organizations (NGOs) during the 1990s and the first decade of this century reversed the curve.

FROM THE JOINT FAO/IAEA PROGRAMME

THE TSETSE FLY ERADICATION PROJECT IN SENEGAL WINS AWARD FOR BEST SUSTAINABLE DEVELOPMENT PRACTICES

Tsetse flies are blood-sucking insect pests threatening food security in the Niayes, a coastal area south east of Dakar. Their bites jeopardize livestock health, and the flies themselves transmit parasites that carry a life-threatening infection to livestock known as African animal trypanosomosis or nagana. A project in Senegal to eradicate tsetse flies that is supported by the FAO/IAEA was recently selected as one of 18 Best Sustainable Development Practices on Food Security at the EXPO Milano 2015. The project was selected among 749 projects for its contribution to furthering sustainable development of small rural communities in marginal areas: it has successfully improved food security and public health in target areas of Senegal through reducing the tsetse fly population by up to 95 percent using nuclear and other techniques.

To manage this problem, the Government of Senegal, with the technical cooperation and financial support of the IAEA, the FAO, the French Agricultural Research Centre for International Development (CIRAD) and the USA under the Peaceful Uses Initiative, set out in 2005 to use insecticides and the sterile insect technique (SIT), a form of insect birth control involving ionizing radiation, to eradicate the tsetse fly in the Niayes.

How it was done

The target area was divided into three blocks, and the project has successfully eradicated the fly population in the first block, and in the second block has reduced the presence of flies by more than 95 percent. The implementation of the technique will also be initiated in the third block late this year or early next year. The overall number of trypanosomosis cases in the region has gone down from 40 percent to 10 percent, paving the way for local farmers to replace their lost bovine herds with a more productive and higher yielding breed of cattle.

The Senegal Minister of Livestock, together with Baba M. Sall of the Directorate of Veterinary Services (DSV) and Momar Seck of the Senegal Institute of Agricultural Research received the award on behalf of the project during the Milan EXPO. The award is part of a programme to raise awareness and spread the best scientific solutions for improving food security and sustainable development, in conjunction with the "Feeding the Planet, Energy for Life", EXPO Milano 2015 — a six-month international showcase highlighting the importance of food security in developing regions and the application of science to achieve these aims.

"We are honoured to be recognized alongside such diverse and innovative projects as part of this important award programme," said Sall. "This award helps us to raise awareness about how nuclear and other techniques can be used to improve food security worldwide. We hope our success with eradicating tsetse flies in the Niayes area continues and can be a source of scientific solutions and inspiration for Senegal and other African countries to achieve their sustainable development goals."

IAEA RECOGNIZED FOR ITS CONTRIBUTION TO TSETSE AND TRYPANOSOMIASIS ERADICATION IN AFRICA

The IAEA was presented with a certificate recognizing the technical support that is provided by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture to member countries of the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC).

The presentation was made at the 14th meeting of PATTEC Coordinators celebrating the 15th anniversary of PATTEC, which was held jointly with the 33rd meeting of the International Scientific Council for Trypanosomiasis Control (ISCTRC) in Ndjamena, Chad, from 14-18 September 2015.

REGIONAL TRAINING COURSE ON FREE OPEN SOURCE SOFTWARE FOR GIS AND DATA MANAGEMENT APPLIED TO TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMMES. FRENCH VERSION

The French version of this regional training course was held in Vienna, Austria, from the 19-30 January 2015. The course was organized jointly by the African Union-Pan African Tsetse and Trypanosomiasis Eradication Campaign (AU-PATTEC), FAO and IAEA and was attended by 15 participants from 10 Member States (Angola, Burkina Faso, Chad, Gabon, Ivory Coast, Mali, Niger, Republic of the Congo, Senegal and Togo).

The course addressed the following topics among others:

- Free open source basics (Quantum GIS), software installation
- GIS basics
- Managing spatial data
- Advanced spatial operations
- FOSS database basics
- Introduction to satellite imagery analysis
- GPS data import
- Map composer software
- Harmonization: data management in PATTEC programmes
- ATLAS on tsetse and animal African trypanosomosis distribution at the country level

Throughout the training, theoretical lessons and practical hands-on sessions were combined. The DVD tutorial "Using open source GIS techniques in insect pest control programmes" was distributed as supporting material.

INTERNSHIP OPPORTUNITIES IN THE INSECT PEST CONTROL LABORATORY

The Insect Pest Control Laboratory of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture offers internship positions from time to time in the tsetse research group. Internships can be in any field of our work, including tsetse biology, behaviour, symbionts and molecular biology. Internships are subject to the normal IAEA terms (https://www.iaea.org/about/employment/internships), which specify that candidates for internships must be taking a university level course or have finished such a course in the past two years. There is no age limit for internships.

Anyone interested in an internship who meets the conditions is invited to apply through the IAEA web site (https://iaea.taleo.net/careersection/interns/jobsearch.ftl?lang=en). Applicants should inform Andrew Parker (a.parker@iaea.org) when they have made an application, from whom more information may be obtained.

FROM DND*i*

SUCCESSFUL COMPLETION OF PHASE I HUMAN CLINICAL TRIALS FOR SCYX-7158

The Drugs for Neglected Diseases initiative (DNDi) announced at the 9th European Congress on Tropical Medicine and International Health (ECTMIH) in Basel, Switzerland, the successful completion of Phase I human clinical trials for SCYX-7158 (AN5568), the first oral drug candidate specifically developed from the earliest drug discovery stage to combat human African trypanosomiasis, or sleeping sickness, a deadly parasitic disease transmitted by the tsetse fly. The Phase I study, conducted in France, assessed the safety, tolerability, pharmacokinetics and pharmacodynamics of SCYX-7158 after single oral ascending doses in 128 healthy human volunteers of sub-Saharan origin. It allowed for the therapeutic dose to be determined at 960 mg administered once as three tablets, with a favourable safety profile. As the drug has a long half-life (400 hours), the study was extended to ensure extensive safety monitoring of the healthy volunteers up to 210 days. This pharmacological finding has the advantage of translating into prolonged exposure with just one dose. These Phase I results confirm that the drug penetrates the brain, which is crucial to treat the late stage of the disease, where the parasite crosses the blood-brain barrier and kills patients if no treatment is given. Based on the results of this study, DND*i* and partners plan to proceed to pivotal Phase IIb/III studies in 2016 at sites in the Democratic Republic of the Congo (DRC), where 90 percent of cases occur.

"We are encouraged by the results of this important milestone for SCYX-7158, which is the fruit of collaboration and hard work of many partners", said Dr. Antoine Tarral, Head of the Human African Trypanosomiasis Clinical Programme, DND*i*. *"The motivation has been the drug candidate's potential of becoming the first ever, oral-only, single-dose treatment for this deadly disease"*. SCYX-7158 was discovered by Anacor Pharmaceuticals, Inc. The compound was identified through DND*i*'s lead optimization programme and successfully progressed through pre-clinical development in 2011. *"We are particularly excited about SCYX-7158 because it is the first drug candidate to come from the early discovery efforts of our lead optimization programme"*, said Dr Rob Don, Discovery & Pre-clinical Director, DND*i*.

Sleeping sickness cases are decreasing but the disease remains persistent in remote, hard-to-reach areas of Africa. One of the major advancements in the treatment of the disease was the introduction of nifurtimox-eflornithine combination therapy (NECT) in 2009, developed by DND*i*, Médecins Sans Frontières/Doctors Without Borders (MSF), and partners. NECT replaced an old, arsenic-based medicine, and today the vast majority of all late-stage sleeping sickness patients receive this combination as first-line treatment. Yet NECT still requires skilled staff in a hospital setting to administer the injections. Patients often travel days to get to health centres. Fexinidazole, administered for ten days with food, is currently being tested in clinical trials as an oral-only treatment that could treat all stages of the disease. SCYX-7158, if successful, would have the additional benefit of its unique single-dose, simple oral tablet administration. Recruitment for patient trials is targeted to begin in 2016 at remote sites in the DRC, where DND*i* has been carrying out fexinidazole clinical trials.

SECTION B – ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

17429. **Acheson, E. S. & Kerr, J. T., 2015.** Looking forward by looking back: using historical calibration to improve forecasts of human disease vector distributions. *Vector Borne Zoonotic Diseases*, **15** (3): 173-183.

Department of Biology, University of Ottawa, Ottawa, Ontario, Canada. [eache094@uottawa.ca].

Arthropod disease vectors, most notably mosquitoes, ticks, tsetse flies, and sandflies, are strongly influenced by environmental conditions and responsible for the vast majority of global vector-borne human diseases. The most widely used statistical models to predict future vector distributions model species niches and project the models forward under future climate scenarios. Although these methods address variations in vector distributions through space, their capacity to predict changing distributions through time is far less certain. Here, we review modelling methods used to validate and forecast future distributions of arthropod vectors under the effects of climate change and outline the uses or limitations of these techniques. We then suggest a validation approach specific to temporal extrapolation models that is gaining momentum in macro-ecological modelling and has great potential for epidemiological modelling of disease vectors. We performed systematic searches in the Web of Science, Science Direct, and Google Scholar to identify peer-reviewed English journal articles that model arthropod disease vector distributions under future environment scenarios. We included studies published up to and including June, 2014. We identified 29 relevant articles for our review. The majority of these studies predicted current species niches and projected the models forward under future climate scenarios without temporal validation. Historically calibrated forecast models improve predictions of changing vector distributions by tracking known shifts through recently observed time periods. With accelerating climate change, accurate predictions of shifts in disease vectors are crucial to target vector control interventions where needs are greatest.

17430. **Anderson, N. E., Mubanga, J., Machila, N., Atkinson, P. M., Dzingirai, V. & Welburn, S. C., 2015.** Sleeping sickness and its relationship with development and biodiversity conservation in the Luangwa Valley, Zambia. *Parasites & Vectors*, **8** (1): 224.

The Royal (Dick) School of Veterinary Studies, Easter Bush Campus, The University of Edinburgh, Roslin, Edinburgh, EH25 9RG, UK; Department of Veterinary Services, Lusaka, Zambia; Division of Pathway Medicine and Centre for Infectious Diseases, School of Biomedical Sciences, College of Medicine and Veterinary Medicine, The University of Edinburgh, Chancellor's Building, 49 Little France Crescent, Edinburgh, EH16 4SB, UK; School of Veterinary Medicine, University of Zambia, Lusaka, Zambia; Geography and Environment, Highfield Campus, University of Southampton, Southampton, SO17 1BJ, UK; Centre for Applied Social Sciences, Faculty of Social Sciences, University of Zimbabwe, Harare, Zimbabwe. [Neil.Anderson@ed.ac.uk].

The Luangwa Valley has a long historical association with human African trypanosomiasis (HAT) and is a recognised geographical focus of this disease. It is also

internationally acclaimed for its high biodiversity and contains many valuable habitats. Local inhabitants of the valley have developed sustainable land use systems in co-existence with wildlife over centuries, based on non-livestock keeping practices largely due to the threat from African animal trypanosomosis. Historical epidemics of human sleeping sickness have influenced how and where communities have settled and have had a profound impact on development in the Valley. Historical attempts to control trypanosomosis have also had a negative impact on conservation of biodiversity. Centralized control over wildlife utilization has marginalised local communities from managing the wildlife resource. To some extent this has been reversed by the implementation of community based natural resource management programmes in the latter half of the 20th century and the Luangwa Valley provides some of the earliest examples of such programmes. More recently, there has been significant uncontrolled migration of people into the mid-Luangwa Valley driven by pressure on resources in the eastern plateau region, encouragement from local chiefs and economic development in the tourist centre of Mfuwe. This has brought changing land-use patterns, most notably agricultural development through livestock keeping and cotton production. These changes threaten to alter the endemically stable patterns of HAT transmission and could have significant impacts on ecosystem health and ecosystem services. In this paper we review the history of HAT in the context of conservation and development and consider the impacts that current changes may have on this complex social-ecological system. We conclude that improved understanding is required to identify specific circumstances where win-win trade-offs can be achieved between the conservation of biodiversity and the reduction of disease in the human population.

17431. **Berkowitz, A. L., Raibagkar, P., Pritt, B. S. & Mateen, F. J., 2015.** Neurologic manifestations of the neglected tropical diseases. *Journal of the Neurological Sciences*, **349** (1-2): 20-32.

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The World Health Organization has identified 17 neglected tropical diseases (NTDs) that disproportionately affect the world's poorest populations. The neurologic aspects of many of these NTDs have received relatively little attention. A search was performed in PubMed (MedLine) for each NTD by disease name, name of its causative organism, and neurology, neurosurgery, neurologist, brain, spinal cord, peripheral nerve, muscle, nervous system, encephalitis, meningitis, encephalopathy, stroke, neuropathy, and myopathy (1968-Sept. 2013). The Oxford Centre for Evidence-based Medicine guidelines were used to determine the level of evidence of neurological involvement and treatment based on the reports identified. Neurologic manifestations were reported for all NTDs except yaws. Neurologic involvement was described in systematic reviews for four NTDs (Chagas disease, echinococcosis, rabies, cysticercosis) (levels 2a-3a), retrospective cohort studies for six (dengue, human African trypanosomiasis, leishmaniasis, leprosy, onchocerciasis, schistosomiasis) (levels 2b-3b), case series for one (food-borne trematodiasis) (level 4), and case reports for five (Buruli ulcer, dracunculiasis, filariasis, soil-transmitted helminthiasis, and trachoma). Level 1 evidence for treatment of neurologic manifestations of NTDs was found for human African trypanosomiasis, leprosy, and cysticercosis and level 2 evidence exists for treatment of neurologic involvement in Chagas disease. For the remaining NTDs, treatment of neurologic complications is described in case series and case reports only. It is concluded that neurologic manifestations of NTDs cause significant morbidity and mortality, although limited evidence

exists on how best to treat these neurologic complications. Increased awareness of neurologic manifestations of the NTDs can increase their early identification and treatment, contributing to ongoing elimination and eradication campaigns.

17432. **Bilbe, G., 2015.** Infectious diseases. Overcoming neglect of kinetoplastid diseases. *Science*, **348** (6238): 974-976.

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Of the 17 neglected tropical diseases listed by the World Health Organization (WHO), three are caused by parasitic kinetoplastid protozoa: human African trypanosomiasis (HAT; also known as sleeping sickness), leishmaniasis, and Chagas disease. The three diseases are responsible for high mortality and morbidity among the world's poorest populations. Although these and other neglected diseases have received increased attention over the past decade, new drugs are still scarce. From 2000 to 2011, only 4 percent of new drugs and vaccines were registered for neglected diseases. However, the drug development pipeline, with sustained resources and research efforts, should see the delivery of new drugs for these diseases over the next decade.

17433. **Büscher, P. & Deborggraeve, S., 2015.** How can molecular diagnostics contribute to the elimination of human African trypanosomiasis? *Expert Review of Molecular Diagnostics*, **15** (5): 607-615.

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A variety of molecular diagnostic tests for human African trypanosomiasis (HAT) (sleeping sickness) has been developed. Some are effectively used for research and confirmation diagnosis in travel medicine, usually following non-standardized protocols. Others have become commercially available as diagnostic kits. WHO aims to eliminate HAT as a public health problem by the year 2020, and zero transmission by the year 2030. This article gives an overview of the recent progress in molecular diagnostics for sleeping sickness, including the most recent data on test performances. Also discussed is how molecular diagnostics can play an important role in the process toward the elimination of HAT.

17434. **Capewell, P., Cooper, A., Clucas, C., Weir, W. & Macleod, A., 2015.** A co-evolutionary arms race: trypanosomes shaping the human genome, humans shaping the trypanosome genome. *Parasitology*, **142 (**Suppl. 1): S108-119.

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Trypanosoma brucei is the causative agent of African sleeping sickness in humans and one of several pathogens that cause the related veterinary disease Nagana. A complex co-evolution has occurred between these parasites and primates that led to the emergence of trypanosome-specific defences and counter-measures. The first line of defence in humans and several other catarrhine primates is the trypanolytic protein apolipoprotein-L1 (APOL1) found within two serum protein complexes, trypanosome lytic factor 1 and 2 (TLF-1 and TLF-2).

Two sub-species of *T. brucei* have evolved specific mechanisms to overcome this innate resistance, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. In *T. b. rhodesiense*, the presence of the serum resistance associated (SRA) gene, a truncated variable surface glycoprotein (VSG), is sufficient to confer resistance to lysis. The resistance mechanism of *T. b. gambiense* is more complex, involving multiple components: reduction in binding affinity of a receptor for TLF, increased cysteine protease activity and the presence of the truncated VSG, *T. b. gambiense*-specific glycoprotein (TgsGP). In a striking example of co-evolution, evidence is emerging that primates are responding to challenge by *T. b. gambiense* and *T. b. rhodesiense*, with several populations of humans and primates displaying resistance to infection by these two sub-species.

17435. **Chritz, K. L., Marshall, F. B., Zagal, M. E., Kirera, F. & Cerling, T. E., 2015.** Environments and trypanosomiasis risks for early herders in the later Holocene of the Lake Victoria basin, Kenya. *Proceedings of the National Academy of Sciences USA.*, **112** (12): 3674-3679.

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Specialized pastoralism developed approximately three thousand years ago (kya) among pastoral Neolithic Elmenteitan herders in eastern Africa. During this time, a mosaic of hunters and herders using diverse economic strategies flourished in southern Kenya. It has been argued that the risk for trypanosomiasis (sleeping sickness), carried by tsetse flies in bushy environments had a significant influence on pastoral diversification and migration out of eastern Africa toward southern Africa approximately two kya. Elmenteitan levels at Gogo Falls (ca. 1.9-1.6 kya) preserve a unique faunal record, including wild mammalian herbivores, domestic cattle and caprines, fish, and birds. It has been suggested that a bushy/woodland habitat that harboured tsetse fly constrained production of domestic herds and resulted in subsistence diversification. Stable isotope analysis of herbivore tooth enamel (n = 86) from this site reveals, instead, extensive C4 grazing by both domesticates and the majority of wild herbivores. Integrated with other ecological proxies (pollen and leaf wax biomarkers), these data imply an abundance of C4 grasses in the Lake Victoria basin at this time, and thus little risk for tsetse-related barriers to specialized pastoralism. These data provide empirical evidence for the existence of a grassy corridor through which small groups of herders could have passed to reach southern Africa.

17436. **Gibson, W., 2015.** Liaisons dangereuses: sexual recombination among pathogenic trypanosomes. *Research in Microbiol*ogy, **166** (6): 459-466

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Sexual recombination between pathogenic microbes has the potential to mobilize genes for harmful traits into new genetic backgrounds creating new pathogen strains. Since 1986 we have known that genetic exchange can occur in trypanosomes, but we are only now starting to

unravel details of the process. In *Trypanosoma brucei* genetic exchange occurs in the tsetse vector, but is not an obligatory part of the life cycle. The process involves meiosis and production of haploid gametes, and thus appears to be true sexual reproduction. This review looks at the experimental evidence concerning genetic exchange and identifies current gaps in our knowledge.

17437. **Gibson, W., Peacock, L., Ferris, V., Fischer, K., Livingstone, J., Thomas, J. & Bailey, M., 2015.** Genetic recombination between human and animal parasites creates novel strains of human pathogen. *PLoS Neglected Tropical Diseases*, **9** (3): e0003665.

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Genetic recombination between pathogens derived from humans and livestock has the potential to create novel pathogen strains, highlighted by the influenza pandemic H1N1/09, which was derived from a re-assortment of swine, avian and human influenza A viruses. Here we investigated whether genetic recombination between subspecies of the protozoan parasite *Trypanosoma brucei (Tbb)*, from humans and animals can generate new strains of human pathogen, *T. b. rhodesiense* (*Tbr*) responsible for sleeping sickness (human African trypanosomiasis, HAT) in East Africa. The trait of human infectivity in *Tbr* is conferred by a single gene, SRA, which is potentially transferable to the animal pathogen *Tbb* by sexual reproduction. We tracked the inheritance of SRA in crosses of *Tbr* and *Tbb* set up by co-transmitting genetically-engineered fluorescent parental trypanosome lines through tsetse flies. SRA was readily transferred into new genetic backgrounds by sexual reproduction between *Tbr* and *Tbb*, thus creating new strains of the human pathogen, *Tbr*. There was no evidence of diminished growth or transmissibility of hybrid trypanosomes carrying SRA. Although expression of SRA is critical to survival of *Tbr* in the human host, we show that the gene exists as a single copy in a representative collection of *Tbr* strains. SRA was found on one homologue of chromosome IV in the majority of *Tbr* isolates examined, but some Ugandan *Tbr* had SRA on both homologues. The mobility of SRA by genetic recombination readily explains the observed genetic variability of *Tbr* in East Africa. We conclude that new strains of the human pathogen *Tbr* are being generated continuously by recombination with the much larger pool of animal-infective trypanosomes. Such novel recombinants present a risk for future outbreaks of HAT.

17438. **Holmes, P., 2015.** On the road to elimination of *rhodesiense* human African trypanosomiasis: first WHO meeting of stakeholders. *PLoS Neglected Tropical Diseases*, **9** (4): e0003571.

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Human African trypanosomiasis (HAT), also known as sleeping sickness, has been a major scourge afflicting populations in Africa in areas where its specific vector, the tsetse fly, thrives. This endemic disease with a very high level of mortality has caused large epidemics in the past and had a major impact on the development of rural populations. Two clinical forms exist, each affecting distinct parts of Africa: a chronic form in West and Central Africa caused by *Trypanosoma brucei gambiense* (>95 percent of current caseload) and an acute form in East

and Southern Africa caused by *T. b. rhodesiense* (<5 percent of current caseload). Confronted with widespread mortality due to HAT, the colonial health systems conducted intensive campaigns during the 1960s that brought HAT under control, with a residual low number of new infections per year. Unfortunately, the ensuing rarity of HAT cases led to a decline in awareness, movement to the bottom of the priority lists, and the neglect of control and surveillance activities. As the disease foci had not been truly eliminated and the ingredients for their transmission were still present, the lack of surveillance allowed HAT to re-emerge and to reach epidemic proportions by the end of the 20th century, when new cases were estimated at around 300 000 per year.

This alarming situation mobilized international efforts to support endemic countries in the revitalization of the fight against the spread of the disease. During the past 15 years, the national sleeping sickness programmes have conducted outstanding work with the support of international groups led by the World Health Organization (WHO) and with the commitment of key pharmaceutical companies and major international donors. Surveillance was reinforced with the deployment of various strategies, most notably mass screening campaigns with treatment of all cases detected. The access to diagnosis and treatment was improved in endemic areas, and the epidemiological knowledge of the disease was advanced, including detailed and dynamic disease mapping in all affected countries. This has involved strong collaboration and coordination of all these stakeholders and the maintenance of a permanent and open dialogue. As a result of these synergistic actions, the general situation improved, and the number of new reported cases has fallen steadily (6 228 in 2013). Based on the observed achievements in the control of the disease, in 2012 the WHO Strategic and Technical Advisory Group for Neglected Tropical Diseases decided to target the elimination of *gambiense* HAT as a public health problem by 2020 and the reduction to zero incidence of the disease by 2030. The 2020 target was included in the WHO roadmap for elimination and control of neglected tropical diseases, and it was defined as the reduction of *gambiense* HAT incidence to less than one new case per 10 000 people at risk, in at least 90 percent of foci, as well as fewer than 2 000 cases reported globally. In 2013, this elimination target was endorsed by the disease-endemic countries, the WHO Expert Committee on Control and Surveillance of HAT, and the London Declaration on Neglected Tropical Diseases. More recently it was adopted by the 66th World Health Assembly in the resolution WHA66.12. In March 2014, WHO convened the first WHO meeting of stakeholders for the elimination of *gambiense* HAT (Geneva, Switzerland), which was attended by participants from national sleeping sickness control programmes, research and development groups, international and non-governmental organizations, and international donors from the public and private sectors. As a result, the participants issued a declaration for the elimination of *gambiense* HAT.

Regarding *rhodesiense* HAT, the acute form of the disease affecting East and Southern Africa, there are important specific characteristics that call for an approach that is different from that of *gambiense* HAT. As opposed to *gambiense* HAT, in which the reservoir is almost exclusively a human being, *rhodesiense* HAT is a zoonotic disease with wild and domestic animals as the main reservoirs, which ensures the maintenance of a population of infected tsetse flies that occasionally transmit the disease to humans. This zoonotic nature, and particularly the existence of a wildlife reservoir, makes disease control objectives more challenging. On 20-22 October 2014, WHO organized in Geneva, Switzerland, the first stakeholders meeting on *rhodesiense* HAT. The meeting reviewed the current epidemiological status of the disease and the challenges for advancing towards elimination. Although recently there has been progress in reducing the incidence of *rhodesiense* HAT, this acute disease with high mortality still holds its epidemic potential. The fight to achieve elimination will need multi-sectoral (One Health) cooperation and coordination at the national, transboundary, regional, and international levels. The participants indicated that greater attention is to be given to the animal reservoir

and the respective roles of livestock and wildlife in different countries and in different ecological situations. They called also for improved and sustained surveillance of infection in humans and animals. The capacity of human resources and infrastructure will need strengthening. The stakeholders also analysed the current status of important technical assets for *rhodesiense* HAT control that are needed to assist in achieving the elimination goal. These include faster adoption and better utilization of new tools that are already available, including improved diagnostics and therapies, as well as the development of new diagnostic methods and treatments, more cost-effective vector control tools, and quality assurance systems. The meeting concluded that the elimination of *rhodesiense* HAT as a public-health problem (defined as less than one case per 10 000 people at risk) by the year 2020 is achievable, provided that the above-mentioned requirements are addressed. The mechanisms of collaboration and coordination among stakeholders were established within the WHO network for HAT elimination, and the meeting looked at procedures needed for monitoring and evaluation of the elimination process as well as confirmation of outcomes.

The meeting concluded by issuing a declaration for the elimination of *rhodesiense* HAT, appealing to the international community at large and to disease-endemic countries in particular for their commitment, political support, and essential resources to achieve the elimination goal. In particular, the appeal calls for the establishment in endemic countries of national coordination bodies that include all sectors concerned with *rhodesiense* HAT transmission and its impact (i.e. human and animal health, wildlife, and tourism) to bring together and synergize efforts. The declaration of the first stakeholders meeting on *rhodesiense* HAT elimination can be viewed at http://who.int/trypanosomiasis_african/meeting_declaration_*rhodesiense*_2014_intro/en/.

17439. **Keating, J., Yukich, J. O., Sutherland, C. S., Woods, G. & Tediosi, F., 2015.** Human African trypanosomiasis prevention, treatment and control costs: a systematic review. *Acta Tropica*, **150**: 4-13.

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The control and eventual elimination of human African trypanosomiasis (HAT) requires the expansion of current control and surveillance activities. A systematic review of the published literature on the costs of HAT prevention, treatment, and control, in addition to the economic burden, was conducted. All studies that contained primary or secondary data on costs of prevention, treatment and control were considered, resulting in the inclusion of 42 papers. The geographically focal nature of the disease and a lack of standardization in the cost data limit the usefulness of the available information for making generalizations across diverse settings. More recent information on the costs of treatment and control interventions for HAT is needed to provide accurate information for analyses and planning. The cost information contained herein can be used to inform rational decision making in control and elimination programmes, and to assess potential synergies with existing vector-borne disease control programmes, but programmes would benefit significantly from new cost data collection.

17440. **Kennedy, P. G., 2015.** Viruses, apoptosis, and neuroinflammation–a double-edged sword. *Journal of Neurovirology*, **21** (1): 1-7.

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Apoptosis, or programmed cell death, is a fundamental and widespread cell biological process that is distinct from cell necrosis and can be induced by a wide variety of stimuli including viral infections. Apoptosis may occur via either the intrinsic or extrinsic pathways and confers several advantages to the virally infected host including the prevention of further viral propagation and the potential inhibition and resolution of inflammatory processes. Several viruses have been shown to have the capacity to induce apoptosis in susceptible cells including herpes simplex virus, Varicella-zoster virus, rabies virus, human immunodeficiency virus, and reovirus. Apoptosis has also been observed in human African trypanosomiasis which is an infection caused by a protozoan parasite. The mechanisms leading to apoptosis may differ depending on the type of infection. Apoptosis has been reported in several neurodegenerative diseases and also psychiatric disorders but the true clinical significance of such observations is not certain, and, though interesting, it is very difficult to ascribe causation in these conditions. The presence of inflammation in the central nervous system in any neurological condition, including those associated with a viral infection, is not necessarily an absolute marker of serious disease and the notion of "good" versus "bad" inflammation is considered to be valid in some circumstances. The precise relationship between viruses, apoptosis, and inflammation is viewed as a complex one requiring further investigation to unravel and understand its nature.

17441. **Khatri, N., Dutt, R. & Madan, A. K., 2015.** Role of moving average analysis for development of multi-target (Q)SAR models. *Mini Reviews in Medicinal Chemistry*, **15** (8): 659-676.

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In the modern drug discovery era, multi target-quantitative structure activity relationship [mt-(Q)SAR] approaches have emerged as novel and powerful alternatives in the field of *in-silico* drug design so as to facilitate the discovery of new chemical entities with multiple biological activities. Amongst various machine learning approaches, moving average analysis (MAA) has frequently exhibited high accuracy of prediction of diverse biological activities against different biological targets and experimental conditions. The role of MAA in developing (Q)SAR models for prediction of single/dual or multi-target activity is briefly reviewed in the present article. Subsequently, MAA was successfully utilized for developing mt-(Q)SAR models for simultaneous prediction of anti-*Plasmodium falciparum* and anti-*Trypanosoma brucei rhodesiense* activities of benzyl phenyl ether derivatives. The statistical significance of models was assessed through inter-correlation analysis, sensitivity, specificity and Matthew's correlation coefficient. Proposed MAA based models were also validated using test sets. High predictability in the order of 80 percent to 95 percent amalgamated with safety (indicated by high selectivity index values) of proposed mt-(Q)SAR models justifies the use of MAA in developing models in order to obtain more realistic and accurate results for prediction of anti-protozoal activity against multiple targets. Activity ranges of the proposed models can play a significant role in the development of novel, potent, versatile and safe anti-protozoal

drugs with improved profiles in terms of both anti-*Plasmodium falciparum* and anti-*Trypanosoma brucei rhodesiense* activities.

17442. **Lun, Z. R., Lai, D. H., Wen, Y. Z., Zheng, L. L., Shen, J. L., Yang, T. B., Zhou, W. L., Qu, L. H., Hide, G. & Ayala, F. J., 2015.** Cancer in the parasitic protozoans *Trypanosoma brucei* and *Toxoplasma gondii*. *Proceedings of the National Academy of Sciences USA*, **112** (29): 8835-8842.

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Cancer is a general name for more than 100 malignant diseases. It is postulated that all cancers start from a single abnormal cell that grows out of control. Untreated cancers can cause serious consequences and deaths. Great progress has been made in cancer research and this has significantly improved our knowledge and understanding of the nature and mechanisms of the disease. However, the origins of cancer are far from being well understood due to the limitations of suitable model systems and to the complexities of the disease. In view of the fact that cancers are found in various species of vertebrates and other metazoa, here we suggest that cancer also occurs in parasitic protozoans such as *Trypanosoma brucei*, a blood parasite, and *Toxoplasma gondii*, an obligate intracellular pathogen. Without treatment, these protozoan cancers may cause severe disease and death in mammals, including humans. The simpler genomes of these single-cell organisms, in combination with their complex life cycles and fascinating life cycle differentiation processes, may help us to better understand the origins of cancers and, in particular, leukemias.

17443. **Matthews, K. R., 2015.** 25 years of African trypanosome research: from description to molecular dissection and new drug discovery. *Molecular & Biochemical Parasitology*, **200** (1-2): 30-40.

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The Molecular Parasitology conference was first held at the Marine Biological laboratory, Woods Hole, USA 25 years ago. Since that first meeting, the conference has evolved and expanded but has remained the showcase for the latest research developments in molecular parasitology. In this perspective, I reflect on the scientific discoveries focussed on African trypanosomes (*Trypanosoma brucei* spp.) that have occurred since the inaugural MPM meeting and discuss the current and future status of research on these parasites.

17444. **Matthews, K. R., McCulloch, R. & Morrison, L. J., 2015.** The within-host dynamics of African trypanosome infections. *Philosophical Transactions of the Royal Society: B Biological Sciences*, **370** (1675).

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African trypanosomes are single-celled protozoan parasites that are capable of long-term survival while living extracellularly in the bloodstream and tissues of mammalian hosts. Prolonged infections are possible because trypanosomes undergo antigenic variation–the expression of a large repertoire of antigenically distinct surface coats, which allows the parasite population to evade antibody-mediated elimination. The mechanisms by which antigen genes become activated influence their order of expression, most likely by influencing the frequency of productive antigen switching, which in turn is likely to contribute to infection chronicity. Superimposed upon antigen switching as a contributor to trypanosome infection dynamics is the density-dependent production of cell-cycle arrested parasite transmission stages, which limit the infection while ensuring parasite spread to new hosts via the bite of blood-feeding tsetse flies. Neither antigen switching nor developmental progression to transmission stages is driven by the host. However, the host can contribute to the infection dynamic through the selection of distinct antigen types, the influence of genetic susceptibility or trypanotolerance and the potential influence of host-dependent effects on parasite virulence, development of transmission stages and pathogenicity. In a zoonotic infection cycle where trypanosomes circulate within a range of host animal populations, and in some cases humans, there is considerable scope for a complex interplay between parasite immune evasion, transmission potential and host factors to govern the profile and outcome of infection.

17445. **Norman, F. F., Monge-Maillo, B., Martinez-Perez, A., Perez-Molina, J. A. & Lopez-Velez, R., 2015.** Parasitic infections in travellers and immigrants: part I protozoa. *Future Microbiology*, **10** (1): 69-86.

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The growth in international commerce, travel and migration contribute to the global emergence of certain parasitic infections. Importation of vectors and food products may contribute to the emergence of protozoan infections in non-endemic countries. Infections such as malaria are potentially fatal, especially in nonimmune patients, and outcome depends largely on timely diagnosis and treatment. Diagnosis/management of imported parasitic infections may be complex especially as some patients may have underlying immunosuppressive conditions such as HIV infection. Major challenges concern the development of improved diagnostic techniques, safer/more effective drug therapies and identification of biological markers of progression and response to treatment. Imported parasitic diseases which may be transmitted vertically or through blood transfusion/organ donation could become a public health priority in the near future. Climate change may affect arthropod distribution and facilitate the spread of

protozoan vector-borne diseases. The first part of this review focuses on protozoan infections in travellers and immigrants.

17446. **Okello, A., Welburn, S. & Smith, J., 2015.** Crossing institutional boundaries: mapping the policy process for improved control of endemic and neglected zoonoses in sub-Saharan Africa. *Health Policy & Planning*, **30** (6): 804-812.

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The recent adoption of the World Health Assembly Resolution 66.12 for neglected tropical diseases (NTDs) in May 2013 is an important turning point for advocacy regarding a number of endemic zoonotic infections, defined by the World Health Organization as the neglected zoonotic diseases (NZDs). In addition to NTD-listed zoonoses such as rabies, echinococcosis (hydatid disease), leishmaniasis, human African trypanosomiasis (sleeping sickness) and *Taenia solium* cysticercosis, the NZDs also include important bacterial zoonoses such as anthrax, bovine tuberculosis and brucellosis. To date, analysis of the processes that prioritize, develop and deliver zoonoses control programmes in many low- and middle-income countries is lacking, despite its potential to highlight significant evidence gaps and institutional constraints to the inter-sectoral approach required for their control. Policy process analysis was conducted via a series of semi-structured interviews with key policy actors within various ministries and institutes in Uganda and Nigeria. The study concluded that despite the rhetoric around “linear” models of health policy development promoting consultation with a wide range of national stakeholders, the decision-making process for zoonotic disease control appears instead overtly influenced by the external political economy of trending pandemic threats, often overlooking national and regional zoonoses priorities. The inclusion of political systems remains a key factor in the zoonoses analysis matrix, enhancing our understanding of the inter-sectoral and trans-disciplinary approaches required for their control. The authors consider policy process analysis to be a fundamental first step of any attempt to holistically strengthen human and animal health systems in a development context, particularly regarding the promotion of integrated control policies for regionally important zoonoses under the growing One Health movement.

17447. **Shaw, W. R., Attardo, G. M., Aksoy, S. & Catteruccia, F., 2015.** A comparative analysis of reproductive biology of insect vectors of human disease. *Current Opinion in Insect Science*, **10**: 142-148.

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Studying the reproductive strategies of insect species that transmit diseases to humans

can identify new exploitable targets for the development of vector control methods. Here we describe shared characteristics and individual features of the reproductive biology of three major disease vectors: *Anopheles gambiae*, *Aedes aegypti* and *Glossina morsitans*. Current studies are identifying (i) species-specific molecular cascades that determine female monandrous behaviour, (ii) core aspects of egg development that could be disrupted for controlling natural populations, and (iii) the increasingly apparent role of resident microbiota in shaping reproductive success and disease transmission potential. The recent completion of multiple genome sequencing projects is allowing comparative genomics studies that not only increase our knowledge of reproductive processes but also facilitate the identification of novel targets for vector control.

17448. **Simarro, P. P., Cecchi, G., Franco, J. R., Paone, M., Diarra, A., Priotto, G., Mattioli, R. C. & Jannin, J. G., 2015.** Monitoring the progress towards the elimination of g*ambiense* human African trypanosomiasis. *PLoS Neglected Tropical Diseases*, **9** (6): e0003785.

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Over the last few years, momentum has gathered around the feasibility and opportunity of eliminating *gambiense* human African trypanosomiasis (g-HAT). Under the leadership of the World Health Organization (WHO), a large coalition of stakeholders is now committed to achieving this goal. A roadmap has been laid out, and indicators and milestones have been defined to monitor the progress of the elimination of g-HAT as a public health problem by 2020. Subsequently, a more ambitious objective was set for 2030: to stop disease transmission. This paper provides a situational update to 2012 for a number of indicators of elimination: number of cases annually reported, geographic distribution of the disease and areas and populations at different levels of risk. Comparing the five-year periods 2003-2007 and 2008-2012, the area at high or very high risk of g-HAT shrank by 60 percent, while the area at moderate risk decreased by 22 percent. These are the areas where g-HAT is still to be considered a public health problem (i.e. > 1 HAT reported case per 10 000 people per annum). This contraction of at-risk areas corresponds to a reduction of 57 percent for the population at high or very high risk (from 4.1 to 1.8 million), and 20 percent for moderate risk (from 14.0 to 11.3 million). Improved data completeness and accuracy of the Atlas of HAT enhanced our capacity to monitor the progress towards the elimination of g-HAT. The trends in the selected indicators suggest that, in recent years, progress has been steady and in line with the elimination goal laid out in the WHO roadmap on neglected tropical diseases.

17449. **Smith, J., Taylor, E. M. & Kingsley, P., 2015.** One World-One Health and neglected zoonotic disease: elimination, emergence and emergency in Uganda. *Social Science & Medicine*, **129**: 12-19.

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This paper traces the emergence and tensions of an internationally constructed and framed One World-One Health (OWOH) approach to control and attempt to eliminate African trypanosomiasis in Uganda. In many respects, trypanosomiasis is a disease that an OWOH approach is perfectly designed to treat, requiring an integrated approach built on effective surveillance in animals and humans, quick diagnosis and targeting of the vector. The reality appears to be that the translation of global notions of OWOH down to national and district levels generates problems, primarily due to interactions between: (i) international, external actors not engaging with the Ugandan state; (ii) actors setting up structures and activities parallel to those of the state; (iii) actors deciding when emergencies begin and end without consultation; (iv) weak Ugandan state capacity to coordinate its own integrated response to disease; (v) limited collaboration between core Ugandan planning activities and a weak, increasingly devolved district health system. These interrelated dynamics result in the global, international interventionalist mode of OWOH undermining the Coordinating Office for Control of Trypanosomiasis in Uganda (COCTU), the body within the Ugandan state mandated expressly with managing a sustainable One Health response to trypanosomiasis outbreaks in Uganda. This does two things, firstly it suggests we need a more grounded, national perspective of OWOH, where states and health systems are acknowledged and engaged with by international actors and initiatives. Secondly, it suggests that more support needs to be given to core coordinating capacity in resource-poor contexts. Supporting national coordinating bodies, focused around One Health, and ensuring that external actors engage with and through those bodies can help develop a sustained, effective OWOH presence in resource-poor countries, where after all most zoonotic disease burden remains.

17450. **Steinmann, P., Stone, C. M., Sutherland, C. S., Tanner, M. & Tediosi, F., 2015.** Contemporary and emerging strategies for eliminating human African trypanosomiasis due to *Trypanosoma brucei gambiense*: review. *Tropical Medicine & International Health*, **20** (6): 707-718.

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This paper reviews current and emerging tools for *gambiense* HAT control and elimination, and proposes strategies that integrate these tools with epidemiological evidence. We reviewed the scientific literature to identify contemporary and emerging tools and strategies for controlling and eliminating *gambiense* HAT. Through an iterative process involving key stakeholders, we then developed comprehensive scenarios leading to elimination, considering both established and new tools for diagnosis, case treatment and vector control. Core components of all scenarios include detecting and treating cases with established or emerging techniques. Relatively more intensive scenarios incorporate vector control. New tools considered include tiny targets for tsetse fly control, use of rapid diagnostic tests and oral treatment with fexinidazole or oxaboroles. Scenarios consider the time when critical new tools are expected to become ready for deployment by national control programmes. Based on a review of the latest epidemiological data, we estimate the various interventions to cover 1 380 600 km^2 and 56 986 000 people. It is concluded that a number of new tools will fill critical gaps in the current armamentarium for diagnosing and treating *gambiense* HAT. Deploying these tools in endemic areas will facilitate the comprehensive and sustainable control of the disease considerably and contribute to the ultimate goal of elimination.

17451. **Sudarshi, D. & Brown, M., 2015.** Human African trypanosomiasis in non-endemic countries. *Clinical Medicine*, **15** (1): 70-73.

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Human African trypanosomiasis (HAT) or sleeping sickness is a parasitic disease acquired by the bite of an infected tsetse fly. In non-endemic countries HAT is rare, and therefore the diagnosis may be delayed leading to potentially fatal consequences. In this article the clinical presentation, diagnosis and treatment of the two forms of HAT are outlined. *Rhodesiense* HAT is an acute illness that presents in tourists who have recently visited game parks in Eastern or Southern Africa, whereas *gambiense* HAT has a more chronic clinical course, in individuals from West or Central Africa.

17452. **Sutcliffe, O. B., Skellern, G. G., Araya, F., Cannavan, A., Sasanya, J. J., Dungu, B., van Gool, F., Munstermann, S. & Mattioli, R. C., 2014.** Animal trypanosomosis: making quality control of trypanocidal drugs possible. *Rev. Sci. Tech.*, [*Scientific and Technical Review of the Office International des Epizooties* (*Paris*)], **33** (3): 813-830.

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African animal trypanosomosis is arguably the most important animal disease impairing livestock agricultural development in sub-Saharan Africa. In addition to vector control, the use of trypanocidal drugs is important in controlling the impact of the disease on animal health and production in most sub-Saharan countries. However, there are no internationally agreed standards (pharmacopoeia-type monographs or documented product specifications) for the quality control of these compounds. This means that it is impossible to establish independent quality control and quality assurance standards for these agents. An international alliance between the Food and Agriculture Organization of the United Nations, the International Federation for Animal Health, the Global Alliance for Livestock Veterinary Medicines, the University of Strathclyde and the International Atomic Energy Agency (with critical support from the World Organisation for Animal Health) was established to develop quality control and quality assurance standards for trypanocidal drugs, with the aim of transferring these methodologies to two control laboratories in sub-Saharan Africa that will serve as reference institutions for their respective regions. The work of the international alliance will allow development of control measures against sub-standard or counterfeit trypanocidal drugs for treatment of trypanosome infection. Monographs on diminazene aceturate (synonym: diminazene diaceturate), isometamidium chloride hydrochloride, homidium chloride and bromide salts and the relevant veterinary formulations for these agents are given in the annex

to this paper. However, the authors do not recommend use of homidium bromide and chloride, because of their proven mutagenic properties in some animal test models and their suspected carcinogenic properties.

17453. **Sutherland, C. S., Yukich, J., Goeree, R. & Tediosi, F., 2015.** A literature review of economic evaluations for a neglected tropical disease: human African trypanosomiasis ("sleeping sickness"). *PLoS Neglected Tropical Diseases*, **9** (2): e0003397.

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Human African trypanosomiasis (HAT) is a disease caused by infection with the parasite *Trypanosoma brucei gambiense* or *T. b. rhodesiense*. It is transmitted to humans via the tsetse fly. Approximately 70 million people worldwide were at risk of infection in 1995, and approximately 20 000 people across Africa are infected with HAT. The objective of this review was to identify existing economic evaluations in order to summarize cost-effective interventions to reduce, control, or eliminate the burden of HAT. The studies included in the review were compared and critically appraised in order to determine if there were existing standardized methods that could be used for economic evaluation of HAT interventions or if innovative methodological approaches are warranted. A search strategy was developed using keywords and was implemented in January 2014 using several databases. The search returned a total of 2 283 articles. After two levels of screening, a total of seven economic evaluations were included and underwent critical appraisal using the Scottish Intercollegiate Guidelines Network (SIGN) Methodology Checklist 6: Economic Evaluations. Results from the existing studies focused on the cost-effectiveness of interventions for the control and reduction of disease transmission. Modelling was a common method to forecast long-term results, and publications focused on interventions by category, such as case detection, diagnostics, drug treatments, and vector control. Most interventions were considered cost-effective based on the thresholds described; however, the current treatment, nifurtimox-eflornithine combination therapy (NECT), has not been evaluated for cost-effectiveness, and considerations for cost-effective strategies for elimination have yet to be completed. Overall, the current evidence highlights the main components that play a role in control; however, economic evaluations of HAT elimination strategies are needed to assist national decision makers, stakeholders, and key funders. These analyses would be of use, as HAT is currently being prioritized as a neglected tropical disease (NTD) to reach elimination by 2020.

17454. **Torr, S. J. & Vale, G. A., 2015.** Know your foe: lessons from the analysis of tsetse fly behaviour. *Trends in Parasitology*, **31** (3): 95-99.

Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK; Warwick Medical School, University of Warwick, Coventry CV4 7AL, UK; Natural Resources Institute, University of Greenwich, Chatham ME4 4TB, UK; South African Centre for Epidemiological Modelling and Analysis, University of Stellenbosch, Stellenbosch 7600, South Africa. [steve.torr@lstmed.ac.uk].

The emergence of new vector-borne diseases requires new methods of vector control. These diseases are often zoonoses associated with wilderness areas, and established methods of vector control used in domestic settings (e.g. indoor-residual spraying, insecticide-treated bed nets) are therefore inappropriate. Similar difficulties are also emerging with the control of "old" vector-borne diseases such as malaria. Understanding the host-finding behaviour of vectors assists the development and application of control methods and aids the understanding of epidemiology. Some general lessons are illustrated by reference to a century of research on the host-finding behaviour of tsetse flies which transmit trypanosomes causing human and animal trypanosomiases, including Rhodesian sleeping sickness, a zoonosis associated with wilderness areas of sub-Saharan Africa.

17455. **Ueno, N. & Lodoen, M. B., 2015.** From the blood to the brain: avenues of eukaryotic pathogen dissemination to the central nervous system. *Current Opinion in Microbiology*, **26**: 53-59.

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Infection of the central nervous system (CNS) is a significant cause of morbidity and mortality, and treatments available to combat the highly debilitating symptoms of CNS infection are limited. The mechanisms by which pathogens in the circulation overcome host immunity and breach the blood-brain barrier are active areas of investigation. In this review, we discuss recent work that has significantly advanced our understanding of the avenues of pathogen dissemination to the CNS for four eukaryotic pathogens of global health importance: *Toxoplasma gondii*, *Plasmodium falciparum*, *Trypanosoma brucei*, and *Cryptococcus neoformans*. These studies highlight the remarkable diversity of pathogen strategies for trafficking to the brain and will ultimately contribute to an improved ability to combat life-threatening CNS disease.

17456. **Welburn, S. C., Beange, I., Ducrotoy, M. J. & Okello, A. L., 2015.** The neglected zoonoses–the case for integrated control and advocacy. *Clinical Microbiology & Infection*, **21** (5): 433-443.

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The neglected zoonotic diseases (NZDs) have been all but eradicated in wealthier countries, but remain major causes of ill-health and mortality across Africa, Asia, and Latin America. This neglect is, in part, a consequence of under-reporting, resulting in an underestimation of their global burden that downgrades their relevance to policy-makers and funding agencies. Increasing awareness about the causes of NZDs and how they can be prevented could reduce the incidence of many endemic zoonoses. Addressing NZDs by targeting the animal reservoir can deliver a double benefit, as enhanced animal health means a reduced risk of infection for humans, as well as improved livelihoods through increased animal productivity. Advocacy for NZD control is increasing, but with it comes a growing awareness that NZD control demands activities both in the short term and over a long period of time. Moreover, despite the promise of cheap, effective vaccines or other control tools, these endemic diseases will not be sustainably controlled in the near future without long-term financial

commitment, particularly as disease incidence decreases and other health priorities take hold. NZD intervention costs can seem high when compared with the public health benefits alone, but these costs are easily outweighed when a full cross-sector analysis is carried out and monetary/non-monetary benefits–particularly regarding the livestock sector–are taken into account. Public-private partnerships have recently provided advocacy for human disease control, and could prove equally effective in addressing endemic zoonoses through harnessing social impact investments. Evidence of the disease burdens imposed on communities by the NZDs and demonstration of the cost-effectiveness of integrated control can strengthen the case for a One Health approach to endemic zoonotic disease control.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

17457. **Pagabeleguem, S., Seck, M. T., Sall, B., Vreysen, M. J., Gimonneau, G., Fall, A. G., Bassene, M., Sidibe, I., Rayaisse, J. B., Belem, A. M. & Bouyer, J., 2015.** Long distance transport of irradiated male *Glossina palpalis gambiensis* pupae and its impact on sterile male yield. *Parasites & Vectors*, **8**: 259.

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The application of the sterile insect technique (SIT) requires mass-production of sterile males of good biological quality. The size of the project area will in most cases determine whether it is more cost effective to produce the sterile flies locally (and invest in a mass-rearing facility) or import the sterile flies from a mass-rearing facility that is located in another country. This study aimed at assessing the effect of long distance transport of sterile male *Glossina palpalis gambiensis* pupae on adult male fly yield. The male pupae were produced at the Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), Bobo-Dioulasso, Burkina Faso, and shipped with a commercial courier service in insulated transport boxes at a temperature of +/-10 ^{0}C to Senegal (+/-36 h of transport). Upon arrival in the insectary in Dakar, the pupae were transferred to an emergence room and the flies monitored for 3-6 days. The results showed that the system of using isothermal boxes that contained phase change material packs (S8) managed to keep the temperature at around 10 ^{o}C which prevented male fly emergence during transport. The emergence rate was significantly higher for pupae from batch 2 (chilled at 4 ^{o}C for one day in the source insectary before transport) than those from batch 1 (chilled at 4 ^{o}C for two days in the source insectary before transport), i.e. an average (+/-SD) of 76.1 +/- 13.2 percent and 72.2 +/- 14.3 percent,

respectively with a small proportion emerging during transport (0.7 +/- 1.7 percent and 0.9 +/- 2.9 percent, respectively). Among the emerged flies, the percentage with deformed (not fully expanded) wings was significantly higher for flies from batch 1 (12.0 +/- 6.3 percent) than from batch 2 (10.7 +/- 7.5 percent). The number of sterile males available for release as a proportion of the total pupae shipped was 65.8 +/- 13.3 percent and 61.7 +/- 14.7 percent for pupal batches 1 and 2, respectively. The results also showed that the temperature inside the parcel must be controlled at around 10 ^{0}C with a maximal deviation of 3 ^{0}C to maximize the male yield.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

[See also **38**: 17436, 17447].

17458. **Childs, S. J., 2015.** An improved temporal formulation of pupal transpiration in *Glossina*. *Mathematical Biosciences*, **262**: 214-229.

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The temporal aspect of a model of pupal dehydration is improved upon. The observed dependence of pupal transpiration on time is attributed to an alternation between two essential modes, for which the deposition of a thin pupal skin inside the puparium and its subsequent demise are thought to be responsible. For each mode of transpiration, the results of the Bursell investigation into pupal dehydration are used as a rudimentary data set. These data are generalized to all temperatures and humidities by invoking the property of multiplicative separability. The problem then, is that as the temperature varies with time, so does the metabolism and the developmental stages to which the model data pertain and must necessarily warp. The puparial-duration formulae of Phelps, Burrows and Hargrove are exploited to facilitate a mapping between the constant temperature time domain of the data and that of some more general cases available. The resulting, *Glossina morsitans* model is extrapolated to other species using their relative surface areas, their relative protected and unprotected transpiration rates and their different fourth instar excretions (drawing to a lesser extent from the data of Buxton and Lewis). In this way the problem of pupal dehydration is formulated as a series of integrals and the consequent survival can be predicted. The discovery of a distinct definition for hygrophilic species within the formulation prompts the investigation of the hypothetical effect of a two-day heat wave on pupae. This leads to the conclusion that the classification of species as hygrophilic, mesophilic and xerophilic is largely true for theirthird and fourth instars and, possibly, the hours shortly before eclosion.

17459. **De Vooght, L., Caljon, G., Van Hees, J. & Van Den Abbeele, J., 2015.** Paternal transmission of a secondary symbiont during mating in the viviparous tsetse fly. *Molecular Biology & Evolution*, **32** (8): 1977-1980.

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Sodalis glossinidius, a maternally inherited secondary symbiont of the tsetse fly, is a bacterium in the early/intermediate state of the transition toward symbiosis, representing an

important model for investigating establishment and evolution of insect-bacteria symbiosis. The absence of phylogenetic congruence in tsetse-*Sodalis* coevolution and the existence of *Sodalis* genotypic diversity in field flies are suggestive of a horizontal transmission route. However, to date no natural mechanism for the horizontal transfer of this symbiont has been identified. Using novel methodologies for the stable fluorescent-labelling and introduction of modified *Sodalis* in tsetse flies, we unambiguously show that male-borne *Sodalis* is horizontally transferred to females during mating and subsequently vertically transmitted to the progeny, that is, paternal transmission. This mixed mode of transmission has major consequences regarding genome evolution as it can lead to coinfections creating opportunities for lateral gene transfer which in turn could affect the interaction with the tsetse host.

17460. **Guerra, L., Stoffolano, J. G., Jr., Belardinelli, M. C., Gambellini, G., Taddei, A. R., Laghezza Masci, V. & Fausto, A. M., 2015.** Disruption of the salivary gland muscle in tsetse, *Glossina pallidipes* Austen, as a result of salivary gland hypertrophy virus infection. *Medical & Veterinary Entomology*, **E Publication ahead of print, 16 July.**

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The secretory region of the salivary glands in *Glossina pallidipes* Austen (Diptera: *Glossinidae*) is characterized by an external muscle layer. Scanning electron microscopy and transmission electron microscopy investigations provide a detailed description of the longitudinal muscle fibres and a comparison of their structure when affected by salivary gland hypertrophy virus. The virus is responsible for hypertrophy of the salivary glands in symptomatic flies, specifically of the muscle fibres, the cytoarchitecture of which is completely altered. Although observations did not reveal viral particles in the muscle cells of either asymptomatic or symptomatic flies, muscle fibres were enlarged and detached from one another and their associated basement membrane only in symptomatic flies. A decrease in type IV collagen labelling in the basement membrane of the muscles in symptomatic flies is reported and is considered a potential cause of the salivary gland muscle alteration and, possibly, myopathy. The maintenance of an organized muscular layer is essential for the normal secretion of saliva and hence its pathology in symptomatic tsetse flies could affect the normal transmission of the trypanosome that develops inside the salivary gland epithelium. Therefore, a better understanding of the possible role of the virus is essential in order to elucidate its impact on salivary deployment in symptomatic flies.

17461**. Hrusa, G., Farmer, W., Weiss, B. L., Applebaum, T., Roma, J. S., Szeto, L., Aksoy, S. & Runyen-Janecky, L. J., 2015.** TonB-dependent heme iron acquisition in the tsetse fly symbiont *Sodalis glossinidius*. *Applied Environmental Microbiology*, **81** (8): 2900-2909.

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Sodalis glossinidius is an intra- and extracellular symbiont of the tsetse fly (*Glossina* sp.), which feeds exclusively on vertebrate blood. *S. glossinidius* resides in a wide variety of tsetse tissues and may encounter environments that differ dramatically in iron content. The *Sodalis* chromosome encodes a putative TonB-dependent outer membrane heme transporter (HemR) and a putative periplasmic/inner membrane ABC heme permease system (HemTUV). Because these gene products mediate iron acquisition processes by other enteric bacteria, we characterized their regulation and physiological role in the *Sodalis*/tsetse system. Our results show that the hemR and tonB genes are expressed by *S. glossinidius* in the tsetse fly. Furthermore, transcription of hemR in *Sodalis* is repressed in a high iron environment by the iron-responsive transcriptional regulator Fur. Expression of the *S. glossinidius* hemR and hemTUV genes in an *Escherichia coli* strain unable to use heme as an iron source stimulated growth in the presence of heme or haemoglobin as the sole iron source. This stimulation was dependent on the presence of either the *E. coli* or *Sodalis* tonB gene. *Sodalis* tonB and hemR mutant strains were defective in their ability to colonize the gut of tsetse flies that lacked endogenous symbionts, while wild-type *S. glossinidius* proliferated in this same environment. Finally, we show that the *Sodalis* HemR protein is localized to the bacterial membrane and appears to bind hemin. Collectively, this study provides strong evidence that TonB-dependent, HemR-mediated iron acquisition is important for the maintenance of symbiont homeostasis in the tsetse fly, and it provides evidence for the expression of bacterial high-affinity iron acquisition genes in insect symbionts.

17462. **Lahondere, C. & Lazzari, C. R., 2015.** Thermal effect of blood feeding in the telmophagous fly *Glossina morsitans morsitans*. *Journal of Thermal Biology*, **48**: 45-50.

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During feeding on warm-blooded hosts, haematophagous insects are exposed to thermal stress due to the ingestion of a meal whose temperature may highly exceed their own body temperature. In order to avoid overheating and its subsequent deleterious effects, these insects respond by setting up molecular protective mechanisms such as heat shock proteins synthesis or by using thermoregulative strategies. Moreover, the duration of contact with the host depends on the way of feeding displayed by the different species (either telmophagous or solenophagous) and thus also impacts their exposure to heat. Solenophagous insects feed directly on blood vessels and are relatively slow feeders while telmophagous insects, by lacerating capillaries, facilitate their access to blood and thus feed more quickly. The aim of this work was to investigate to what extent strictly telmophagous insects such as tsetse flies are exposed to thermal stress during feeding and consequently to evaluate the impact of the feeding strategy on the exposition to overheating in haematophagous insects in general. Real time thermographic analysis during feeding revealed that the flies' body significantly heat up quite homogeneously. At the end of feeding, however, a marked regional heterothermy occurs as a consequence of the alary muscles warm up that precedes take-off. Feeding strategies, either solenophagy or telmophagy, thus appear to have a great impact on both exposure to predation risks and to thermal stress.

17463. **Ooi, C. P., Haines, L. R., Southern, D. M., Lehane, M. J. & Acosta-Serrano, A., 2015.** Tsetse GmmSRPN10 has anti-complement activity and is important for successful establishment of trypanosome infections in the fly midgut. *PLoS Neglected Tropical Diseases*, **9** (1): e3448.

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The complement cascade in mammalian blood can damage the alimentary tract of haematophagous arthropods. As such, these animals have evolved their own repertoire of complement-inactivating factors, which are inadvertently exploited by blood-borne pathogens to escape complement lysis. Unlike the bloodstream stages, the procyclic (insect) stage of *Trypanosoma brucei* is highly susceptible to complement killing, which is puzzling considering that a tsetse takes a blood meal every 2-4 days. In this study, we identified four tsetse (*Glossina morsitans morsitans*) serine protease inhibitors (serpins) from a midgut expressed sequence tag (EST) library (GmmSRPN3, GmmSRPN5, GmmSRPN9 and GmmSRPN10) and investigated their role in modulating the establishment of a *T. brucei* infection in the midgut. Although not having evolved in a common blood-feeding ancestor, all four serpins have an active site sharing remarkable homology with the human complement C1-inhibitor serpin, SerpinG1. RNAi knockdown of individual GmmSRPN9 and GmmSRPN10 genes resulted in a significantly decreased rate of infection by procyclic form *T. brucei*. Furthermore, recombinant GmmSRPN10 was both able to inhibit the activity of human complement-cascade serine proteases, C1s and Factor D, and to protect the *in vitro* killing of procyclic trypanosomes when incubated with complement-activated human serum. Thus, the secretion of serpins, which may be part of a blood meal complement inactivation system in tsetse, is used by procyclic trypanosomes to evade an influx of fresh trypanolytic complement with each blood meal. This highlights another facet of the complicated relationship between *T. brucei* and its tsetse vector, where the parasite takes advantage of tsetse physiology to further its chances of propagation and transmission.

17464. **Snyder, A. K. & Rio, R. V., 2015.** *Wigglesworthia morsitans* folate (vitamin B_9) biosynthesis contributes to tsetse host fitness. *Applied Environmental Microbiology*, **81** (16): 5375-5386.

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Closely related ancient endosymbionts may retain minor genomic distinctions through evolutionary time, yet the biological relevance of these small pockets of unique loci remains unknown. The tsetse fly (Diptera: *Glossinidae*), the sole vector of lethal African trypanosomes (*Trypanosoma* spp.), maintains an ancient and obligate mutualism with species belonging to the gammaproteobacterium *Wigglesworthia*. Extensive concordant evolution with associated *Wigglesworthia* species has occurred through tsetse species radiation. Accordingly, the retention of unique symbiont loci between *Wigglesworthia* genomes may prove instrumental toward host species-specific biological traits. Genome distinctions between *Wigglesworthia morsitans* (harboured within *Glossina morsitans* bacteriomes) and the basal species *Wigglesworthia glossinidia* (harboured within *Glossina brevipalpis* bacteriomes) include the retention of chorismate and downstream folate (vitamin B_9) biosynthesis capabilities, contributing to distinct symbiont metabolomes. Here, we demonstrate that these *W. morsitans* pathways remain functionally intact, with folate likely being systemically disseminated through a synchronously expressed tsetse folate transporter within bacteriomes. The folate produced by *W. morsitans* is demonstrated to be pivotal for *G. morsitans* sexual maturation and

reproduction. Modest differences between ancient symbiont genomes may still play key roles in the evolution of their host species, particularly if loci are involved in shaping host physiology and ecology. Enhanced knowledge of the *Wigglesworthia*-tsetse mutualism may also provide novel and specific avenues for vector control.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

[See also **38**: 17429].

17465. **Albert, M., Wardrop, N. A., Atkinson, P. M., Torr, S. J. & Welburn, S. C., 2015.** Tsetse fly (*G. f. fuscipes*) distribution in the Lake Victoria basin of Uganda. *PLoS Neglected Tropical Diseases*, **9** (4): e0003705.

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Tsetse flies transmit trypanosomes, the causative agent of human and animal African trypanosomiases. The tsetse vector is extensively distributed across sub-Saharan Africa. Trypanosomiasis maintenance is determined by the interrelationship of three elements: vertebrate host, parasite and the vector responsible for transmission. Mapping the distribution and abundance of tsetse flies assists in predicting trypanosomiasis distributions and developing rational strategies for disease and vector control. Given scarce resources to carry out regular full scale field tsetse surveys to up-date existing tsetse maps, there is a need to devise inexpensive means for regularly obtaining dependable area-wide tsetse data to guide control activities. In this study we used spatial epidemiological modelling techniques (logistic regression) involving 5 000 field-based tsetse-data (*G. f. fuscipes*) points over an area of 40 000 km^2, with satellite-derived environmental surrogates composed of precipitation, temperature, land cover, normalised difference vegetation index (NDVI) and elevation at the sub-national level. We used these extensive tsetse data to analyse the relationships between presence of tsetse (*G. f. fuscipes*) and environmental variables. The strength of the results was enhanced through the application of a spatial autologistic regression model (SARM). Using the SARM we showed that the probability of tsetse presence increased with the proportion of forest cover and riverine vegetation. The key outputs are a predictive tsetse distribution map for the Lake Victoria basin of Uganda and an improved understanding of the association between tsetse presence and environmental variables. The predicted spatial distribution of tsetse in the Lake Victoria basin of Uganda will provide significant new information to assist with the spatial targeting of tsetse and trypanosomiasis control.

17466**. Bass, B., Traore, D., Diakité, M., Diall, Y. G., Mariko, I., Bengaly, S., Boiré, S., Traore, K., Kone, F., Diarra, C. O., Sissoko, M., Samake, T., Diarra, A. & Fofana, A., 2015**. Updating of data on the distribution of tsetse in the cotton area of Kita in Mali. *Bulletin de la santé et de la production Animales en Afrique* (**in press).**

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Housed in the transition area between the semi-arid zone in the north and the wet zone in the south, the cotton zone of Kita suffered strong anthropogenic and climatic pressures between 1990 and 2000. Drought, desertification and economic activities based on agriculture and livestock have contributed to the degradation of the area. An entomological survey in the study area between June and November 2013 led to the capture of two tsetse species: *Glossina palpalis gambiensis* and *Glossina tachinoides.* In an earlier study of tsetse distribution in Mali, Djiteye *et al*. (1997) noted the presence of three tsetse species in the cotton zone of Kita: *Glossina palpalis gambiensis*, *Glossina tachinoides*, and *Glossina morsitans submorsitans.* However, *Glossina palpalis gambiensis* no longer exists in the localities of Manacoura, Djidian and Sandiabougou, representing a decline of more than 120 km compared with that found by Djiteye *et al.* (1997). Also, *Glossina tachinoides* which was formerly present in the localities of Beyon and Bougarbaya is now absent, indicating a decline of more than 80 km. Likewise, *Glossina morsitans submorsitans* is absent in the study localities while it was captured by Djiteye *et al.* (1997). Results of the present study show that through their actions on the environment and climatic changes, humans have reduced the distribution area of tsetse towards the south and caused the disappearance of *Glossina morsitans submorsitans.*

17467. **Bass, B., Bagayoko, M., Traore, D. & Kone, F., 2115.** Survey of *Glossina* and other biting flies in the circles of Sikasso and Kadiolo (Mali) as a prelude to a control campaign. *Bulletin de la santé et de la production Animales en Afrique* (**in press).**

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The objective of the FAO-TCP project MLI/3402I is to initiate an effective campaign for long-lasting protection of cattle against African animal trypanosomosis (AAT) through control of the tsetse fly combined with strategic treatments of animals to reduce resistance to trypanocidal drugs. Before implementing these activities, a baseline entomological survey was initiated by the Central Veterinary Laboratory. Thirty five villages were canvased in the circles of Kadiolo (20 villages in nine municipalities) and Sikasso (15 villages in four municipalities). For the study, 350 Challier-Laveissière biconical traps were placed in galleries and at the level of the contact points of various streams, their geography referenced by means of GPS. The entomological survey led to the capture of two *Glossina* species: *Glossina palpalis gambiensis* and *Glossina tachinoides*. In total, 786 *Glossina* were captured, of which 405 were males and 381 females. Six hundred and ninety one *Glossina palpalis gambiensis* were captured, of which 349 were males and 342 females, and 95 *Glossina tachinoides* among which 56 males and 39 females. The rate of infection of the tsetse flies in the circle of Kadiolo only was 43 percent (175/402 *Glossina*).

17468. **Cecchi, G., Paone, M., Argiles Herrero, R., Vreysen, M. J. & Mattioli, R. C., 2015.** Developing a continental atlas of the distribution and trypanosomal infection of tsetse flies (*Glossina* species). *Parasites & Vectors*, **8**: 284.

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Tsetse flies (Genus: *Glossina*) are the sole cyclical vectors of African trypanosomoses. Despite their economic and public health impacts in sub-Saharan Africa, it has been decades since the latest distribution maps at the continental level were produced. The Food and Agriculture Organization of the United Nations is trying to address this shortcoming through the Atlas of tsetse and African animal trypanosomosis. For the tsetse component of the Atlas, a geospatial database is being assembled which comprises information on the distribution and trypanosomal infection of *Glossina* species. Data are identified through a systematic literature review. Field data collected since January 1990 are included, with a focus on occurrence, apparent density and infection rates of tsetse flies. Mapping is carried out at the level of site/location. For tsetse distribution, the database includes such ancillary information items as survey period, trap type, attractant (if any), number of traps deployed in the site and the duration of trapping (in days). For tsetse infection, the sampling and diagnostic methods are also recorded. As a proof of concept, tsetse distribution data for three pilot countries (Ethiopia, Kenya and Uganda) were compiled from 130 peer-reviewed publications, which enabled tsetse occurrence to be mapped in 1 266 geographic locations. Maps were generated for eight tsetse species (i.e. *G. brevipalpis*, *G. longipennis*, *G. fuscipes fuscipes*, *G. tachinoides*, *G. pallidipes*, *G. morsitans submorsitans*, *G. austeni* and *G. swynnertoni*). For tsetse infection rates, data were identified in 25 papers, corresponding to 91 sites. A methodology was developed to assemble a geo-spatial database on the occurrence, apparent density and trypanosomal infection of *Glossina* species, which will enable continental maps to be generated. The methodology is suitable for broad brush mapping of all tsetse species of medical and veterinary public health importance. For a few tsetse species, especially those having limited economic importance and circumscribed geographic distribution (e.g. *fusca* group), recently published information is scanty or non-existent. Tsetse-infested countries can adopt and adapt this approach to compile national atlases, which ought to draw also on the vast amount of unpublished information.

17469. **De Meeus, T., Bouyer, J., Ravel, S. & Solano, P., 2015.** Ecotype evolution in *Glossina palpalis* subspecies, major vectors of sleeping sickness. *PLoS Neglected Tropical Diseases*, **9** (3): e0003497.

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The role of environmental factors in driving adaptive trajectories of living organisms is still being debated. This is even more important to understand when dealing with important neglected diseases and their vectors. In this paper, we analysed genetic divergence, computed from seven microsatellite loci, of 614 tsetse flies (*Glossina palpalis gambiensis* and *Glossina palpalis palpalis*, major vectors of animal and human trypanosomes) from 28 sites of West and Central Africa. We found that the two subspecies are so divergent that they deserve the species

status. Controlling for geographic and time distances that separate these samples, which have a significant effect, we found that *G. p. gambiensis* from different landscapes (Niayes of Senegal, savannah and coastal environments) were significantly genetically different and thus represent different ecotypes or subspecies. We also confirm that *G. p. palpalis* from Ivory Coast, Cameroon and DRC are strongly divergent. These results provide an opportunity to examine whether new tsetse fly ecotypes might display different behaviour, dispersal patterns, host preferences and vectorial capacities. This work also urges a revision of taxonomic status of *Glossina palpalis* subspecies and highlights again how fast ecological divergence can be, especially in host-parasite-vector systems.

17470. **De Meeus, T., Ravel, S., Rayaisse, J. B., Kaba, D., Courtin, F., Bouyer, J., Dayo, G. K., Camara, M. & Solano, P., 2014.** Genetic correlations within and between isolated tsetse populations: what can we learn? *Acta Tropica*, **138** Suppl: S6-11.

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Isolated tsetse populations constitute a target for tsetse control programmes in endemic countries, since their isolation, if demonstrated, allows control without reinvasion risk from neighbouring populations. Population genetic parameters, such as the fixation index, have proven useful to assess isolation status, and should also give important information on the divergence time since isolation. We gathered results obtained from different datasets regarding several examples of putatively totally isolated tsetse populations of different tsetse species: *Glossina palpalis gambiensis* in Guinea, in the Niayes of Senegal, and in the sacred wood of Bama in Burkina Faso; and *G. tachinoides* from Bitou and Pama in south-east Burkina Faso. The different levels of isolation were compared to differentiation between the two subspecies *G. p. gambiensis* and *G. p. palpalis* which both occur allopatrically along the Comoe River in Ivory Coast. We also use some historical evidence to calibrate differentiation speed and give estimates of time since separation for the different cases studied. Discrepancies mostly come from underestimates of effective population sizes, and we propose improving sampling design and genetic markers quality to circumvent such caveats.

17471**. Getahun, M. N., Cecchi, G. & Seyoum, E., 2014.** Population studies of *Glossina pallidipes* in Ethiopia: emphasis on cuticular hydrocarbons and wing morphometric analysis. *Acta Tropica*, **138** Suppl: S12-21.

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Tsetse flies, like many insects, use pheromones for inter- and intra-specific

communication. Several of their pheromones are cuticular hydrocarbons (CHCs) that are perceived by contact at close range. We hypothesized that for successful implementation of the sterile insect technique (SIT), along with proper identification of target area and target species, the target tsetse populations and the sterile flies must chemically communicate with each other. To study the population structuring of *Glossina pallidipes* in Ethiopia, CHCs were extracted and analysed from three tsetse belts. As a comparative approach, wing morphometric analysis was performed. The analysis of the relative abundance of CHCs revealed that populations of *G. pallidipes* from the Rift Valley tsetse belt showed a distinct clustering compared with populations from the other two belts. The spatial pattern of CHC differences was complemented by the wing morphometric analysis. Our data suggest that CHCs of known biological and ecological role, when combined with wing morphometric data, will provide an alternative means for the study of population structuring of *Glossina* populations. This could aid the planning of area wide control strategies using SIT, which is dependent on sexual competence.

17472. **Hargrove, J. W. & Ackley, S. F., 2015.** Mortality estimates from ovarian age distributions of the tsetse fly *Glossina pallidipes* Austen sampled in Zimbabwe suggest the need for new analytical approaches. *Bulletin of Entomological Research*, **105** (3): 294-304.

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Mortality estimates are central to understanding tsetse fly population dynamics, but are difficult to acquire from wild populations. They can be obtained from age distribution data but, with limited data, it is unclear whether the assumptions required to make the estimates are satisfied and, if not, how violations affect the estimates. We evaluate the assumptions required for existing mortality estimation techniques using long-term longitudinal ovarian dissection data from 144 106 female tsetse, *Glossina pallidipes* Austen, captured in Zimbabwe between 1988 and 1999. At the end of the hot-dry season each year, mean ovarian ages peaked, and maximum-likelihood mortality estimates declined to low levels, contrary to mark-recapture estimates, suggesting violations of the assumptions underlying the estimation technique. We demonstrate that age distributions are seldom stable for *G. pallidipes* at our study site, and hypothesize that this is a consequence of a disproportionate increase in the mortality of pupae and young adults at the hottest times of the year. Assumptions of age-independent mortality and capture probability are also violated, the latter bias varying with capture method and with pregnancy and nutritional status. As a consequence, mortality estimates obtained from ovarian dissection data are unreliable. To overcome these problems we suggest simulating female tsetse populations, using dynamical modelling techniques that make no assumptions about the stability of the age distribution.

17473. **Hargrove, J. W. & Muzari, M. O., 2015.** Artificial warthog burrows used to sample adult and immature tsetse (*Glossina* spp.) in the Zambezi Valley of Zimbabwe. *PLoS Neglected Tropical Diseases*, **9** (3): e0003565.

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The biology of adult tsetse (*Glossina* spp.), vectors of trypanosomiasis in Africa has been extensively studied, but little is known about larviposition in the field. In September-November 1998, in the hot-dry season in Zimbabwe's Zambezi Valley, we used artificial warthog burrows to capture adult females as they deposited larvae. Females were subjected to ovarian dissection and were defined as perinatal flies, assumed to have entered burrows to larviposit, if oocyte sizes indicated > 95 percent pregnancy completion. Perinatal flies were defined as full-term pregnant if there was a late third instar larva *in utero*, or postpartum if the uterus was empty. All other females were defined as pre-full-term pregnant (pre-FT). Of 845 *G. m. morsitans* captured, 91 percent (765) were female and 295/724 (41 percent) of females dissected were perinatal flies. By contrast, of 2 805 *G. pallidipes* captured only 71 percent (2 003) were female and only 33 percent (596/1 825) of females were perinatal. Among all perinatal females 67 percent (596/891) were *G. pallidipes*. Conversely, in burrows not fitted with traps–such that flies were free to come and go–1 834 (59 percent) of pupae deposited were *G. m. morsitans* and only 1 297 (41 percent) were *G. pallidipes*. Thus, while more full-term pregnant *G. pallidipes* enter burrows, greater proportions of *G. m. morsitans* larviposit in them, reflecting a greater discrimination among *G. pallidipes* in choosing larviposition sites. Catches of males and pre-FT females increased strongly with temperatures above 32 ^{0}C, indicating that these flies used burrows as refuges from high ambient temperatures. Conversely, catches of perinatal females changed little with maximum temperature but declined from late September through November: females may anticipate that burrows will be inundated during the forthcoming wet season. Ovarian age distributions of perinatal and pre-FT females were similar, consistent with all ages of females larvipositing in burrows with similar probability. It is concluded that artificial warthog burrows provide a novel method for collecting tsetse pupae, studying tsetse behaviour at larviposition, assessing the physiological status of female tsetse and their larvae, and of improving understanding of the physiological dynamics of terminal pregnancy, and population dynamics generally, with a view to improving methods of trypanosomiasis control.

17474. **Kato, A. B., Hyseni, C., Okedi, L. M., Ouma, J. O., Aksoy, S., Caccone, A. & Masembe, C., 2015.** Mitochondrial DNA sequence divergence and diversity of *Glossina fuscipes fuscipes* in the Lake Victoria basin of Uganda: implications for control. *Parasites & Vectors*, **8** (1): 385.

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Glossina fuscipes fuscipes is the main vector of African trypanosomiasis affecting both humans and livestock in Uganda. The human disease (sleeping sickness) manifests itself in two forms: acute and chronic. The Lake Victoria basin in Uganda has the acute form and a history of tsetse re-emergence despite concerted efforts to control tsetse. The government of Uganda has targeted the basin for tsetse eradication. To provide empirical data for this initiative, we screened tsetse flies from the basin for genetic variation at the mitochondrial DNA cytochrome oxidase II (mtDNA COII) gene with the goal of investigating genetic diversity and gene flow among tsetse, tsetse demographic history, and comparing these results with results from a previous study based on microsatellite loci data in the same area. We collected 429 *Gff* tsetse

fly samples from 14 localities in the entire Ugandan portion of the Lake Victoria coast, covering 40 000 km^2. We performed genetic analyses on them and added data collected for 56 *Gff* individuals from four additional sampling sites in the basin. The 529 bp partial mitochondrial DNA cytochrome oxidase II (mtDNA COII) sequences totalling 485 were analysed for genetic differentiation, structuring and demographic history. The results were compared with findings from a previous study based on microsatellite loci data from the basin. The differences within sampling sites explained a significant proportion of the genetic variation. We found three very closely related mtDNA population clusters, which co-occurred in multiple sites. Although Phi ST (0 - 0.592; $p < 0.05$) and Bayesian analyses suggest some level of weak genetic differentiation, there is no correlation between genetic divergence and geographic distance ($r = 0.109$, $p = 0.185$), and demographic tests provide evidence of locality-based demographic history. The mtDNA data analysed here complement inferences made in a previous study based on microsatellite data. Given the differences in mutation rates, mtDNA afforded a look further back in time than microsatellites and revealed that *Gff* populations were more connected in the past. Microsatellite data revealed more genetic structuring than mtDNA. The differences in connectedness and structuring over time could be related to vector control efforts. Tsetse re-emergence after control interventions may be due to re-invasions from outside the treated areas, which emphasizes the need for an integrated area-wide tsetse eradication strategy for sustainable removal of the tsetse and trypanosomiasis problem from this area.

17475. **Melachio, T. T., Njiokou, F., Ravel, S., Simo, G., Solano, P. & De Meeus, T., 2015.** Effect of sampling methods, effective population size and migration rate estimation in *Glossina palpalis palpalis* from Cameroon. *Infection, Genetics & Evolution*, **33**: 150-157.

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Human and animal trypanosomiases are two major constraints to development in Africa. These diseases are mainly transmitted by tsetse flies in particular by *Glossina palpalis palpalis* in Western and Central Africa. To set up an effective vector control campaign, prior population genetics studies have proved useful. Previous studies on population genetics of *G. p. palpalis* using microsatellite loci showed high heterozygote deficits, as compared to Hardy-Weinberg expectations, mainly explained by the presence of null alleles and/or the mixing of individuals belonging to several reproductive units (Wahlund effect). In this study we implemented a system of trapping, consisting of a central trap and two to four satellite traps around the central one to evaluate a possible role of the Wahlund effect in tsetse flies from three Cameroon human and animal African trypanosomiases foci (Campo, Bipindi and Fontem). We also estimated effective population sizes and dispersal. No differences were observed between the values of allelic richness, genetic diversity and Wright's F_{IS}, in the samples from central and from satellite traps, suggesting an absence of Wahlund effect. Partitioning of the samples with Bayesian methods showed numerous clusters of 2-3 individuals as expected from a population at demographic equilibrium with two expected offspring per reproducing female. As previously shown, null alleles appeared as the most probable factor inducing these heterozygote deficits

in these populations. Effective population sizes varied from 80 to 450 individuals while immigration rates were between 0.05 and 0.43, showing substantial genetic exchanges between different villages within a focus. These results suggest that "suppression" with establishment of physical barriers may be the best strategy for a vector control campaign in this forest context.

17476. **Rayaisse, J. B., Salou, E., Kiema, S., Akoudjin, M., Kaba, D., Kagbadouno, M., Djohan, V., Camara, M., Dayo, G. K., Courtin, F., Solano, P. & Bouyer, J., 2015.** Tsetse diversity and abundance in southern Burkina Faso in relation with the vegetation. *Parasitology Research*, **114***:* 3357-3363.

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The increase of human population, combined with climatic changes, contributed to the modification of spatial distribution of tsetse flies, the main vector of trypanosomiasis. In order to establish and compare tsetse presence and its relationship with vegetation, an entomological survey was performed using biconical traps deployed in transects simultaneously with a phyto-sociological study, on the Comoe river at its source in the village of Moussodougou, and in the semi-protected area of Folonzo, both localities being in southern Burkina Faso. In Folonzo, the survey revealed a diversity of tsetse with four species occurring with apparent densities as follows: *Glossina tachinoides* (8.9 tsetse/trap/day); *G. morsitans submorsitans* (1.8 tsetse/trap/day); *G. palpalis gambiensis* (0.6 tsetse/trap/day) and *G. medicorum* (0.15 tsetse/trap/day). In Moussodougou, a highly anthropized area, mainly *G. p. gambiensis* was caught (2.06 tsetse/trap/day), and rarely *G. tachinoides*. The phyto-sociological study allowed discrimination of six types of vegetation in both localities, with three concordances that are riparian forest, shrubby and woody savannah. In Moussodougou, all tsetse were caught in the riparian forest. That was also the case in Folonzo where a great proportion (95 to 99 percent depending on the season) of *G. p. gambiensis* and *G. tachinoides* were caught in the gallery, while *G. m. submorsitans* occurred both in the gallery and in the savannah, and *G. medicorum* in the forest gallery. This study showed that although *G. tachinoides* and *G. p. gambiensis* are both riparian, they do not have the same preference in terms of biotope.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also **38:** 17473, 17474].

17477. **Bauer, B. & Baumann, M. P., 2015.** Laboratory evaluation of efficacy and persistence of a 1 percent w/w fipronil pour-on formulation (Topline®) against *Glossina palpalis gambiensis*, Diptera: *Glossinidae*. *Parasitology Research*, **114** (8): 2919-2923.

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One Zebu bull of 365 kg live weight was treated along the back line with 36 mL of fipronil as a pour-on formulation. Long-lasting mortalities of *Glossina palpalis gambiensis* were recorded despite exposure to sunlight and regular rinsing with 50 L of water during the following five months. Significantly higher mortalities were still observed even 140, 170 and 190 days after treatment following their triple releases or triple feeding of caged tsetse on the treated bull. Mortalities of 70, 80 and 44 percent, respectively, were recorded after 15 days of observation. This contrasted with the mortalities of control flies that were released in the

presence of the untreated bull or fed in cages on the animal, amounting to 20 and twice 10 percent after 170 and 190 days. The feeding successes of the released or caged flies were higher than 95 percent and did not differ between control and experimental groups, indicating no repulsive or irritant effects of fipronil. The findings of this study are discussed, particularly in view of the potential of fipronil as an effective means for tsetse control.

17478. **Bett, M. K., Saini, R. K. & Hassanali, A., 2015.** Repellency of tsetse-refractory waterbuck (*Kobus defassa*) body odour to *Glossina pallidipes* (Diptera: *Glossinidae*): assessment of relative contribution of different classes and individual constituents. *Acta Tropica*, **146**: 17-24.

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Our earlier studies on the comparative behavioural responses of caged savanna tsetse (*Glossina morsitans morsitans* and *Glossina pallidipes*) on a preferred host (ox) and a non-host (waterbuck) suggested the presence of allomonal constituents on the latter. Follow up comparison of the compositions of odours of waterbuck with those of ox and buffalo led to the identification of a series of compounds (15) specific to waterbuck, including straight chain carboxylic acid (C5-C10), phenols (guaiacol and carvacrol), 2-alkanone homologues (C8-C12), geranylacetone and delta-octalactone. Behavioural studies in a wind tunnel in the laboratory suggested that *G. m. morsitans* was repelled by a synthetic blend of waterbuck-specific constituents. In the present study, the effects of different blends of these compounds on catches of mixed sexes of *G. pallidipes* in attractant-baited NG2G traps were evaluated in the field. Each multicomponent class of constituents (acids, ketones and phenols) was found to reduce fly catches, but a 14-component blend of all these compounds was more effective (reduced catches by 79-85 percent), indicating that each of these classes of compounds contributes incrementally to the repellency of the waterbuck odour. However, subtractive assays showed some redundancy within each class of compound, with some even demonstrating attractive properties. Addition of (RS)-delta-octalactone to the 14-component significantly increased the repellency of the resulting blend. A five-component blend of compounds selected on the basis of their relative performance in subtractive assays (delta-octalactone, guaiacol, geranylacetone, hexanoic and pentanoic acid) showed substantial reduction in fly catches (84 percent) relative to the baited control. In separate sets of experiments involving an ox tethered in the middle of an incomplete ring of electric screens in the presence or absence of 15-component or five-component blends, comparable levels in the reduction of fed flies (94 and 96 percent, respectively) were obtained with the two blends. The chemo-ecological significance and practical implication of these results are highlighted.

17479. **Muhanguzi, D., Okello, W. O., Kabasa, J. D., Waiswa, C., Welburn, S. C. & Shaw, A. P., 2015.** Cost analysis of options for management of African animal trypanosomosis using interventions targeted at cattle in Tororo District; south-eastern Uganda. *Parasites & Vectors*, **8** (1): 387.

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Tsetse-transmitted African trypanosomes cause both nagana (African animal trypanosomiasis-AAT) and sleeping sickness (human African Trypanosomiasis - HAT) across sub-Saharan Africa. Vector control and chemotherapy are the contemporary methods of tsetse and trypanosomosis control in this region. In most African countries, including Uganda, veterinary services have been decentralised and privatised. As a result, livestock keepers meet the costs of most of these services. To be sustainable, AAT control programmes need to tailor tsetse control to the inelastic budgets of resource-poor small scale farmers. To guide the process of tsetse and AAT control toolkit selection, that now, more than ever before, needs to optimise resources, the costs of different tsetse and trypanosomiasis control options need to be determined. A detailed costing of the restricted application protocol (RAP) for African trypanosomosis control in Tororo District was undertaken between June 2012 and December 2013. A full cost calculation approach was used; including all overheads, delivery costs, depreciation and netting out transfer payments to calculate the economic (societal) cost of the intervention. Calculations were undertaken in Microsoft Excel without incorporating probabilistic elements. The cost of delivering RAP to the project was US$ 6.89 per animal per year while that of four doses of a curative trypanocide per animal per year was US$ 5.69. However, effective tsetse control does not require the application of RAP to all animals. Protecting cattle from trypanosome infections by spraying 25 percent, 50 percent or 75 percent of all cattle in a village costs US$ 1.72, 3.45 and 5.17 per animal per year respectively. Alternatively, a year of a single dose of curative or prophylactic trypanocide treatment plus 50 percent RAP would cost US$ 4.87 and US$ 5.23 per animal per year. Pyrethroid insecticides and trypanocides represent 22.4 and 39.1 percent of the cost of RAP and chemotherapy respectively. It is concluded that cost analyses of low cost tsetse control options should include full delivery costs since they constitute 77.6 percent of all project costs. The relatively low cost of RAP for AAT control and its collateral impact on tick control make it an attractive option for livestock management by smallholder livestock keepers.

17480. **Rayaisse, J. B., Salou, E., Courtin, F., Yoni, W., Barry, I., Dofini, F., Kagbadouno, M., Camara, M., Torr, S. J. & Solano, P., 2015.** Baited-boats: an innovative way to control riverine tsetse, vectors of sleeping sickness in West Africa. *Parasites & Vectors*, **8**: 236.

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Human African trypanosomiasis (HAT) is an important neglected tropical disease caused by *Trypanosoma* spp. parasites transmitted by species of tsetse fly (*Glossina* spp). The most important vectors of HAT are riverine tsetse and these can be controlled by attracting them to stationary baits such as insecticide-impregnated traps or targets deployed along the banks of

rivers. However, the geographical nature of some riverine habitats, particularly mangroves but also extensive lake and river networks, makes deployment of baits difficult and limits their efficacy. It is known that tsetse are attracted by the movement of their hosts. Our hypothesis was that mounting a target on canoes typically used in Africa ("pirogues") would produce an effective means of attracting-and-killing riverine tsetse in extensive wetland habitats. In Folonzo, southern Burkina Faso, studies were made of the numbers of tsetse attracted to a target (75 x 50 cm) of blue cloth and netting mounted on a pirogue moving along a river, versus the same target placed on the riverbank. The targets were covered with a sticky film which caught tsetse as they contacted the target. The pirogue-mounted target caught twice as many *G. tachinoides* and *G. p. gambiensis*, and eight times more *G. morsitans submorsitans* than the stationary one ($p < 0.001$). In conclusion, pirogues are common vehicle for navigating the rivers, lakes and swamps of West Africa. The demonstration that tsetse can be attracted to targets mounted on such boats suggests that pirogues might provide a cost-effective and convenient platform for deploying targets to control tsetse in the mangrove systems of West Africa where HAT persists. Further studies to assess the impact of pirogue-mounted targets on tsetse populations in HAT foci and the protective value of targets for pirogue passengers are recommended.

17481. **Shaw, A. P., Tirados, I., Mangwiro, C. T., Esterhuizen, J., Lehane, M. J., Torr, S. J. & Kovacic, V., 2015.** Costs of using "tiny targets" to control *Glossina fuscipes fuscipes*, a vector of *gambiense* sleeping sickness in Arua District of Uganda. *PLoS Neglected Tropical Diseases*, **9** (3): e0003624.

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To evaluate the relative effectiveness of tsetse control methods, their costs need to be analysed alongside their impact on tsetse populations. Very little has been published on the costs of methods specifically targeting human African trypanosomiasis. In northern Uganda, a 250 km^2 field trial was undertaken using small (0.5 x 0.25 m) insecticide-treated targets ("tiny targets"). Detailed cost recording accompanied every phase of the work. Costs were calculated for this operation as if managed by the Ugandan vector control services: removing purely research components of the work and applying local salaries. This calculation assumed that all resources are fully used, with no spare capacity. The full cost of the operation was assessed at US$ 85.4 per km^2, of which US$ 55.7 or 65.2 percent were field costs, made up of three component activities (target deployment: 34.5 percent, trap monitoring: 10.6 percent and target maintenance: 20.1 percent). The remaining US$ 29.7 or 34.8 percent of the costs were for preliminary studies and administration (tsetse surveys: 6.0 percent, sensitisation of local populations: 18.6 percent and office support: 10.2 percent). Targets accounted for only 12.9 percent of the total cost, other important cost components were labour (24.1 percent) and transport (34.6 percent). Comparison with the updated cost of historical HAT vector control projects and recent estimates indicates that this work represents a major reduction in cost levels. This is attributed not just to the low unit cost of tiny targets but also to the organisation of delivery, using local labour with bicycles or motorcycles. Sensitivity analyses were undertaken, investigating key prices and assumptions. It is believed that these costs are generalizable to

other HAT foci, although in more remote areas, with denser vegetation and fewer people, costs would increase, as would be the case for other tsetse control techniques.

17482. **Shaw, A. P., Wint, G. R., Cecchi, G., Torr, S. J., Mattioli, R. C. & Robinson, T. P., 2015.** Mapping the benefit-cost ratios of interventions against bovine trypanosomosis in Eastern Africa. *Preventive Veterinary Medicine.* **E Publication ahead of print 18 June.**

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This study builds upon earlier work mapping the potential benefits from bovine trypanosomosis control and analysing the costs of different approaches. Updated costs were derived for five intervention techniques: trypanocides, targets, insecticide-treated cattle, aerial spraying and the release of sterile males. Two strategies were considered: continuous control and elimination. For mapping the costs, cattle densities, environmental constraints, and the presence of savannah or riverine tsetse species were taken into account. These were combined with maps of potential benefits to produce maps of benefit-cost ratios. The results illustrate a diverse picture, and they clearly indicate that no single technique or strategy is universally profitable. For control using trypanocide prophylaxis, returns are modest, even without accounting for the risk of drug resistance but, in areas of low cattle densities, this is the only approach that yields a positive return. Where cattle densities are sufficient to support it, the use of insecticide-treated cattle stands out as the most consistently profitable technique, widely achieving benefit-cost ratios above five. In parts of the high-potential areas such as the mixed farming, high-oxen-use zones of western Ethiopia, the fertile crescent north of Lake Victoria and the dairy production areas in western and central Kenya, all tsetse control strategies achieve benefit-cost ratios from two to over 15, and for elimination strategies, ratios from five to over 20. By contrast, in some areas, notably where cattle densities are below 20 per km^2, the costs of interventions against tsetse match or even outweigh the benefits, especially for control scenarios using aerial spraying or the deployment of targets where both savannah and riverine flies are present. If the burden of human African trypanosomiasis was factored in, the benefit-cost ratios of some of the low-return areas would be considerably increased. Comparatively, elimination strategies give rise to higher benefit-cost ratios than do those for continuous control. However, the costs calculated for elimination assume problem-free, large scale operations, and they rest on the outputs of entomological models that are difficult to validate in the field. Experience indicates that the conditions underlying successful and sustained elimination campaigns are seldom met. By choosing the most appropriate thresholds for benefit-cost ratios, decision-makers and planners can use the maps to define strategies, assist

in prioritising areas for intervention, and help choose among intervention techniques and approaches. The methodology would have wider applicability in analysing other disease constraints with a strong spatial component.

17483. **Vale, G. A., Hargrove, J. W., Lehane, M. J., Solano, P. & Torr, S. J., 2015.** Optimal strategies for controlling riverine tsetse flies using targets: a modelling study. *PLoS Neglected Tropical Diseases*, **9** (3): e0003615.

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Tsetse flies occur in much of sub-Saharan Africa where they transmit the trypanosomes that cause the diseases of sleeping sickness in humans and nagana in livestock. One of the most economical and effective methods of tsetse control is the use of insecticide-treated screens, called targets, that simulate hosts. Targets have been ~1 m^2, but recently it was shown that those tsetse that occupy riverine situations, and which are the main vectors of sleeping sickness, respond well to targets only ~0.06 m^2. The cheapness of these tiny targets suggests the need to reconsider what intensity and duration of target deployments comprise the most cost-effective strategy in various riverine habitats. A deterministic model, written in Excel spreadsheets and managed by Visual Basic for Applications, simulated the births, deaths and movement of tsetse confined to a strip of riverine vegetation composed of segments of habitat in which the tsetse population was either self-sustaining, or not sustainable unless supplemented by immigrants. Results suggested that in many situations the use of tiny targets at high density for just a few months per year would be the most cost-effective strategy for rapidly reducing tsetse densities by the ~90 percent expected to have a great impact on the incidence of sleeping sickness. Local elimination of tsetse becomes feasible when targets are deployed in isolated situations, or where the only invasion occurs from populations that are not self-sustaining. In conclusion, seasonal use of tiny targets deserves field trials. The ability to recognise habitats that contain tsetse populations which are not self-sustaining could improve the planning of all methods of tsetse control, against any species, in riverine, savannah or forest situations. Criteria to assist such recognition are suggested.

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

[See also **38:** 17454, 17463, 17468].

17484. **Aksoy, S., Weiss, B. L. & Attardo, G. M., 2014.** Trypanosome transmission dynamics in tsetse. *Current Opinion in Insect Science*, **3**: 43-49.

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Tsetse flies (Diptera: *Glossinidae*) are vectors of African trypanosomes. Tsetse undergo viviparous reproductive biology, and depend on their obligate endosymbiont (genus

Wigglesworthia) for the maintenance of fecundity and immune system development. Trypanosomes establish infections in the midgut and salivary glands of the fly. Tsetse's resistance to trypanosome infection increases as a function of age. Among the factors that mediate resistance to parasites are antimicrobial peptides (AMPs) produced by the immune deficiency (Imd) signalling pathway, peptidoglycan recognition protein (PGRP) LB, tsetse-EP protein and the integrity of the midgut peritrophic matrix (PM) barrier. The presence of obligate *Wigglesworthia* during larval development is essential for adult immune system maturation and PM development. Thus, *Wigglesworthia* prominently influences the vector competency of its tsetse host.

17485. **Alibu, V. P., Enyaru, J. C. K., Matovu, E., Malele, I. I., Chisi, J. E., Mbongo, N., Mansinsa, P., Intisar, E. R., Mohammed, Y., Abdelrahman, M. M., Ochi, E. B. & Lukaw, Y. S., 2015.** Molecular xenomonitoring of trypanosomes in tsetse flies. *Journal of Parasitology & Vector Biology,* **7**(6): 108-114.

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Monitoring trypanosomes infections in wild-caught tsetse flies in a given area is important for predicting epidemic outbreaks and spread of disease, and could help focus control programmes on areas requiring immediate attention in order to limit disease transmission and spread. The main objective of this study was to evaluate the recently developed RIME LAMP and PanTryp LAMP for screening large numbers of tsetse flies for trypanosomes and to assess their sensitivities and specificities for trypanosomes in endemic areas. Wild-caught tsetse flies were dissected and the mid-guts examined by microscopy. The mid-guts were pooled in fives (including one infected gut where applicable), homogenised and DNA extracted by Quiagen kits. TBR- and ITS-PCRs were carried out and examined under ethidium bromide stained agarose gels while RIMELAMP and PanTryp LAMP were carried out and stained with SYBR green and also observed under ethidium bromide stained agarose gels. A total of 14 912 tsetse flies identified as *Glossina fuscipes fuscipes, Glossina pallidipes, Glossina morsitans, Glossina swynnertoni* and *Glossina fuscipes quazensis* were trapped from the six different countries. Of these, 8 789 were dissected. Both males and female tsetse flies had equal infection rates (12.2 percent) although overall infection rates varied with country. The highest number of infected tsetse flies was obtained by PanTryp LAMP followed by RIME LAMP, ITS-PCR, TBR-PCR and microscopy respectively. PanTryp LAMP was the most sensitive method followed by ITS-PCR, RIME LAMP and TBR-PCR respectively. However, ITS-PCR was the most specific followed by TBR-PCR, RIME LAMP and PanTryp LAMP respectively. Carrying out LAMP tests in the field provides the simplest and quickest means to estimate trypanosome infection rates in the vector tsetse flies.

17486. **Birhanu, H., Fikru, R., Mussa, S., Weldu, K., Tadesse, G., Ashenafi, H., Tola, A.,**

Tesfaye, D., Berkvens, D., Goddeeris, B. M. & Buscher, P., 2015. Epidemiology of *Trypanosoma evansi* and *Trypanosoma vivax* in domestic animals from selected districts of Tigray and Afar regions, northern Ethiopia. *Parasites & Vectors*, **8** (1): 212.

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African animal trypanosomosis, transmitted cyclically by tsetse flies or mechanically by other biting flies, causes serious inflictions to livestock health. This study investigates the extent of non-tsetse transmitted animal trypanosomosis (NTTAT) by *Trypanosoma* (*T.*) *evansi* and *T. vivax* in domestic animals in the tsetse-free regions of northern Ethiopia, Afar and Tigray. A cross sectional study was conducted on 754 dromedary camels, 493 cattle, 264 goats, 181 sheep, 84 donkeys, 25 horses and 10 mules. The micro haematocrit centrifugation technique was used as the parasitological test. Plasma was collected for serodiagnosis with CATT/*T. evansi* and RoTat 1.2 immune trypanolysis (ITL) while buffy coat specimens were collected for molecular diagnosis with *T. evansi* type A specific RoTat 1.2 PCR, *T. evansi* type B specific EVAB PCR and *T. vivax* specific TvPRAC PCR. The parasitological prevalence was 4.7 percent in Tigray and 2.7 percent in Afar and was significantly higher ($p = 0.011$) in cattle (7.3 percent) than in the other hosts. Seroprevalence using CATT/*T. evansi* was 24.6 percent in Tigray and 13.9 percent in Afar and was significantly higher ($p < 0.001$) in cattle (37.3 percent) than in the other hosts. On the other hand, seroprevalence assessed by ITL was only 1.9 percent suggesting cross reaction of CATT/*T. evansi* with *T. vivax* or other trypanosome infections. Molecular prevalence of *T. evansi* type A was 8.0 percent in Tigray and in Afar and varied from 28.0 percent in horses to 2.2 percent in sheep. It was also significantly higher ($p < 0.001$) in camel (11.7 percent) than in cattle (6.1 percent), donkey (6 percent), goat (3.8 percent), and sheep (2.2 percent). Four camels were positive for *T. evansi* type B. Molecular prevalence of *T. vivax* was 3.0 percent and was similar in Tigray and Afar. It didn't differ significantly among the host species except that it was not detected in horses and mules. In conclusion, NTTAT caused by *T. vivax* and *T. evansi*, is an important threat to animal health in Tigray and Afar. For the first time, we confirm the presence of *T. evansi* type B in Ethiopian camels. Unexplained results obtained with the current diagnostic tests in bovines warrant particular efforts to isolate and characterise trypanosome strains that circulate in Northern Ethiopia.

17487. **Caljon, G., Hussain, S., Vermeiren, L. & Van Den Abbeele, J., 2015.** Description of a nanobody-based competitive immunoassay to detect tsetse fly exposure. *PLoS Neglected Tropical Diseases*, **9** (2): e0003456.

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Tsetse flies are the main vectors of human and animal African trypanosomes. The Tsal proteins in tsetse fly saliva were previously identified as suitable biomarkers of bite exposure. A new competitive assay was conceived based on nanobody (Nb) technology to ameliorate the detection of anti-Tsal antibodies in mammalian hosts. A camelid-derived Nb library was generated against the *Glossina morsitans morsitans* sialome and exploited to select Tsal specific Nbs. One of the three identified Nb families (family III, TsalNb-05 and TsalNb-11) was found suitable for anti-Tsal antibody detection in a competitive ELISA format. The competitive ELISA was able to detect exposure to a broad range of tsetse species (*G. morsitans morsitans*, *G. pallidipes*, *G. palpalis gambiensis* and *G. fuscipes*) and did not cross-react with the other haematophagous insects (*Stomoxys calcitrans* and *Tabanus yao*). Using a collection of plasmas from tsetse-exposed pigs, the new test characteristics were compared with those of the previously described *G. m. morsitans* and rTsal1 indirect ELISAs, revealing equally good specificities (> 95 percent) and positive predictive values (> 98 percent) but higher negative predictive values and hence increased sensitivity (> 95 percent) and accuracy (> 95 percent). In conclusion, we have developed a highly accurate Nb-based competitive immunoassay to detect specific anti-Tsal antibodies induced by various tsetse fly species in a range of hosts. We propose that this competitive assay provides a simple serological indicator of tsetse fly presence without the requirement of test adaptation to the vertebrate host species. In addition, the use of monoclonal Nbs for antibody detection is innovative and could be applied to other tsetse fly salivary biomarkers in order to achieve a multi-target immunoprofiling of hosts. In addition, this approach could be broadened to other pathogenic organisms for which accurate serological diagnosis remains a bottleneck.

17488. **Dicko, A. H., Percoma, L., Sow, A., Adam, Y., Mahama, C., Sidibe, I., Dayo, G. K., Thevenon, S., Fonta, W., Sanfo, S., Djiteye, A., Salou, E., Djohan, V., Cecchi, G. & Bouyer, J., 2015.** A spatio-temporal model of African animal trypanosomosis risk. *PLoS Neglected Tropical Diseases*, **9** (7): e0003921.

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African animal trypanosomosis (AAT) is a major constraint to sustainable development of cattle farming in sub-Saharan Africa. The habitat of the tsetse fly vector is increasingly

fragmented owing to demographic pressure and shifts in climate, which leads to heterogeneous risk of cyclical transmission both in space and time. In Burkina Faso and Ghana, the most important vectors are riverine species, namely *Glossina palpalis gambiensis* and *G. tachinoides*, which are more resilient to human-induced changes than the savannah and forest species. Although many authors studied the distribution of AAT risk both in space and time, spatio-temporal models allowing predictions of it are lacking. We used datasets generated by various projects, including two baseline surveys conducted in Burkina Faso and Ghana within national PATTEC (Pan African Tsetse and Trypanosomosis Eradication Campaign) initiatives. We computed the entomological inoculation rate (EIR) or tsetse challenge using a range of environmental data. The tsetse apparent density and infection rate were separately estimated and subsequently combined to derive the EIR using a "one layer-one model" approach. The estimated EIR was then projected into suitable habitats. This risk index was finally validated against data on bovine trypanosomosis. It allowed a good prediction of the parasitological status (r^2 = 67 percent), showed a positive correlation but less predictive power than serological status (r^2 = 22 percent) aggregated at the village level but was not related to the illness status (r^2 = 2 percent). The presented spatio-temporal model provides a fine-scale picture of the dynamics of AAT risk in sub-humid areas of West Africa. The estimated EIR was high in the proximity of rivers during the dry season and more widespread during the rainy season. The present analysis is a first step in a broader framework for efficient risk management of climate-sensitive vector-borne diseases.

17489. **Djohan, V., Kaba, D., Rayaisse, J. B., Dayo, G. K., Coulibaly, B., Salou, E., Dofini, F., Kouadio Ade, M., Menan, H. & Solano, P., 2015.** Detection and identification of pathogenic trypanosome species in tsetse flies along the Comoe River in Côte d'Ivoire. *Parasite*, **22**: 18.

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In order to identify pathogenic trypanosomes responsible for African trypanosomiasis, and to better understand tsetse-trypanosome relationships, surveys were undertaken in three sites located in different eco-climatic areas in Côte d'Ivoire during the dry and rainy seasons. Tsetse flies were caught during five consecutive days using biconical traps, dissected and microscopically examined to look for trypanosome infection. Samples from infected flies were tested by PCR using specific primers for *Trypanosoma brucei* s.l., *T. congolense* savannah type, *T. congolense* forest type and *T. vivax*. Of 1 941 tsetse flies caught including four species, i.e. *Glossina palpalis palpalis*, *G. p. gambiensis*, *G. tachinoides* and *G. medicorum*, 513 (26 percent) were dissected and 60 (12 percent) were found positive by microscopy. Up to 41 percent of the infections were due to *T. congolense* savannah type, 30 percent to *T. vivax*, 20 percent to *T. congolense* forest type and 9 percent due to *T. brucei* s.l. All four trypanosome species and subgroups were identified from *G. tachinoides* and *G. p. palpalis*, while only two were isolated from *G. p. gambiensis* (*T. brucei* s.l., *T. congolense* savannah type) and *G. medicorum* (*T. congolense* forest, savannah types). Mixed infections were found in 25 percent of cases and all involved *T. congolense* savannah type with another trypanosome species. The simultaneous occurrence of *T. brucei* s.l., and tsetse from the *palpalis* group may suggest that human trypanosomiasis can still be a constraint in these localities, while high rates of *T. congolense* and *T. vivax* in the area suggest a potential risk of animal trypanosomosis along the Comoe River.

17490. **Duguma, R., Tasew, S., Olani, A., Damena, D., Alemu, D., Mulatu, T., Alemayehu, Y., Yohannes, M., Bekana, M., Hoppenheit, A., Abatih, E., Habtewold, T., Delespaux, V. & Duchateau, L., 2015.** Spatial distribution of Glossina sp. and Trypanosoma sp. in south-western Ethiopia. Parasites & Vectors, 8: 430.

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Accurate information on the distribution of the tsetse fly is of paramount importance to better control animal trypanosomosis. Entomological and parasitological surveys were conducted in the tsetse belt of south-western Ethiopia to describe the prevalence of trypanosomosis (PoT), the abundance of tsetse flies (AT) and to evaluate the association with potential risk factors. The study was conducted between 2009 and 2012. The parasitological survey data were analysed by a random effects logistic regression model, whereas the entomological survey data were analysed by a Poisson regression model. The percentage of animals with trypanosomosis was regressed on the tsetse fly count using a random effects logistic regression model. The following six risk factors were evaluated for PoT: (i) altitude: significant and inverse correlation with trypanosomosis, (ii) annual variation of PoT: no significant difference between years, (iii) regional state: compared to Benishangul-Gumuz (18.0 percent), the three remaining regional states showed significantly lower PoT, (iv) river system: the PoT differed significantly between the river systems, (iv) sex: male animals (11.0 percent) were more affected than females (9.0 percent), and finally (vi) age at sampling: no difference between the considered classes. Observed trypanosome species were T. congolense (76.0 percent), T. vivax (18.1 percent), T. b. brucei (3.6 percent), and mixed T. congolense/vivax (2.4 percent). The first four risk factors listed above were also evaluated for AT, and all had a significant effect on AT. In the multivariable model only altitude was retained, with AT decreasing with increasing altitude. Four different Glossina species were identified i.e. G. tachinoides (52.0 percent), G. pallidipes (26.0 percent), G. morsitans submorsitans (15.0 percent) and G. fuscipes fuscipes (7.0 percent). Significant differences in catches/trap/day between districts were observed for each species. No association could be found between the tsetse fly counts and trypanosomosis prevalence. In conclusion, trypanosomosis remains a constraint to livestock production in south-western Ethiopia. Four Glossina and three Trypanosoma species were observed. Altitude had a significant impact on AT and PoT. PoT was not associated with AT, which could be explained by the importance of mechanical transmission. This needs to be investigated further as it might jeopardize control strategies that target the tsetse fly population.

17491. **Echodu, R., Sistrom, M., Bateta, R., Murilla, G., Okedi, L., Aksoy, S., Enyioha, C.,**

Enyaru, J., Opiyo, E., Gibson, W. & Caccone, A., 2015. Genetic diversity and population structure of *Trypanosoma brucei* in Uganda: implications for the epidemiology of sleeping sickness and Nagana. *PLoS Neglected Tropical Diseases*, **9** (2): e0003353.

Faculty of Science, Gulu University, Gulu, Uganda; Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut, USA; Trypanosomiasis Research Centre, Kenya Agricultural Research Institute, Kikuyu, Kenya; National Agricultural Research Organisation, National Livestock Resources Research Institute, Tororo, Uganda; Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, Connecticut, USA; School of Biological Sciences, Makerere University, Kampala, Uganda; School of Biological Sciences, University of Bristol, Bristol, UK. [mark.sistrom@yale.edu].

While human African trypanosomiasis (HAT) is in decline on the continent of Africa, the disease still remains a major health problem in Uganda. There are recurrent sporadic outbreaks in the traditionally endemic areas in south-east Uganda, and continued spread to new unaffected areas in central Uganda. We evaluated the evolutionary dynamics underpinning the origin of new foci and the impact of host species on parasite genetic diversity in Uganda. We genotyped 269 *Trypanosoma brucei* isolates collected from different regions in Uganda and south western Kenya at 17 microsatellite loci, and checked for the presence of the SRA gene that confers human infectivity to *T. b. rhodesiense*. Both Bayesian clustering methods and Discriminant Analysis of Principal Components partition *Trypanosoma brucei* isolates obtained from Uganda and south western Kenya into three distinct genetic clusters. Clusters 1 and 3 include isolates from central and southern Uganda, while cluster 2 contains mostly isolates from south western Kenya. These three clusters are not sorted by subspecies designation (*T. b. brucei* vs *T. b. rhodesiense*), host or date of collection. The analyses also show evidence of genetic admixture among the three genetic clusters and long-range dispersal, suggesting recent and possibly on-going gene flow between them. Our results show that the expansion of the disease to the new foci in central Uganda occurred from the northward spread of *T. b. rhodesiense* (*Tbr*). They also confirm the emergence of the human infective strains (*Tbr*) from non-infective *T. b. brucei* (*Tbb*) strains of different genetic backgrounds, and the importance of cattle as a *Tbr* reservoir, as confounders that shape the epidemiology of sleeping sickness in the region.

17492. **Geiger, A., Hamidou Soumana, I., Tchicaya, B., Rofidal, V., Decourcelle, M., Santoni, V. & Hem, S., 2015.** Differential expression of midgut proteins in *Trypanosoma brucei gambiense*-stimulated vs. non-stimulated *Glossina palpalis gambiensis* flies. *Frontiers in Microbiology*, **6**: 444.

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The unicellular pathogenic protozoan *Trypanosoma brucei gambiense* is responsible for the chronic form of sleeping sickness. This vector-borne disease is transmitted to humans by the tsetse fly of the group *Glossina palpalis*, including the subspecies *G. p. gambiensis*, in which the parasite completes its developmental cycle. Sleeping sickness control strategies can

therefore target either the human host or the fly vector. Indeed, suppression of one step in the parasite developmental cycle could abolish parasite transmission to humans, with consequences on the spreading of the disease. In order to develop this type of approach, we have identified, at the proteome level, events resulting from the tripartite interaction between the tsetse fly *G. p. gambiensis*, its microbiome, and the trypanosome. Proteomes were analysed from four biological replicates of midguts from flies sampled three days post-feeding on either a trypanosome-infected (stimulated flies) or a non-infected (non-stimulated flies) blood meal. Over 500 proteins were identified in the midguts of flies from both feeding groups, 13 of which were shown to be differentially expressed in trypanosome-stimulated vs. non-stimulated flies. Functional annotation revealed that several of these proteins have important functions that could be involved in modulating the fly infection process by trypanosomes (and thus fly vector competence), including anti-oxidant and anti-apoptotic, cellular detoxifying, trypanosome agglutination, and immune stimulating or depressive effects. The results show a strong potential for diminishing or even disrupting fly vector competence, and their application holds great promise for improving the control of sleeping sickness.

17493. **Imhof, S., Vu, X. L., Butikofer, P. & Roditi, I., 2015.** A glycosylation mutant of *Trypanosoma brucei* links social motility defects *in vitro* to impaired colonization of tsetse flies *in vivo*. *Eukaryotic Cell*, **14** (6): 588-592.

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Transmission of African trypanosomes by tsetse flies requires that the parasites migrate out of the midgut lumen and colonize the ectoperitrophic space. Early procyclic culture forms correspond to trypanosomes in the lumen; on agarose plates they exhibit social motility, migrating *en masse* as radial projections from an inoculation site. We show that an Rft1(-/-) mutant needs to reach a greater threshold number before migration begins, and that it forms fewer projections than its wild-type parent. The mutant is also up to four times less efficient at establishing midgut infections. Ectopic expression of Rft1 rescues social motility defects and restores the ability to colonize the fly. These results are consistent with social motility reflecting movement to the ectoperitrophic space, implicate N-glycans in the signalling cascades for migration *in vivo* and *in vitro*, and provide the first evidence that parasite-parasite interactions determine the success of transmission by the insect host.

17494. **Lelisa, K., Shimeles, S., Bekele, J. & Sheferaw, D., 2014.** Bovine trypanosomosis and its fly vectors in three selected settlement areas of Hawa-Gelan district, western Ethiopia. *Onderstepoort Journal of Veterinary Research*, **81** (1) Art. 715.

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A cross-sectional study aimed at investigating the species diversity of fly vectors and estimating the prevalence of bovine trypanosomosis was carried out from October 2009 to May 2010 in selected settlement areas of the Hawa-Gelan district in the western Wollega zone of Ethiopia. Standard methods of sampling and identification were employed for both entomological and parasitological examination. Three species of the genus *Glossina* (*Glossina pallidipes*, *Glossina morsitans* submorsitans and *Glossina fuscipes*) and two genera of biting

flies (*Stomoxys* and *Tabanus*) were caught and identified. The overall apparent density of *Glossina* species caught was 10.5 flies per trap per day, with a higher proportion of female flies (57.2 percent). Out of a total 389 cattle examined, 42 (10.8 percent; 95 percent CI: 7.89 percent - 14.3 percent) were found infected with trypanosomes. Three trypanosome species were detected in the study area, namely *Trypanosoma congolense* (54.8 percent), *Trypanosoma brucei* (23.8 percent) and *Trypanosoma vivax* (21.4 percent). The prevalence of trypanosomosis was found to be significantly ($p < 0.05$) higher in cattle with poor body condition. There was an association between mean packed cell volume (PCV) and the occurrence of parasitaemia ($chi^2 = 49.5$, $p < 0.05$). About 95.2 percent of cattle that were positive for trypanosomes had a PCV less than the lower limit for cattle. Considering the current result, bovine trypanosomosis seems to be a serious constraint for agricultural activities in the settlement areas of the Hawa-Gelan district and seems to be associated with the presence of *Glossina* species. Therefore, application of control methods through community involvement to reduce the *Glossina* species infestation level is likely to increase animal productivity.

17495. **Mbewe, N. J., Mweempwa, C., Guya, S. & Wamwiri, F. N., 2015.** Microbiome frequency and their association with trypanosome infection in male *Glossina morsitans centralis* of Western Zambia. *Veterinary Parasitology*, **211** (1-2): 93-98.

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Tsetse flies (Diptera: *Glossinidae*) are considered primary cyclical vectors that transmit pathogenic trypanosomes in Africa. They harbour a variety of microbes including *Wolbachia*, *Sodalis* and the salivary gland hypertrophy virus (SGHV) which are all vertically transmitted. Knowledge on tsetse microbiome and their interactions may identify novel strategies for tsetse fly and trypanosomiasis control. Area-wide application of such strategies requires an understanding of the natural microbiome frequency in the different species and subspecies of *Glossina* in their geographical populations. Consequently, this study determined the prevalence of *Sodalis*, *Wolbachia*, SGHV and trypanosome infections in *Glossina morsitans centralis* from two sites of Western Zambia. We also explored possible associations of the microbes with trypanosome infections. Male *G. morsitans centralis* samples were collected from two sites (Lyoni and Lusinina) in Western Zambia. The age structure of the flies at each site was determined using the wing fray method. DNA was extracted from the samples and analysed for *Wolbachia, Sodalis*, SGHV and trypanosome presence using PCR. Associations and measures of associations between trypanosome infection and microbes in the fly were determined. The flies from the two locations (Lusinina, $n = 45$ and Lyoni, $n = 24$) had a similar age structure with their median fray category not being significantly different ($p = 0.698$). The overall prevalence of *Wolbachia* was 72.5 percent (95 percent CI: 61.6-83.3 percent), *Sodalis* was 15.9 percent (95 percent CI: 7.1-24.8 percent), SGHV was 31.9 percent (95 percent CI: 20.6-43.2 percent) and *Trypanosoma* species was 23.2 percent (95 percent CI: 13-33.4 percent). The prevalence of *Wolbachia* was significantly higher in Lusinina than Lyoni ($p = 0.000$). However, this was not the case for *Sodalis*, SGHV and *Trypanosoma* species. Despite the low number of flies that were positive for both trypanosome and *Sodalis* (six; 8.7 percent), a statistically significant association ($p = 0.013$; 95 percent CI: 1.5-25.8) was observed in *G. morsitans centralis*. The study showed that the prevalence of microbiota may vary within the same species of the tsetse depending on the geographical location as was the case of *Wolbachia*.

Further it showed that infection with *Sodalis* could affect vector competence. The study concludes that *Sodalis* could be an ideal candidate for symbiont-mediated trypanosomiasis control interventions in *G. morsitans centralis*.

17496. **Mweempwa, C., Marcotty, T., Claudia De Pus, C., Penzhorn, B.L., Dicko, A.H., Bouyer, J. & Reginald De Deken, R., 2015.** Impact of habitat fragmentation on tsetse populations and trypanosomosis risk in Eastern Zambia. *Parasites & Vectors*, **8**: 406.

Department of Veterinary and Livestock Development, Zambia; Animal Health Department, Institute of Tropical Medicine, Antwerp, 2000, Belgium; Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa; West African Science Service in Climate Change and Adapted Land Use (WASCAL), Cheikh Anta Diop University, Dakar, BP 5683, Senegal; Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Unité Mixte de Recherche Contrôle des Maladies Animales Exotiques et Émergentes, Campus International de Baillarguet, Montpellier, 34398, France; Institut Sénégalais de Recherches Agricoles, Laboratoire National d'Elevage et de Recherches Vétérinaires, Service de Parasitologie, Dakar-Hann, BP 2057, Sénégal; VERDI-R&D, Louveigné, 4141, Belgium. [cmweempwa@yahoo.com].

Fragmentation of tsetse habitat in eastern Zambia is largely due to encroachments by subsistence farmers into new areas in search of new agricultural land. The impact of habitat fragmentation on tsetse populations is not clearly understood. This study was aimed at establishing the impact of habitat fragmentation on physiological and demographic parameters of tsetse flies in order to enhance the understanding of the relationship between fragmentation and African animal trypanosomosis (AAT) risk. A longitudinal study was conducted to establish the age structure, abundance, proportion of females and trypanosome infection rate of *Glossina morsitans morsitans* Westwood (Diptera: *Glossinidae*) in areas of varying degrees of habitat fragmentation in Eastern Zambia. Black screen fly rounds were used to sample tsetse populations monthly for one year. Logistic regression was used to analyse age, proportion of females and infection rate data. Flies got significantly older as fragmentation increased ($p < 0.004$). The proportion of old flies, i.e. above ovarian category four, increased significantly ($p < 0.001$) from 25.9 percent (CI 21.4-31.1) at the least fragmented site (Lusandwa) to 74.2 percent (CI 56.8-86.3) at the highly fragmented site (Chisulo). In the most fragmented area (Kasamanda), tsetse flies had almost disappeared. In the highly fragmented area a significantly higher trypanosome infection rate in tsetse ($p < 0.001$) than in areas with lower fragmentation was observed. Consequently a comparatively high trypanosomosis incidence rate in livestock was observed there despite lower tsetse density ($p < 0.001$). The overall proportion of captured female flies increased significantly ($p < 0.005$) as fragmentation reduced. The proportion increased from 0.135 (CI 0.10-0.18) to 0.285 (CI 0.26-0.31) at the highly and least fragmented sites, respectively. Habitat fragmentation creates conditions to which tsetse populations respond physiologically and demographically thereby affecting tsetse-trypanosome interactions and hence influencing trypanosomosis risk. Temperature rise due to fragmentation coupled with dominance of old flies in populations increases infection rate in tsetse and hence creates a high risk of trypanosomosis in fragmented areas. Possibilities of how correlations between the biological characteristics of populations and the degree of fragmentation can be used to structure populations based on their well-being, using integrated GIS and remote sensing techniques are discussed.

17497. **Rock, K. S., Stone, C. M., Hastings, I. M., Keeling, M. J., Torr, S. J. & Chitnis, N.,**

2015. Mathematical models of human African trypanosomiasis epidemiology. *Advances in Parasitology*, **87**: 53-133.

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Human African trypanosomiasis (HAT), commonly called sleeping sickness, is caused by *Trypanosoma* spp. and transmitted by tsetse flies (*Glossina* spp.). HAT is usually fatal if untreated and transmission occurs in foci across sub-Saharan Africa. Mathematical modelling of HAT began in the 1980s using extensions of the Ross-Macdonald malaria model and has since consisted, with a few exceptions, of similar deterministic compartmental models. These models have captured the main features of HAT epidemiology and provided insight on the effectiveness of the two main control interventions (treatment of humans and tsetse fly control) in eliminating transmission. However, most existing models have overestimated the prevalence of infection and ignored transient dynamics. There is a need for properly validated models, evolving with improved data collection that can provide quantitative predictions to help guide control and elimination strategies for HAT.

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

17498. **Lumbala, C., Simarro, P. P., Cecchi, G., Paone, M., Franco, J. R., Kande Betu Ku Mesu, V., Makabuza, J., Diarra, A., Chansy, S., Priotto, G., Mattioli, R. C. & Jannin, J. G., 2015.** Human African trypanosomiasis in the Democratic Republic of the Congo: disease distribution and risk. *International Journal of Health Geography*, **14** (1): 20.

National Sleeping Sickness Control Programme, Kinshasa, Democratic Republic of the Congo; World Health Organization, Control of Neglected Tropical Diseases, Innovative and Intensified Disease Management, 1211, Geneva, 27, Switzerland; Food and Agriculture Organization of the United Nations, Subregional Office for Eastern Africa, Addis Ababa, Ethiopia; Food and Agriculture Organization of the United Nations, Animal Production and Health Division, Viale delle Terme di Caracalla, 00153, Rome, Italy; Neglected Tropical Diseases Department, Ministry of Public Health, Kinshasa, Democratic Republic of the Congo; World Health Organization, Regional Office for Africa, Inter-country Support Team, Libreville, Gabon. [diarraam@who.afro.int].

For the past three decades, the Democratic Republic of the Congo (DRC) has been the country reporting the highest number of cases of human African trypanosomiasis (HAT). In 2012, DRC continued to bear the heaviest burden of *gambiense* HAT, accounting for 84 percent of all cases reported at the continental level (i.e. 5 968/7 106). This paper reviews the status of sleeping sickness in the DRC between 2000 and 2012, with a focus on spatio-temporal patterns. Epidemiological trends at the national and provincial levels are presented. The number of HAT

cases reported yearly from the DRC decreased by 65 percent from 2000 to 2012, i.e. from 16 951 to 5 968. At the provincial level a more complex picture emerges. Whilst HAT control in the Equateur province has had a spectacular impact on the number of cases (97 percent reduction), the disease has proved more difficult to tackle in other provinces, most notably in Bandundu and Kasai, where despite substantial progress, HAT remains entrenched. HAT prevalence presents its highest values in the northern part of the Province Orientale, where a number of constraints hinder surveillance and control. Significant coordinated efforts by the National Sleeping Sickness Control Programme and the World Health Organization in data collection, reporting, management and mapping, culminating in the Atlas of HAT, have enabled HAT distribution and risk in DRC to be known with more accuracy than ever before. Over 18 000 locations of epidemiological interest have been geo-referenced (average accuracy approximately 1.7 km), corresponding to 93.6 percent of reported cases (period 2000-2012). The population at risk of contracting sleeping sickness has been calculated for two five-year periods (2003-2007 and 2008-2012), resulting in estimates of 33 and 37 million people respectively. The progressive decrease in HAT cases reported since 2000 in the DRC is likely to reflect a real decline in disease incidence. If this result is to be sustained, and if further progress is to be made towards the goal of HAT elimination, the ongoing integration of HAT control and surveillance into the health system needs to be closely monitored and evaluated, and active case-finding activities maintained, especially in those areas where the risk of infection remains high and where resurgence could occur.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **38**: 17431, 17440, 17455].

17499. **Checchi, F., Funk, S., Chandramohan, D., Haydon, D. T. & Chappuis, F., 2015.** Updated estimate of the duration of the meningo-encephalitic stage in *gambiense* human African trypanosomiasis. *BMC Research Notes*, **8**: 292.

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The duration of the stages of HAT is an important factor in epidemiological studies and intervention planning. Previously, we published estimates of the duration of the haemo-lymphatic stage 1 and meningo-encephalitic stage 2 of the *gambiense* form of human African trypanosomiasis (HAT), in the absence of treatment. Here we revise the estimate of stage 2 duration, based on data from Uganda and South Sudan, by adjusting observed infection prevalence for incomplete case detection coverage and diagnostic inaccuracy. The revised best estimate for the mean duration of stage 2 is 252 days (95 percent CI 171-399), about half of our initial best estimate, giving a total mean duration of untreated *gambiense* HAT infection of approximately 2 years and 2 months. Our new estimate provides improved information on the transmission dynamics of this neglected tropical disease in Uganda and South Sudan. We stress that there remains considerable variability around the estimated mean values, and that one must

be cautious in applying these results to other foci.

17500. **Kato, C. D., Alibu, V. P., Nanteza, A., Mugasa, C. M. & Matovu, E., 2015.** Interleukin (IL)-6 and IL-10 are up-regulated in late stage *Trypanosoma brucei rhodesiense* sleeping sickness. *PLoS Neglected Tropical Diseases*, **9** (6): e0003835.

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Sleeping sickness due to *Trypanosoma brucei rhodesiense* has a wide spectrum of clinical presentations coupled with differences in disease progression and severity across East and Southern Africa. The disease progresses from an early (haemo-lymphatic) stage to the late (meningo-encephalitic) stage characterized by presence of parasites in the central nervous system. We hypothesized that disease progression and severity of the neurological response are modulated by cytokines. A total of 55 sleeping sickness cases and 41 healthy controls were recruited passively at Lwala hospital, in Northern Uganda. A panel of six cytokines (IFN-gamma, IL1-beta, TNF-alpha, IL-6, TGF-beta and IL-10) were assayed from paired plasma and cerebrospinal fluid (CSF) samples. Cytokine concentrations were analysed in relation to disease progression, clinical presentation and severity of neurological responses. Median plasma levels (pg/mL) of IFN-gamma (46.3), IL-6 (61.7), TGF-beta (8 755) and IL-10 (256.6) were significantly higher in cases compared to controls ($p < 0.0001$). When early stage and late stage CSF cytokines were compared, IL-10 and IL-6 were up-regulated in late stage patients and were associated with a reduction in tremors and cranioneuropathy. IL-10 had a higher staging accuracy with a sensitivity of 85.7 percent (95 percent CI, 63.7 percent - 97 percent) and a specificity of 100 percent (95 percent CI, 39.8 percent - 100 percent) while for IL-6, a specificity of 100 percent (95 percent CI, 47.8 percent - 100 percent) gave a sensitivity of 83.3 percent (95 percent CI, 62.2 percent - 95.3 percent). Our study demonstrates the role of host inflammatory cytokines in modulating the progression and severity of neurological responses in sleeping sickness. We demonstrate here an up-regulation of IL-6 and IL-10 during the late stage with a potential as adjunct stage biomarkers. Given that both cytokines could potentially be elevated by other CNS infections, our findings should be further validated in a large cohort of patients including those with other inflammatory diseases such as cerebral malaria.

17501. **Kato, C. D., Nanteza, A., Mugasa, C., Edyelu, A., Matovu, E. & Alibu, V. P., 2015.** Clinical profiles, disease outcome and co-morbidities among *T. b. rhodesiense* sleeping sickness patients in Uganda. *PLoS One*, **10** (2): e0118370.

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The acute form of human African trypanosomiasis (HAT, also known as sleeping sickness) caused by *Trypanosoma brucei rhodesiense* has been shown to have a wide spectrum of focus-specific clinical presentation and severity in East and Southern Africa. Indeed HAT occurs in regions endemic for other tropical diseases; however data on how these co-morbidities might complicate the clinical picture and affect disease outcome remains largely scanty. We describe here the clinical presentation, presence of co-infections, and how the latter

impact on HAT prognosis. We carried out a retrospective analysis of clinical data from 258 sleeping sickness patients reporting to Lwala hospital between 2005 and 2012. The mean patient age was 28.6 years with a significant number of cases below 18 years ($p < 0.0001$). About 93.4 percent of the cases were diagnosed as late stage ($p < 0.0001$). The case fatality rate was 10.5 percent with post treatment reactive encephalopathy reported in 7.9 percent of the cases, of whom 36.8 percent eventually died. Fever was significantly ($p = 0.045$) higher in patients under 18 years. Of the early stage patients, 26.7 percent and 6.7 percent presented with late stage signs of sleep disorder and mental confusion respectively. Among the co-infections, malaria was significantly more prevalent (28.9 percent; $p < 0.0001$) followed by urinary tract infections (4.2 percent). Co-infections were present in 14.3 percent of in-hospital deaths, 38.5 percent of which were recorded as malaria. Malaria was significantly more common in patients under 18 years (45.5 percent; $p < 0.02$), and was reported in 60 percent of the fatal cases in this age group. We show a wide spectrum of sleeping sickness clinical presentation and disease outcomes that were apparently not significantly influenced by concurrent infections. It would thus be interesting to determine the host and/or parasite factors that might be responsible for the observed diverse clinical presentation.

(c) TREATMENT

[See also **38**: 17438, 17439, 17446, 17448, 17449, 17451].

17502. **Ilboudo, H., Camara, O., Ravel, S., Bucheton, B., Lejon, V., Camara, M., Kabore, J., Jamonneau, V. & Deborggraeve, S., 2015.** *Trypanosoma brucei gambiense* spliced leader RNA is a more specific marker for cure of human African trypanosomiasis than *T. b. gambiense* DNA. *Journal of Infectious Diseases*. **E Publication ahead of print, June 16.**

Centre International de Recherche-Développement sur l'Élevage en zone Subhumide, Unité de Recherches sur les Bases Biologiques de la Lutte Intégrée, Ministère de la Santé et de l'Hygiène Publique, Programme National de Lutte contre la Trypanosomose Humaine Africaine, Conakry, Guinea; Unité Mixte de Recherche IRD-CIRAD 177, Institut de Recherche pour le Développement, Montpellier, France; Université Polytechnique de Bobo-Dioulasso, Burkina Faso; Biomedical Sciences Department, Institute of Tropical Medicine Antwerp, Belgium. [sdeborggraeve@itg.be].

To assess the efficacy of treatment for human African trypanosomiasis, accurate tests that can discriminate relapse from cure are needed. We report the first data that the spliced leader (SL) RNA is a more specific marker for cure of human African trypanosomiasis than parasite DNA. In blood samples obtained from 61 patients in whom human African trypanosomiasis was cured, SL RNA detection had specificities of 98.4 percent - 100 percent, while DNA detection had a specificity of only 77 percent. Data from our proof-of-concept study show that SL RNA detection has high potential as a test of cure.

17503. **Mpanya, A., Hendrickx, D., Baloji, S., Lumbala, C., da Luz, R. I., Boelaert, M. & Lutumba, P., 2015.** From health advice to taboo: community perspectives on the treatment of sleeping sickness in the Democratic Republic of Congo, a qualitative study. *PLoS Neglected Tropical Diseases*, **9** (4): e0003686.

Programme National de Lutte contre la Trypanosomiase Humaine Africaine, Kinshasa, Democratic Republic of Congo; Institute of Tropical Medicine, Antwerp, Belgium;

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Socio-cultural and economic factors constitute real barriers for uptake of screening and treatment of human African trypanosomiasis (HAT) in the Democratic Republic of Congo (DRC). Better understanding of and addressing these barriers may enhance the effectiveness of HAT control. We performed a qualitative study consisting of semi-structured interviews and focus group discussions in the Bandundu and Kasai Oriental provinces, two provinces lagging behind in the HAT elimination effort. Our study population included current and former HAT patients, as well as healthcare providers and managers of the national HAT control programme. All interviews and discussions were voice recorded on a digital device and data were analysed with the ATLAS.ti software. Health workers and community members quoted a number of prohibitions that have to be respected for six months after HAT treatment: no work, no sexual intercourse, no hot food, not walking in the sun. Violating these restrictions is believed to cause serious, and sometimes deadly, complications. These strong prohibitions are well known by the community and lead some people to avoid HAT screening campaigns for fear of having to observe such taboos in case of diagnosis. The restrictions originally aimed to mitigate the severe adverse effects of the melarsoprol regimen, but are not evidence-based and became obsolete with the new safer drugs. Correct health information regarding HAT treatment is essential. Health providers should address the perspective of the community in a constant dialogue to keep abreast of unintended transformations of meaning.

6. ANIMAL TRYPANOSOMOSIS

(a) SURVEY AND DISTRIBUTION

17504. **Bass, B., Diall, Y. G., Diarra, M., Boiré, S., Samake, N'T., Diarra, A. & Fofana, A., 2015.** Prevalence of bovine trypanosomosis in the circles of Kadiolo and Sikasso in Mali before initiating a control campaign. *Bulletin de la santé et de la production Animales en Afrique.* (**in press**).

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The objective of the FAO-TCP project MLI/3402I is to initiate a campaign for effective and long-lasting protection of cattle against trypanosomosis by eliminating resistance to trypanocides through controlling tsetse flies and strategic treatment of animals. Before implementing these activities a parasitological baseline survey was carried out by the Central Veterinary Laboratory. Blood samples (1 208) were taken from cattle. Fifteen samples were positive for trypanosomes (nine in Kadiolo and six in Sikasso). All trypanosomes identified were *Trypanosoma vivax*. Trypanosome prevalence was 1.12 percent in the circle of Kadiolo and 1.2 percent in the circle of Sikasso. For the whole zone covered by the study, the prevalence was 1.16 percent. Average haematocrits were 28.9 percent in the circle of Kadiolo, 33.4 percent in the circle of Sikasso, and 31.2 percent overall.

17505. **Camargo, R., Izquier, A., Uzcanga, G. L., Perrone, T., Acosta-Serrano, A., Carrasquel, L., Arias, L. P., Escalona, J. L., Cardozo, V. & Bubis, J., 2015.** Variant surface glycoproteins from Venezuelan trypanosome isolates are recognized by sera from animals infected with either *Trypanosoma evansi* or *Trypanosoma vivax*. *Veterinary Parasitology*, **207** (1-2): 17-33.

Fundacion Instituto de Estudios Avanzados IDEA, Caracas, Venezuela; Universidad Simon Bolivar, Departamento de Biologia Celular, Caracas, Venezuela; Universidad Central de Venezuela, Instituto de Ciencia y Tecnologia de Alimentos, Caracas, Venezuela; Fundacion Instituto de Estudios Avanzados IDEA, Caracas, Venezuela; Instituto Venezolano de Investigaciones Cientificas IVIC, Centro de Biofisica y Bioquimica, Caracas, Venezuela; Parasitology Department, Liverpool School of Tropical Medicine, Liverpool, UK; Universidad Simon Bolivar, Departamento de Quimica, Caracas, Venezuela. [jbubis@usb.ve].

Salivarian trypanosomes sequentially express only one variant surface glycoprotein (VSG) on their cell surface from a large repertoire of VSG genes. Seven cryopreserved animal trypanosome isolates known as TeAp-ElFrio01, TEVA1 (or TeAp-N/D1), TeGu-N/D1, TeAp-Mantecal01, TeGu-TerecayTrino, TeGu-Terecay03 and TeGu-Terecay323, which had been isolated from different hosts identified in several geographical areas of Venezuela were expanded using adult albino rats. Soluble forms of predominant VSGs expressed during the early infection stages were purified and corresponded to concanavalin A-binding proteins with molecular masses of 48-67 kDa by sodium dodecyl sulphate-polyacrylamide gel electropohoresis, and pI values between 6.1 and 7.5. The biochemical characterization of all purified soluble VSGs revealed that they were dimers in their native form and represented different gene products. Sequencing of some of these proteins yielded peptides homologous to VSGs from *Trypanosoma* (*Trypanozoon*) *brucei* and *Trypanosoma* (*Trypanozoon*) *evansi* and established that they most likely are mosaics generated by homologous recombination. Western blot analysis showed that all purified VSGs were cross-reacting antigens that were recognized by sera from animals infected with either *T. evansi* or *Trypanosoma* (*Dutonella*) *vivax*. The VSG glycosyl-phosphatidylinositol cross-reacting determinant epitope was only partially responsible for the cross-reactivity of the purified proteins, and antibodies appeared to recognize cross-reacting conformational epitopes from the various soluble VSGs. ELISA experiments were performed using infected bovine sera collected from cattle in a Venezuelan trypanosome-endemic area. In particular, soluble VSGs from two trypanosome isolates, TeGu-N/D1 and TeGu-TeracayTrino, were recognized by 93.38 percent and 73.55 percent of naturally *T. vivax*-infected bovine sera, respectively. However, approximately 70 percent of the sera samples did not recognize all seven purified proteins. Hence, the use of a combination of various VSGs for the diagnosis of animal trypanosomosis is recommended.

17506. **Haji, I. J., Malele, I. & Namangala, B., 2014.** Occurrence of haemoparasites in cattle in Monduli district, northern Tanzania. *Onderstepoort Journal of Veterinary Research*, **81** (1): doi: 10.4102/ojvr.v81i1.843.

Department of Economic and Productive Sectors, Regional Commissioner's Office, Tanzania; Department of Paraclinical Studies, University of Zambia, Zambia; Tsetse and Trypanosomosis Research Institute, Tanga, Tanzania; Department of Paraclinical Studies, University of Zambia, Zambia. [boniface_1020@yahoo.com].

Haemoparasitic infections are among the most economically important cattle diseases in

sub-Saharan Africa. The present study investigated the occurrence of haemoparasites in 295 indigenous cattle from five villages (Mswakini, Lake Manyara, Naitolia, Makuyuni and Nanja) of the Monduli district, a wildlife-domestic animal-human interface area in northern Tanzania. The data showed that the overall occurrence of haemoparasites in the sampled cattle was 12.5 percent (95 percent CI: 8.7 percent - 16.3 percent), involving single and mixed infections with *Theileria parva*, *Anaplasma marginale*, *Babesia bovis*, *Trypanosoma vivax* and *Trypanosoma brucei*. The highest haemoparasite occurrence was recorded in Lake Manyara (18.3 percent; 95 percent CI: 8.5 percent - 28.1 percent), and the lowest was recorded in Nanja (6.5 percent; 95 percent CI: 0.4 percent - 12.6 percent). This preliminary study, furthermore, provided evidence of the possible arthropod vectors (ticks and tsetse flies) that may be involved in the transmission of haemoparasites to cattle in the Monduli district. It is envisaged that this survey will stimulate more studies to determine the prevalence of haemoparasites in livestock by using more sensitive molecular techniques.

17507. **Haji, I. J., Sugimoto, C., Kajino, K., Malele, I., Simukoko, H., Chitambo, H. & Namangala, B., 2015.** Determination of the prevalence of trypanosome species in cattle from Monduli district, northern Tanzania, by loop mediated isothermal amplification. *Tropical Animal Health & Production*, **47** (6): 1139-1143.

Department of Economic and Productive Sectors, Region Commissioner's Office, P.O. Box 3050, Arusha, Tanzania. [b.namangala@unza.zm].

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

17508. **Ntantiso, L., de Beer, C., Marcotty, T. & Latif, A. A., 2014.** Bovine trypanosomosis prevalence at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. *Onderstepoort Journal of Veterinary Research*, **81** (1). doi: 10.4102/ojvr.v81i1.762.

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The northern KwaZulu-Natal (NKZN) region of South Africa is the southern limit of the African tsetse belt. Entomological information on *Glossina brevipalpis* and *Glossina austeni* was generated following the outbreak of trypanosomosis in cattle in 1990. However, these data have not been supported by parallel studies on the epidemiology of the disease and therefore there has been no control policy in place. This study represents the first intensive investigation to address the epidemiology of trypanosomosis in NKZN. Tsetse abundance, trypanosome herd average prevalence (HAP), herd average anaemia (HAA) and herd average packed cell volume (HA-PCV) were investigated at three communal dip tanks located at the edge of Hluhluwe-iMfolozi Park by monthly sampling from June 2006-November 2007. Seasonal trypanosome surveys were conducted at seven other communal dip tanks. *Glossina brevipalpis* prevalence was high at two of the dip tanks, Mvutshini and Ekuphindisweni, but low at Ocilwane, whilst *G. austeni* was only collected from Mvutshini. This high and low tsetse challenges presented different disease scenarios. Cattle at Mvutshini and Ekuphindisweni had the highest HAP of 12.3 percent and 8.9 percent respectively, both significantly different ($p = 0.001$) from the HAP obtained from cattle at Ocilwane (2.9 percent). These two cattle herds also had the highest HAA, 27.7 percent and 33.4 percent respectively, whilst cattle at Ocilwane had the lowest, 11.1 percent ($p = 0.001$). Conversely, cattle at Ocilwane had the highest HA-PCV, ranging between 29.0 percent and 32.0 percent, whilst cattle at Mvutshini and Ekuphindisweni had the lowest HA-PCV (24.0 percent-29.0 percent). By combining the data from the three dip tanks (1 318 observations), 62.0 percent of the infected cattle were found anaemic, compared with 20.0 percent in the uninfected group. Trypanosome seasonal surveys showed that cattle at all the seven dip tanks were infected with trypanosomes; mean HAP, HAA and HA-PCV values were 10.2 percent, 46.6 percent and 23.7 percent, respectively. This study generated information on the epidemiological factors related to the widespread presence of trypanosome-infected cattle and tsetse flies and it can be concluded that trypanosomosis is a disease of economic importance impacting the livelihood of resource-poor farmers in NKZN.

17509. **Nyimba, P. H., Komba, E. V., Sugimoto, C. & Namangala, B., 2015.** Prevalence and species distribution of caprine trypanosomosis in Sinazongwe and Kalomo districts of Zambia. *Veterinary Parasitology*, **210** (3-4): 125-130.

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African animal trypanosomosis is one of the key livestock diseases hindering full exploitation of livestock production potential covering 37 countries across sub-Saharan Africa. Many studies have been carried out to investigate the prevalence of the disease in cattle and humans in many tropical African countries but very little attention has been directed towards generating the disease prevalence rates in goats. The current study was conducted between December 2013 and January 2014 to establish the prevalence of caprine trypanosomosis in Sinazongwe and Kalomo districts, southern Zambia. It involved 422 goats which were first examined by palpation for possible enlargement of superficial lymph nodes. Blood samples were then collected from the goats and subjected to laboratory diagnosis using the microscope and loop mediated isothermal amplification (LAMP). None of the examined goats displayed enlargement of superficial lymph nodes. On microscopy only one goat was found to be positive. The results of investigations using the LAMP method showed that 100 goats were infected with

trypanosomes giving an overall prevalence rate of 23.7 percent. The prevalence of infection in Sinazongwe was 22.4 percent (n = 183) while in Kalomo it was 24.7 percent (n = 239); and the difference between the two districts was statistically significant ($p < 0.05$). *Trypanosoma brucei*, *Trypanasoma vivax* and *Trypanasoma congolense* were detected in 82.0 percent, 31.0 percent and 23.0 percent of the infected goats, respectively. Mixed infections were detected among 33.0 percent of the positive samples. The high prevalence rate of trypanosomes detected in the study area confirms earlier reports that trypanosomosis is re-emerging in the areas previously aerial sprayed by Government. The detection of trypanosomes in naturally infected goats confirms the important role goats play in the epidemiology of African animal trypanosomosis.

(b) PATHOLOGY AND IMMUNOLOGY

(c) TRYPANOTOLERANCE

17510. **Berthier, D., Peylhard, M., Dayo, G. K., Flori, L., Sylla, S., Bolly, S., Sakande, H., Chantal, I. & Thevenon, S., 2015.** A comparison of phenotypic traits related to trypanotolerance in five West African cattle breeds highlights the value of shorthorn taurine breeds. *PLoS One*, **10** (5): e0126498.

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Animal African trypanosomosis particularly affects cattle and dramatically impairs livestock development in sub-Saharan Africa. African Zebu (AFZ) or European taurine breeds usually die of the disease in the absence of treatment, whereas West African taurine breeds (AFT), considered trypanotolerant, are able to control the pathogenic effects of trypanosomosis. Up to now, only one AFT breed, the longhorn N'Dama (NDA), has been largely studied and is considered as the reference trypanotolerant breed. Shorthorn taurine trypanotolerance has never been properly assessed and compared to NDA and AFZ breeds. This study compared the trypanotolerant/susceptible phenotype of five West African local breeds that differ in their demographic history. Thirty-six individuals belonging to the longhorn taurine NDA breed, two shorthorn taurine Lagune (LAG) and Baoulé (BAO) breeds, the Zebu Fulani (ZFU) and the Borgou (BOR), an mixed breed between AFT and AFZ, were infected with *Trypanosoma congolense* IL1180. All the cattle were genetically characterized using dense SNP markers, and parameters linked to parasitaemia, anaemia and leukocytes were analysed using synthetic variables and mixed models. We showed that LAG, followed by NDA and BAO, displayed the best control of anaemia. ZFU showed the greatest anaemia and the BOR breed had an intermediate value, as expected from its admixed origin. Large differences in leukocyte counts were also observed, with higher leukocytosis for AFT. Nevertheless, no differences in parasitaemia were found, except a tendency to take longer to display detectable parasites in ZFU. We demonstrated that LAG and BAO are as trypanotolerant as NDA. This study highlights the value of shorthorn taurine breeds, which display strong local adaptation to trypanosomosis. Thanks to further analyses based on comparisons of the genome or transcriptome of the breeds, these results open up the way for better knowledge of host-pathogen interactions and, furthermore, for identifying key biological pathways.

(d) TREATMENT

[See also **38**: 17452].

17511. **Alingu, R. A., Muhanguzi, D., MacLeod, E., Waiswa, C. & Fyfe, J., 2014.** Bovine trypanosome species prevalence and farmers' trypanosomiasis control methods in south-western Uganda. *Journal of the South African Veterinary Association*, **85** (1): 1094.

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A cross-sectional study was conducted in Mbarara district, south-western Uganda in May 2012 to determine the burden of African animal trypanosomosis (AAT) in the semi-intensive dairy production systems where pyrethroid acaricides are frequently used in the control of tick-borne diseases (TBDs). A total of 295 cattle blood samples were taken and analysed using a single pair of primers previously designed to amplify internal transcribed spacer (ITS1) of trypanosome ribosomal deoxyribonucleic acid (rDNA). A structured questionnaire was carried out with 55 participating livestock farmers to generate data on acaricide and trypanocidal drug usage. The overall prevalence of trypanosome species was 2.4 percent (95 percent CI; 1.0 percent - 4.8 percent); *Trypanosoma vivax* was the most predominant species (2.0 percent; 95 percent CI; 0.7 percent - 4.4 percent). A single mixed infection of *T. vivax* and *Trypanosoma brucei* s.l. was detected. All the participating farmers used acaricides for tsetse and TBD control; 89.1 percent of the acaricides used were pyrethroids. About half of the farmers used trypanocidal drugs, mainly diminazene formulations (Berenil®). The low prevalence of trypanosomes in examined samples is most likely related to the frequent use of pyrethroid insecticides, trypanocides and restricted grazing (paddocking and tethering). These rigorous management practices are geared towards optimising production of exotic dairy breeds kept in this region that are highly susceptible to TBDs and AAT.

17512. **Bass, B., Traore, D., Bengaly, S., Diallo, D., Diakite,, B., Sidibe, I., Fonton, N., Kone, F., Issa Traore, I. & Samake, T., 2015**. Evaluation of the efficiency of the solution 8 percent neem oil in the fight against tsetse flies and African animal trypanosomose in the circle of Dioila (Mali). *Bulletin de la Santé et de la Production Animales en Afrique* (**in press**).

Laboratoire Central Vétérinaire, Bamako, Mali; Département Médecine Traditionnelle de l'Institut National de Recherche en Santé Publique, Bamako, Mali; Centre International de Recherche-Développement sur l'Élevage en zone Subhumide, Bobo Dioulasso, Burkina Faso; Université Abomey-Calavi, Benin. [drbassml1@yahoo.fr].

The present work had the objective of seeking for alternative solutions to the expensive chemical use of insecticides for the agro-breeders of the circle of Dioila. The study was divided into: (i) determination of the physico-chemical constituents of neem oil; (ii) evaluation of the efficacy of the oil on *Glossina palpalis gambiensis* in the laboratory; and (iii) evaluation of its efficacy on cattle under practical farming conditions. We optimized the physico-chemical characteristics of the neem oil by extracting the bioactive compounds and determining the fatty acid composition. In the second part, we showed that a 4 percent solution of neem oil has insecticidal and repulsive effects on tsetse. By taking into account other factors in the field

(sun, wind, rain), an 8 percent solution was used in the field as a "pour on" treatment on cattle. This reduced the fly prevalence to zero and resulted in an average cattle PCV of 43.5.

17513. **Mbewe, N. J., Sitali, L., Namangala, B. & Michelo, C., 2015.** Adherence to the Food and Agriculture Organization guidelines on trypanocide usage among cattle farmers in Itezhi tezhi, Central Zambia. *Veterinary Parasitology*, **209** (1-2): 43-49.

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Trypanocides will continue to play an important role in the control of tsetse fly transmitted trypanosomosis now and in the near future. The drugs are mostly administered by farmers without any veterinary supervision leading to misuse and under dosing of medication, and these could be factors that promote trypanocidal drug resistance (TDR) development. In order to delay or prevent TDR, the Food and Agriculture Organization (FAO) recommended guidelines on trypanocide use. It is not known if these recommended guidelines are adhered to in the Itezhi tezhi district of Zambia. A survey was undertaken to examine how socio-economic and environmental factors were associated with adherence to the recommended guidelines on trypanocide use in Itezhi tezhi, Central Zambia. Ninety farmers who use trypanocides were interviewed using a questionnaire to collect their socio-economic characteristics (age, education in years, cattle herd size, competence on trypanocide use and their access to extension on trypanocide use) and trypanocide usage practices while crush pens which they use were stratified according to location, whether in the game management area (GMA) (Mutenda, Itumbi, Kapulwe and Banachoongo) or non-GMA (Iyanda, New Ngoma and Shinampamba) as an environmental factor. Associations and measures of associations to adherence of FAO guidelines were determined. The results showed that 25.6 percent of the farmers adhered to the guidelines by FAO on trypanocide use and that none of the socio-economic factors under investigation was significantly associated with it. Further the likelihood of adhering to the FAO guidelines on trypanocide use was 80 percent lower in farmers who used crush pens in the GMA than those whot used crush pens in the non-GMA (AOR 0.20, 95 percent CI: 0.05-0.81, p = 0.02). There was low adherence to the recommended FAO guidelines on trypanocide use and it was associated with the location of the crush pen whether in the GMA or not, as an environmental factor. With farmers in the GMA less likely to adhere to FAO guidelines than those in the non-GMA, we recommend an integrated approach of measures to control trypanosomosis in the GMA of Itezhi tezhi to lessen overuse of trypanocides by the farmers.

17514. **Moti, Y., De Deken, R., Thys, E., Van Den Abbeele, J., Duchateau, L. & Delespaux, V., 2015.** PCR and microsatellite analysis of diminazene aceturate resistance of bovine trypanosomes correlated to knowledge, attitude and practice of livestock keepers in south-western Ethiopia. *Acta Tropica*, **146**: 45-52.

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African animal trypanosomosis is threatening agricultural production and cattle breeding more severely than any other livestock disease in the continent, even more since the advent of drug resistance. A longitudinal study was conducted from November 2012 to May 2013 in the Ghibe valley to evaluate diminazene aceturate (DA) resistance and assess livestock owners' perceptions of trypanocidal drug use. Four peasant associations (PAs) were purposively selected and the cattle randomly sampled in each PA. At the beginning of the study (t0), 106 bovines positive for trypanosomes by the haematocrit centrifugation technique (HCT) and 119 negative control animals were recruited for six months follow-up using HCT, 18S-PCR-RFLP, DpnII-PCR-RFLP and microsatellite analysis. Prevalence of trypanosomosis was 18.1 percent based on the HCT technique and the mean PCV value was 23.6+/-5.1 percent for the 587 sampled cattle. Out of the 106 HCT positive, 64 (60.4 percent) were positive for the presence of trypanosomes using the 18S-PCR-RFLP. Species detection showed 38 (59.4 percent) *Trypanosoma congolense* savannah, 18 (28.1 percent) *Trypanosoma vivax*, five (7.8 percent) *Trypanosoma theileri* and three (4.7 percent) *T. congolense* Kilifi. Among the *T. congolense* savannah samples, 31 (81.6 percent) showed a DA resistant RFLP profile, two (5.3 percent) a mixed profile and five did not amplify using the DpnII-PCR-RFLP. A positive HCT had a significant effect on PCV ($p < 0.001$) with the mean PCV value equal to 24.4+/-0.2 percent in the absence of trypanosomes and to 20.9+/-0.3 percent in the presence of trypanosomes. PCV increased significantly ($p < 0.001$) with 4.4+/-0.5 percent one month after treatment. All *T. congolense* savannah type were analysed using microsatellite markers TCM1, TCM3 and TCM4. The main events were new infections (40.0 percent) and relapses (37.5 percent) with cures lagging at 22.5 percent. In 10 purposively selected PAs a semi-structured questionnaire was used. The average herd size was the highest in Abelti PA (6.7+/-1.8 TLU) and the mean herd size was statistically different ($p = 0.01$) in the 10 PAs. Trypanosomosis was designated as the main disease affecting cattle by 97 percent of the respondents. DA was used by 95.5 percent of the farmers though more than half of them (51.9 percent) were not familiar with isometamidium (ISM). There was a trend to overdose young small animals and to underdose large ones. Oxen were treated very frequently (nearly 20 times/year) and calves almost never. To improve the situation in the Ghibe valley, extension messages should be delivered to promote a rational drug use, improved livestock management and the application of strategic vector control methods.

7. EXPERIMENTAL TRYPANOSOMOSIS

(a) DIAGNOSTICS

[See also **38**: 17433, 17487, 17502, 17506].

17515. **Ahmed, H. A., MacLeod, E. T., Welburn, S. C. & Picozzi, K., 2015.** Development of real time PCR to study experimental mixed infections of *T. congolense* savannah and *T. b. brucei* in *Glossina morsitans morsitans*. *PLoS One*, **10** (3): e0117147.

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Tsetse flies are able to acquire mixed infections naturally or experimentally either

simultaneously or sequentially. Traditionally, natural infection rates in tsetse flies are estimated by microscopic examination of different parts of the fly after dissection, together with the isolation of the parasite *in vivo*. However, until the advent of molecular techniques it was difficult to speciate trypanosomes infections and to quantify trypanosome numbers within tsetse flies. Although more expensive, qPCR allows the quantification of DNA and is less time consuming due to real time visualization and validation of the results. The current study evaluated the application of qPCR to quantify the infection load of tsetse flies with *T. b. brucei* and *T. congolense* savannah and to study the possibility of competition between the two species. The results revealed that the two qPCR reactions are of acceptable efficiency (99.1 percent and 95.6 percent, respectively), sensitivity and specificity and can be used for quantification of infection load with trypanosomes in experimentally infected *Glossina morsitans morsitans*. The mixed infection of laboratory *Glossina* species and quantification of the infection suggest the possibility that a form of competition exists between the isolates of *T. b. brucei* and *T. congolense* savannah that we used when they co-exist in the fly midgut.

17516. **Enyaru, C. K. J., Njuguna, J., Alibu, V. P., Matovu, E., Malele, I. I., Chisi, E. J., Mbongo, N., Mansinsa, P., Intisar, E. l. R., Mohammed, Y., Mubarak, M., Ochi, E. & Nantulya, V., 2015.** Development and evaluation of lateral flow test for the detection of trypanosomes in tsetse flies. *Journal of Parasitology & Vector Biology*, **6**(12) 181-188.

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A lateral flow test (LFT) which is based on antibodies raised against the *Trypanosoma brucei* peptide was evaluated for its analytical sensitivity, specificity, positive predictive value and negative predictive values in order to assess its utility for surveillance of trypanosome infections in tsetse flies. The diagnostic test agreement of the FLT and microscopy was 91.0 percent and kappa value of 80.5 percent at the confidence interval 0.72 to 0.80 which is a high level of test agreement. Furthermore, the relative diagnostic sensitivity and specificity of lateral flow test were 83.0 and 96.0 percent, respectively. Both the positive predictive and the negative predictive values were high at 92.7 and 90.0 percent, respectively. The LFT is, therefore, recommended for surveillance of trypanosomes in tsetse flies in order to: (i) indicate areas with tsetse infected with trypanosomes; (ii) indicate tsetse infected with potentially human pathogenic trypanosomes and (iii) guide in prioritizing control strategies for human African trypanosomiasis (HAT).

17517. **Hayashida, K., Kajino, K., Hachaambwa, L., Namangala, B. & Sugimoto, C., 2015.** Direct blood dry LAMP: a rapid, stable, and easy diagnostic tool for human African trypanosomiasis. *PLoS Neglected Tropical Diseases*, **9** (3): e0003578.

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Loop-mediated isothermal amplification (LAMP) is a rapid and sensitive tool used for the diagnosis of a variety of infectious diseases. One of the advantages of this method over the polymerase chain reaction is that DNA amplification occurs at a constant temperature, usually between 60 and 65 ^{0}C; therefore, expensive devices are unnecessary for this step. However, LAMP still requires complicated sample preparation steps and a well-equipped laboratory to produce reliable and reproducible results, which limits its use in resource-poor laboratories in most developing countries. In this study, we made several substantial modifications to the technique to carry out on-site diagnosis of human African trypanosomiasis (HAT) in remote areas using LAMP. The first essential improvement was that LAMP reagents were dried and stabilized in a single tube by incorporating trehalose as a cryoprotectant to prolong shelf life at ambient temperature. The second technical improvement was achieved by simplifying the sample preparation step so that DNA or RNA could be amplified directly from detergent-lysed blood samples. With these modifications, diagnosis of HAT in local clinics or villages in endemic areas becomes a reality, which could greatly impact on the application of diagnosis not only for HAT but also for other tropical diseases.

17518. **Jamonneau, V., Camara, O., Ilboudo, H., Peylhard, M., Koffi, M., Sakande, H., N'Dri, L., Sanou, D., Dama, E., Camara, M. & Lejon, V., 2015.** Accuracy of individual rapid tests for serodiagnosis of *gambiense* sleeping sickness in West Africa. *PLoS Neglected Tropical Diseases*, **9** (2): e0003480.

Centre International de Recherche-Développement sur l'Elevage en zones Subhumide (CIRDES), Bobo-Dioulasso, Burkina Faso; Institut de Recherche pour le Développement (IRD), Unité Mixte de Recherche IRD-CIRAD 177, Montpellier, France; Programme National de Lutte contre la Trypanosomose Humaine Africaine, Conakry, Guinea; Université Jean Lorougnon GUEDE (UJLoG), UFR Environnement, Laboratoire des Interactions Hôte-Microorganismes-Environnement et Evolution (LIHME), Daloa, Côte d'Ivoire; Institut Pierre Richet (IPR), Unité de Recherche Trypanosomoses, Bouaké, Côte d'Ivoire. [veerle.lejon@ird.fr].

Individual rapid tests for serodiagnosis (RDT) of human African trypanosomiasis (HAT) are particularly suited for passive screening and surveillance. However, so far, no large scale evaluation of RDTs has been performed for diagnosis of *Trypanosoma brucei gambiense* HAT in West Africa. The objective of this study was to assess the diagnostic accuracy of two commercial HAT-RDTs on stored plasma samples from West Africa. SD Bioline HAT and HAT Sero-K-Set were performed on 722 plasma samples originating from Guinea and Côte d'Ivoire, including 231 parasitologically confirmed HAT patients, 257 healthy controls, and 234 unconfirmed individuals whose blood tested antibody positive in the card agglutination test but negative by parasitological tests. Immune trypanolysis was performed as a reference test for the presence of trypanosome specific antibody. Sensitivities in HAT patients were respectively 99.6 percent for SD Bioline HAT, and 99.1 percent for HAT Sero-K-Set, specificities in healthy controls were respectively 87.9 percent and 88.3 percent. Considering combined positivity in both RDTs, increased the specificity significantly ($p </= 0.0003$) to 93.4 percent, while 98.7 percent sensitivity was maintained. Specificities in controls were 98.7-99.6 percent for the combination of one or two RDTs with trypanolysis, maintaining a sensitivity of at least 98.1 percent. The observed specificity of the single RDTs was relatively low. Serial

application of SD Bioline HAT and HAT Sero-K-Set might offer superior specificity compared with a single RDT, maintaining high sensitivity. The combination of one or two RDTs with trypanolysis seems promising for HAT surveillance.

17519. **Obishakin, E., Stijlemans, B., Santi-Rocca, J., Vandenberghe, I., Devreese, B., Muldermans, S., Bastin, P. & Magez, S., 2014.** Generation of a nanobody targeting the paraflagellar rod protein of trypanosomes. *PLoS One*, **9** (12): e115893.

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Trypanosomes are protozoan parasites that cause diseases in humans and livestock for which no vaccines are available. Disease eradication requires sensitive diagnostic tools and efficient treatment strategies. Immunodiagnostics based on antigen detection are preferable to antibody detection because the latter cannot differentiate between active infection and cure. Classical monoclonal antibodies are inaccessible to cryptic epitopes (based on their size - 150 kDa), costly to produce and require cold chain maintenance, a condition that is difficult to achieve in trypanosomiasis endemic regions, which are mostly rural. Nanobodies are recombinant, heat-stable, small-sized (15 kDa), antigen-specific, single-domain, variable fragments derived from heavy chain-only antibodies in camelids. Because of numerous advantages over classical antibodies, we investigated the use of nanobodies for the targeting of trypanosome-specific antigens and diagnostic potential. An alpaca was immunized using lysates of *Trypanosoma evansi*. Using phage display and bio-panning techniques, a cross-reactive nanobody (Nb392) targeting all trypanosome species and isolates tested was selected. Immunoblotting, immunofluorescence microscopy, immunoprecipitation and mass spectrometry assays were combined to identify the target recognized. Nb392 targets paraflagellar rod protein (PFR1) of *T. evansi*, *T. brucei*, *T. congolense* and *T. vivax*. Two different RNAi mutants with defective PFR assembly (PFR2RNAi and KIF9BRNAi) were used to confirm its specificity. In conclusion, using a complex protein mixture for alpaca immunization, we generated a highly specific nanobody (Nb392) that targets a conserved trypanosome protein, i.e. PFR1 in the flagella of trypanosomes. Nb392 is an excellent marker for the PFR and can be useful in the diagnosis of trypanosomiasis. In addition, as demonstrated, Nb392 can be a useful research or PFR protein isolation tool.

17520. **Rudramurthy, G. R., Sengupta, P. P., Metilda, B., Balamurugan, V., Prabhudas, K. & Rahman, H., 2015.** Development of an enzyme immunoassay using recombinant invariant surface glycoprotein (rISG) 75 for serodiagnosis of bovine trypanosomosis. *Indian Journal of Experimental Biology*, **53** (1): 7-15.

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Trypanosomosis or surra is caused by the haemoflagellate parasite, *Trypanosoma evansi* and is an important disease of animals, including domestic and wild herbivores and carnivores,

in tropical countries. The invariant surface glycoproteins (ISGs) are bloodstream stage specific and are uniformly distributed over the entire surface of the trypanosomes. In the present study, the extracellular domain (ED) region of ISG-75 from *T. evansi* encoding a polypeptide of 440 amino acids, has been heterologously expressed in *Escherichia coli*. Further, the immunoreactivity of recombinant ISG-75 (rISG-75) was characterized in immunoblot and ELISA using *T. evansi* hyperimmune sera raised in experimental animals. The protein was found immunoreactive when compared with a panel of antigens (VSG RoTat 1.2 and whole cell lysate) using bovine serum samples from the field. The diagnostic potential of rISG-75 was evaluated in ELISA with a large number of bovine field serum samples. The optimum sensitivity and specificity were 98.47 and 99.1, respectively. The present finding showed that the expressed protein has potential use in the serodiagnosis of trypanosomosis.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **38**: 17585, 17592, 17615, 17642].

17521. **Castillo-Acosta, V. M., Ruiz-Perez, L. M., Van Damme, E. J., Balzarini, J. & Gonzalez-Pacanowska, D., 2015.** Exposure of *Trypanosoma brucei* to an N-acetylglucosamine-binding lectin induces VSG switching and glycosylation defects resulting in reduced infectivity. *PLoS Neglected Tropical Diseases*, **9** (3): e0003612.

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17522. **Cheng, D., Weckerle, A., Yu, Y., Ma, L., Zhu, X., Murea, M., Freedman, B. I., Parks, J. S. & Shelness, G. S., 2015.** Biogenesis and cytotoxicity of APOL1 renal-risk variant proteins in hepatocytes and hepatoma cells. *Journal of Lipid Research,* **56** (8):1583-93.

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Two apolipoprotein L1 (APOL1) gene variants, which likely evolved to protect individuals from African sleeping sickness, are strongly associated with non-diabetic kidney disease in individuals with recent African ancestry. Consistent with its role in trypanosome killing, the pro-death APOL1 protein is toxic to most cells, but its mechanism of cell death is poorly understood and little is known regarding its intracellular trafficking and secretion. Because the liver appears to be the main source of circulating APOL1, we examined its secretory behaviour and mechanism of toxicity in hepatoma cells and primary human hepatocytes. APOL1 is poorly secreted *in vitro*, even in the presence of chemical chaperones; however, it is efficiently secreted in wild-type transgenic mice, suggesting that APOL1 secretion has specialized requirements that cultured cells fail to support. In hepatoma cells, inducible expression of APOL1 and its risk variants promoted cell death, with the G1 variant displaying the highest degree of toxicity. To explore the basis for APOL1-mediated cell toxicity, ER stress, pyroptosis, autophagy, and apoptosis were examined. Our results suggest that autophagy represents the predominant mechanism of APOL1-mediated cell death. Overall,

these results increase our understanding of the basic biology and trafficking behaviour of circulating APOL1 from the liver.

17523. **Cnops, J., De Trez, C., Stijlemans, B., Keirsse, J., Kauffmann, F., Barkhuizen, M., Keeton, R., Boon, L., Brombacher, F. & Magez, S., 2015.** NK-, NKT- and CD8-derived IFN-gamma drives myeloid cell activation and erythrophagocytosis, resulting in trypanosomosis-associated acute anaemia. *PLoS Pathogens*, **11** (6): e1004964.

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African trypanosomes are the causative agents of human African trypanosomosis (HAT/sleeping sickness) and animal African trypanosomosis (AAT/nagana). A common hallmark of African trypanosome infections is inflammation. In murine trypanosomosis, the onset of inflammation occurs rapidly after infection and is manifested by an influx of myeloid cells in both liver and spleen, accompanied by a burst of serum pro-inflammatory cytokines. Within 48 hours after reaching peak parasitaemia, acute anaemia develops and the percentage of red blood cells drops by 50 percent. Using a newly developed *in vivo* erythrophagocytosis assay, we recently demonstrated that activated cells of the myeloid phagocytic system display enhanced erythrophagocytosis causing acute anaemia. Here, we aimed to elucidate the mechanism and immune pathway behind this phenomenon in a murine model for trypanosomosis. Results indicate that IFN-gamma plays a crucial role in the recruitment and activation of erythrophagocytic myeloid cells, as mice lacking the IFN-gamma receptor were partially protected against trypanosomosis-associated inflammation and acute anaemia. NK and NKT cells were the earliest source of IFN-gamma during *T. b. brucei* infection. Later in infection, CD8+ and to a lesser extent CD4+ T cells become the main IFN-gamma producers. Cell depletion and transfer experiments indicated that during infection the absence of NK, NKT and CD8+ T cells, but not CD4+ T cells, resulted in a reduced anaemic phenotype similar to trypanosome infected IFN-gamma R-/- mice. Collectively, this study shows that NK, NKT and CD8+ T cell-derived IFN-gamma is a critical mediator in trypanosomosis-associated pathology, driving enhanced erythrophagocytosis by myeloid phagocytic cells and the induction of acute inflammation-associated anaemia.

17524. **Coles, J. A., Myburgh, E., Ritchie, R., Hamilton, A., Rodgers, J., Mottram, J. C., Barrett, M. P. & Brewer, J. M., 2015.** Intravital imaging of a massive lymphocyte response in the cortical dura of mice after peripheral infection by trypanosomes. *PLoS Neglected Tropical Diseases*, **9** (4): e0003714.

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Peripheral infection by *Trypanosoma brucei*, the protozoan responsible for sleeping sickness, activates lymphocytes, and, at later stages, causes meningoencephalitis. We have videoed the cortical meninges and superficial parenchyma of C56BL/6 reporter mice infected

with *T. b. brucei*. By use of a two-photon microscope to image through the thinned skull, the integrity of the tissues was maintained. We observed a 47-fold increase in CD2+ T cells in the meninges by 12 days post infection (dpi). CD11c+ dendritic cells also increased, and extravascular trypanosomes, made visible either by expression of a fluorescent protein, or by intravenous injection of furamidine, appeared. The likelihood that invasion will spread from the meninges to the parenchyma will depend strongly on whether the trypanosomes are below the arachnoid membrane, or above it, in the dura. Making use of optical signals from the skull bone, blood vessels and dural cells, we conclude that up to 40 dpi, the extravascular trypanosomes were essentially confined to the dura, as were the great majority of the T cells. Inhibition of T cell activation by intraperitoneal injection of abatacept reduced the numbers of meningeal T cells at 12 dpi and their mean speed fell from 11.64 +/- 0.34 μm/min (mean +/- SEM) to 5.2 +/- 1.2 μm/min ($p = 0.007$). The T cells occasionally made contact lasting tens of minutes with dendritic cells, indicative of antigen presentation. The population and motility of the trypanosomes tended to decline after about 30 dpi. We suggest that the lymphocyte infiltration of the meninges may later contribute to encephalitis, but have no evidence that the dural trypanosomes invade the parenchyma.

17525. **De Trez, C., Katsandegwaza, B., Caljon, G. & Magez, S., 2015.** Experimental African trypanosome infection by needle passage or natural tsetse fly challenge thwarts the development of collagen-induced arthritis in DBA/1 prone mice via an impairment of antigen specific B cell autoantibody titres. *PLoS One*, **10** (6): e0130431.

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Collagen-induced arthritis (CIA) is a B cell-mediated autoimmune disease. Recently published studies have demonstrated that in some rare cases pathogens can confer protection from autoimmunity. *Trypanosoma brucei* parasites are tsetse fly transmitted extracellular protozoans causing sleeping sickness disease in humans and Nagana in livestock in sub-Saharan endemic areas. In the past, we demonstrated that trypanosome infections impair B cell homeostasis and abolish vaccine-induced protection against unrelated antigens. Hence, here we hypothesized that trypanosome infection can affect the onset of CIA by specifically dampening specific B-cell responses and type II collagen antibody titres in DBA/1 prone mice. We observed a substantial delay in the onset of collagen-induced arthritis in *T. brucei*-infected DBA/1 mice that correlates with a drastic decrease of type II collagen titres of the different IgG isotypes in the serum. Treatment of infected mice with Berenil, a trypanocidal drug, restored the development of CIA-associated clinical symptoms. Interestingly, these data were confirmed by the challenge of immunized DBA/1 prone mice with *T. brucei*-infected tsetse flies. Together, these results demonstrate that T. *brucei* infection is impairing the maintenance of the antigen specific plasma B cell pool driving the development of CIA in DBA/1 prone mice.

17526. **Eze, J. I., Ayogu, L. C., Abonyi, F. O. & Eze, U. U., 2015.** The beneficial effect of dietary zinc supplementation on anaemia and immunosuppression in *Trypanosoma brucei* infected rats. *Experimental Parasitology*, **154**: 87-92.

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Zinc is an essential trace element crucial for normal development and function of cells mediating non-specific immunity and protects bio-molecules from oxidative damage. This study was designed to assess the effects of dietary zinc supplementation on anaemia and immunity of trypanosome-infected rats. From the results, dietary zinc supplementation can be useful in the management of anaemia and immunosuppression caused by trypanosomes in rats.

17527. **Jirku, M., Votypka, J., Petrzelkova, K. J., Jirku-Pomajbikova, K., Kriegova, E., Vodicka, R., Lankester, F., Leendertz, S. A., Wittig, R. M., Boesch, C., Modry, D., Ayala, F. J., Leendertz, F. H. & Lukes, J., 2015.** Wild chimpanzees are infected by *Trypanosoma brucei*. *International Journal for Parasitology: Parasites & Wildlife*, **4** (3): 277-282.

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Although wild chimpanzees and other African great apes live in regions endemic for African sleeping sickness, very little is known about their trypanosome infections, mainly due to major difficulties in obtaining their blood samples. In the present work, we established a diagnostic ITS1-based PCR assay that allows detection of the DNA of all four *Trypanosoma brucei* subspecies (*Trypanosoma brucei brucei*, *Trypanosoma brucei rhodesiense*, *Trypanosoma brucei gambiense*, and *Trypanosoma brucei evansi*) in faeces of experimentally infected mice. Next, using this assay we revealed the presence of trypanosomes in the faecal samples of wild chimpanzees and this finding was further supported by results obtained using a set of primate tissue samples. Phylogenetic analysis of the ITS1 region showed that the majority of the sequences obtained fell into the robust *T. brucei* group, providing strong evidence that these infections were caused by *T. b. rhodesiense* and/or *T. b. gambiense*. The optimized technique of trypanosome detection in faeces will improve our knowledge about the epidemiology of trypanosomes in primates and possibly also other endangered mammals, from which blood and tissue samples cannot be obtained. Finally, we demonstrated that the mandrill serum was able to efficiently lyse *T. b. brucei* and *T. b. rhodesiense*, and to some extent *T. b. gambiense*, while the chimpanzee serum failed to lyse any of these subspecies.

17528. **Liu, G., Sun, D., Wu, H., Zhang, M., Huan, H., Xu, J., Zhang, X., Zhou, H. & Shi, M., 2015.** Distinct contributions of CD4+ and CD8+ T cells to pathogenesis of *Trypanosoma brucei* infection in the context of gamma interferon and interleukin-10. *Infection & Immunity*, **83** (7): 2785-2795.

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Although gamma interferon (IFN-gamma) and interleukin-10 (IL-10) have been shown to be critically involved in the pathogenesis of African trypanosomiasis, the contributions to this disease of CD4(+) and CD8 (+) T cells, the major potential producers of the two cytokines, are incompletely understood. Here we show that, in contrast to previous findings, IFN-gamma

was produced by CD4 (+), but not CD8 (+), T cells in mice infected with *Trypanosoma brucei*. Without any impairment in the secretion of IFN-gamma, infected CD8 (-/-) mice survived significantly longer than infected wild-type mice, suggesting that CD8 (+) T cells mediated mortality in an IFN-gamma-independent manner. The increased survival of infected CD8 (-/-) mice was significantly reduced in the absence of IL-10 signalling. Interestingly, IL-10 was also secreted mainly by CD4 (+) T cells. Strikingly, depletion of CD4 (+) T cells abrogated the prolonged survival of infected CD8 (-/-) mice, demonstrating that CD4 (+) T cells mediated protection. Infected wild-type mice and CD8(-/-) mice depleted of CD4(+) T cells had equal survival times, suggesting that the protection mediated by CD4(+) T cells was counteracted by the detrimental effects of CD8(+) T cells in infected wild-type mice. Interestingly, CD4 (+) T cells also mediated the mortality of infected mice in the absence of IL-10 signalling, probably via excessive secretion of IFN-gamma. Finally, CD4 (+), but not CD8 (+), T cells were critically involved in the synthesis of IgG antibodies during *T. brucei* infections. Collectively, these results highlight distinct roles of CD4 (+) and CD8 (+) T cells in the context of IFN-gamma and IL-10 during *T. brucei* infections.

17529. **McCarroll, C. S., Rossor, C. L., Morrison, L. R., Morrison, L. J. & Loughrey, C. M., 2015.** A pre-clinical animal model of *Trypanosoma brucei* infection demonstrating cardiac dysfunction. *PLoS Neglected Tropical Diseases*, **9** (5): e0003811.

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African trypanosomiasis (AT) caused by *Trypanosoma brucei* species, results in both neurological and cardiac dysfunction and can be fatal if untreated. Research on the pathogenesis and treatment of the disease has centred to date on the characteristic neurological symptoms, whereas cardiac dysfunction (e.g. ventricular arrhythmias) in AT remains largely unstudied. Animal models of AT demonstrating cardiac dysfunction similar to that described in field cases of AT are critically required to transform our understanding of AT-induced cardiac pathophysiology and identify future treatment strategies. We have previously shown that *T. brucei* can interact with heart muscle cells (cardiomyocytes) to induce ventricular arrhythmias in *ex vivo* adult rat hearts. However, it is unknown whether the arrhythmias observed *ex vivo* are also present during *in vivo* infection in experimental animal models. Here we show for the first time the characterisation of ventricular arrhythmias *in vivo* in two animal models of AT infection using electrocardiographic (ECG) monitoring. The first model utilised a commonly used monomorphic laboratory strain, *Trypanosoma brucei brucei* Lister 427, whilst the second model used a pleomorphic laboratory strain, *T. b. brucei* TREU 927, which demonstrates a similar chronic infection profile to clinical cases. The frequency of ventricular arrhythmias and heart rate (HR) was significantly increased at the endpoint of infection in the TREU 927 infection model, but not in the Lister 427 infection model. At the end of infection, hearts from both models were isolated and Langendorff perfused *ex vivo* with increasing concentrations of the beta-adrenergic agonist isoproterenol (ISO). Interestingly, the increased frequency of arrhythmias observed *in vivo* in the TREU 927 infection model was lost upon isolation of the heart *ex vivo*, but re-emerged with the addition of ISO. Our results demonstrate

that TREU 927 infection modifies the substrate of the myocardium in such a way as to increase the propensity for ventricular arrhythmias in response to a circulating factor *in vivo* or beta-adrenergic stimulation *ex vivo*. The TREU 927 infection model provides a new opportunity to accelerate our understanding of AT-related cardiac pathophysiology and importantly has the required sensitivity to monitor adverse cardiac-related electrical dysfunction when testing new therapeutic treatments for AT.

17530. **McCulloch, R. & Field, M. C., 2015.** Quantitative sequencing confirms VSG diversity as central to immune evasion by *Trypanosoma brucei*. *Trends in Parasitology*, **31**(8): 346-349.

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Antigenic variation is central to the virulence of African trypanosomes, where the VSG coat is used to evade the host immune system. Recent advances in technology have now allowed more secrets of this system to emerge, with the surprising insight that a broad repertoire of VSGs is rapidly expressed. This has major implications for how the parasite must evade the host immune response.

17531. **McCulloch, R., Morrison, L. J. & Hall, J. P., 2015.** DNA recombination strategies during antigenic variation in the African trypanosome. *Microbiology Spectrum*, **3** (2): 1-25.

Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK; Roslin Institute, University of Edinburgh, UK; Department of Biology, University of York, York, UK. [Richard.McCulloch@glasgow.ac.uk].

Survival of the African trypanosome in its mammalian hosts has led to the evolution of antigenic variation, a process for evasion of adaptive immunity that has independently evolved in many other viral, bacterial and eukaryotic pathogens. The essential features of trypanosome antigenic variation have been understood for many years and comprise a dense, protective variant surface glycoprotein (VSG) coat, which can be changed by recombination-based and transcription-based processes that focus on telomeric VSG gene transcription sites. However, it is only recently that the scale of this process has been truly appreciated. Genome sequencing of *Trypanosoma brucei* has revealed a massive archive of >1 000 VSG genes, the huge majority of which are functionally impaired but are used to generate far greater numbers of VSG coats through segmental gene conversion. This chapter discusses the implications of such VSG diversity for immune evasion by antigenic variation, and considers how this expressed diversity can arise, drawing on a growing body of work that has begun to examine the proteins and sequences through which VSG switching is catalysed. Most studies of trypanosome antigenic variation have focused on *T. brucei*, the causative agent of human sleeping sickness. Other work has begun to look at antigenic variation in animal-infective trypanosomes, and compare the findings that are emerging, as well as consider how antigenic variation relates to the dynamics of host-trypanosome interaction.

17532. **Mony, B. M. & Matthews, K. R., 2015.** Assembling the components of the quorum sensing pathway in African trypanosomes. *Molecular Microbiology*, **96** (2): 220-232.

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African trypanosomes, parasites that cause human sleeping sickness, undergo a density-dependent differentiation in the bloodstream of their mammalian hosts. This process is driven by a released parasite-derived factor that causes parasites to accumulate in G1 and become quiescent. This is accompanied by morphological transformation to “stumpy” forms that are adapted to survival and further development when taken up in the blood meal of tsetse flies, the vector for trypanosomiasis. Although the soluble signal driving differentiation to stumpy forms is unidentified, a recent genome-wide RNAi screen identified many of the intracellular signalling and effector molecules required for the response to this signal. These resemble components of nutritional starvation and quiescence pathways in other eukaryotes, suggesting that parasite development shares similarities with the adaptive quiescence of organisms such as yeasts and *Dictyostelium* in response to nutritional starvation and stress. Here, the trypanosome signalling pathway is discussed in the context of these conserved pathways and the possible contributions of opposing “slender retainer” and “stumpy inducer” arms described. As evolutionarily highly divergent eukaryotes, the organisation and conservation of this developmental pathway can provide insight into the developmental cycle of other protozoan parasites, as well as the adaptive and programmed developmental responses of all eukaryotic cells.

17533. **Morias, Y., Abels, C., Laoui, D., Van Overmeire, E., Guilliams, M., Schouppe, E., Tacke, F., deVries, C. J., De Baetselier, P. & Beschin, A., 2015.** Ly6C- monocytes regulate parasite-induced liver inflammation by inducing the differentiation of pathogenic Ly6C+ monocytes into macrophages. *PLoS Pathogens*, **11** (5): e1004873.

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Monocytes consist of two well-defined subsets, the Ly6C+ and Ly6C- monocytes. Both CD11b+ myeloid cells populations have been proposed to infiltrate tissues during inflammation. While infiltration of Ly6C+ monocytes is an established pathogenic factor during hepatic inflammation, the role of Ly6C- monocytes remains elusive. Mice suffering experimental African trypanosome infection die from systemic inflammatory response syndrome (SIRS) that is initiated by phagocytosis of parasites by liver myeloid cells and culminates in apoptosis/necrosis of liver myeloid and parenchymal cells that reduces host survival. C57BL/6 mice are considered as trypanotolerant to *Trypanosoma congolense* infection. We have reported that in these animals, IL-10, produced among others by myeloid cells, limits the liver damage caused by pathogenic TNF-producing Ly6C+ monocytes,

ensuring prolonged survival. Here, the heterogeneity and dynamics of liver myeloid cells in *T. congolense*-infected C57/BL6 mice were further dissected. Moreover, the contribution of Ly6C- monocytes to trypanotolerance was investigated. By using FACS analysis and adoptive transfer experiments, we found that the accumulation of Ly6C- monocytes and macrophages in the liver of infected mice coincided with a drop in the pool of Ly6C+ monocytes. Pathogenic TNF mainly originated from Ly6C+ monocytes while Ly6C- monocytes and macrophages were major and equipotent sources of IL-10 within myeloid cells. Moreover, Nr4a1 (Nur77) transcription factor-dependent Ly6C- monocytes exhibited IL-10-dependent and cell contact-dependent regulatory properties contributing to trypanotolerance by suppressing the production of TNF by Ly6C+ monocytes and by promoting the differentiation of the latter cells into macrophages. Thus, Ly6C- monocytes can dampen liver damage caused by an extensive Ly6C+ monocyte-associated inflammatory immune response in *T. congolense* trypanotolerant animals. In a more general context, Ly6C- or Ly6C+ monocyte targeting may represent a therapeutic approach in liver pathogenicity induced by chronic infection.

17534. **Muchiri, M. W., Ndung'u, K., Kibugu, J. K., Thuita, J. K., Gitonga, P. K., Ngae, G. N., Mdachi, R. E. & Kagira, J. M., 2015.** Comparative pathogenicity of *Trypanosoma brucei rhodesiense* strains in Swiss white mice and *Mastomys natalensis* rats. *Acta Tropica*, **150**: 23-28.

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We evaluated *Mastomys natelensis* rat as an animal model for Rhodesian sleeping sickness. Parasitaemia, clinical and pathological characteristics induced by *T. b. rhodesiense* isolates, KETRI 3439, 3622 and 3637 were compared in *Mastomys* rats and Swiss white mice. Each isolate was intra-peritoneally injected in mice and rat groups ($n = 12$) at 1×10^4 trypanosomes/0.2 mL. Pre-patent period (PP) ranges for the KETRI 3439 and KETRI 3622-groups was 3-6 days for mice and 4-5 days for rats while for KETRI 3637-infected mice and rats they were 5-9 and 4-12 days, respectively. Pairwise comparison between PP of mice and rats separately infected with any one of the isolates showed no significant difference ($p > 0.05$). The PP's of KETRI 3637-infected mice were significantly ($p > 0.01$) longer than those infected with KETRI 3439 or KETRI 3622, a trend also observed in rats. The second parasitaemic wave was more prominent in mice. Clinical signs included body weakness, dyspnoea, peri-orbital oedema and extreme emaciation which were more common in rats. Survival times for KETRI 3439 and 3622-infected groups were significantly ($p < 0.05$) longer in mice than rats but similar in KETRI 3637-infected groups. Inflammatory lesions were more severe in rats than mice. All mice and KETRI 3622-infected rats had splenomegaly, organ congestion with rats additionally showing prominent lymphadenopathy. KETRI 3439-infected rats showed haemorrhagic pneumonia, enteritis with moderate splenomegaly and lymphadenopathy. KETRI 3637-infected rats had the most severe lesions characterized by prominent splenomegaly, lymphadenopathy, hepatomegaly, enlarged adrenal glands, organ congestion, generalized oedemas, gastroenteritis, pneumonia and brain congestion. KETRI 3637-infected *Mastomys* is a suitable model for studying pathophysiology of HAT.

17535. **Mugnier, M. R., Cross, G. A. & Papavasiliou, F. N., 2015.** The *in vivo* dynamics of antigenic variation in *Trypanosoma brucei*. *Science*, **347** (6229): 1470-1473.

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Trypanosoma brucei, a causative agent of African sleeping sickness, constantly changes its dense variant surface glycoprotein (VSG) coat to avoid elimination by the immune system of its mammalian host, using an extensive repertoire of dedicated genes. However, the dynamics of VSG expression in *T. brucei* during an infection are poorly understood. We have developed a method, based on *de novo* assembly of VSGs, for quantitatively examining the diversity of expressed VSGs in any population of trypanosomes, and monitored VSG population dynamics *in vivo*. Our experiments revealed unexpected diversity within parasite populations and a mechanism for diversifying the genome-encoded VSG repertoire. The interaction between *T. brucei* and its host is substantially more dynamic and nuanced than previously expected.

17536. **Onyilagha, C., Jia, P., Jayachandran, N., Hou, S., Okwor, I., Kuriakose, S., Marshall, A. & Uzonna, J. E., 2015.** The B cell adaptor molecule Bam32 is critically important for optimal antibody response and resistance to *Trypanosoma congolense* infection in mice. *PLoS Neglected Tropical Diseases*, **9** (4): e0003716.

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Bam32, a 32 kDa adaptor molecule, plays an important role in B cell receptor signalling, T cell receptor signalling and antibody affinity maturation in germinal centres. Since antibodies against trypanosome variant surface glycoproteins (VSGs) are critically important for control of parasitaemia, we hypothesized that Bam32 deficient (Bam32-/-) mice would be susceptible to *T. congolense* infection. We found that *T. congolense*-infected Bam32-/- mice successfully control the first wave of parasitaemia but then fail to control subsequent waves and ultimately succumb to their infections unlike wild type (WT) C57BL6 mice which are relatively resistant. Although infected Bam32-/- mice had significantly greater hepatomegaly and splenomegaly, their serum AST and ALT levels were not different, suggesting that increased liver pathology may not be responsible for the increased susceptibility of Bam32-/- mice to *T. congolense*. Using direct *ex vivo* flow cytometry and ELISA, we show that CD4+ T cells from infected Bam32-/- mice produced significantly increased amounts of disease-exacerbating pro-inflammatory cytokines (including IFN-gamma, TNF-alpha and IL-6). However, the percentages of regulatory T cells and IL-10-producing CD4+ cells were similar in infected WT and Bam32-/- mice. While serum levels of parasite-specific IgM antibodies were normal, the levels of parasite-specific IgG, (particularly IgG1 and IgG2a) were significantly lower in Bam32-/- mice throughout infection. This was associated with impaired germinal centre response in Bam32-/- mice despite increased numbers of T follicular helper (Tfh) cells. Adoptive transfer studies indicate that an intrinsic B cell defect was responsible for the enhanced susceptibility of Bam32-/- mice to *T. congolense* infection. Collectively, our data show that Bam32 is important for optimal anti-trypanosome IgG antibody response and suppression of disease-promoting pro-inflammatory cytokines and its deficiency leads to an inability to control *T. congolense* infection in mice.

17537. **Palomba, M., Seke-Etet, P. F., Laperchia, C., Tiberio, L., Xu, Y. Z., Colavito, V., Grassi-Zucconi, G. & Bentivoglio, M., 2015.** Alterations of orexinergic and melanin-

concentrating hormone neurons in experimental sleeping sickness. *Neuroscience*, **290**: 185-195.

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Human African trypanosomiasis or sleeping sickness is a severe, neglected tropical disease caused by the extracellular parasite *Trypanosoma brucei*. The disease, which leads to chronic neuroinflammation, is characterized by sleep and wake disturbances, documented also in rodent models. In rats and mice infected with *Trypanosoma brucei brucei*, we here tested the hypothesis that the disease could target neurons of the lateral hypothalamus (LH) containing orexin (OX)-A or melanin-concentrating hormone (MCH), implicated in sleep/wake regulation. In the cerebrospinal fluid of infected rats, the OX-A level was significantly decreased early after parasite neuroinvasion, and returned to the control level at an advanced disease stage. The number of immunohistochemically characterized OX-A and MCH neurons decreased significantly in infected rats during disease progression and in infected mice at an advanced disease stage. A marked reduction of the complexity of dendritic arborisations of OX-A neurons was documented in infected mice. The evaluation of NeuN-immunoreactive neurons did not reveal significant neuronal loss in the LH of infected mice, thus suggesting a potential selective vulnerability of OX-A and MCH neurons. Immunophenotyping and quantitative analysis showed marked activation of microglial cells surrounding OX-A neurons in infected mice. Day/night oscillation of c-Fos baseline expression was used as a marker of OX-A neuron activity in mice. In control animals, c-Fos was expressed in a higher proportion of OX-A neurons in the night (activity) phase than in the day (rest) phase. Interestingly, in infected mice the diurnal spontaneous c-Fos oscillation was reversed, with the proportion of OX-A/Fos neurons significantly higher at daytime than at night time. Altogether the findings reveal a progressive decrease of OX-A and MCH neurons and dysregulation of OX-A neuron diurnal activity in rodent models of sleeping sickness. The data point to the involvement of these peptidergic neurons in the pathogenesis of sleep/wake alterations in the disease and to their vulnerability to inflammatory signalling.

17538. **Stijlemans, B., Beschin, A., Magez, S., Van Ginderachter, J. A. & De Baetselier, P., 2015.** Iron homeostasis and *Trypanosoma brucei* associated immunopathogenicity development: a battle/quest for iron. *Biomedical Research International*, **2015**: Article ID 819389.

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African trypanosomosis is a chronic debilitating disease affecting the health and economic well-being of developing countries. The immune response during African trypanosome infection consisting of a strong proinflammatory M1-type activation of the myeloid phagocyte system (MYPS) results in iron deprivation for these extracellular parasites. Yet, the persistence of M1-type MYPS activation causes the development of anaemia (anaemia of chronic disease, ACD) as a most prominent pathological parameter in the mammalian host, due to enhanced erythrophagocytosis and retention of iron within the MYPS thereby depriving iron for erythropoiesis. In this review we give an overview of how parasites acquire iron from

the host and how iron modulation of the host MYPS affects trypanosomosis-associated anaemia development. Finally, we also discuss different strategies at the level of both the host and the parasite that can/might be used to modulate iron availability during African trypanosomosis.

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[See also **38**: 17441, 17443, 17583, 17584, 17628].

17539. **Adeyemi, O. S. & Sulaiman, F. A., 2015.** Evaluation of metal nanoparticles for drug delivery systems. *Journal of Biomedical Research*, **29** (2): 145-149.

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Diminazene aceturate is a trypanocide with unwanted toxicity and limited efficacy. It was reasoned that conjugating diminazene aceturate to functionalized nanoparticles would lower untoward toxicity while improving selectivity and therapeutic efficacy. Silver and gold nanoparticles were evaluated for their capacities to serve as carriers for diminazene aceturate. The silver and gold nanoparticles were synthesized, functionalized and coupled to diminazene aceturate following established protocols. The nanoparticle conjugates were characterized. The free diminazene aceturate and drug conjugated nanoparticles were subsequently evaluated for cytotoxicity *in vitro*. The characterizations by transmission electron microscopy or UV/Vis spectroscopy revealed that conjugation of diminazene aceturate to silver or gold nanoparticles was successful. Evaluation for cytotoxic actions *in vitro* demonstrated no significance difference between free diminazene aceturate and the conjugates. Our data suggest that surface modified metal nanoparticles could be optimized for drug delivery systems.

17540. **Alves, M. A., de Queiroz, A. C., Alexandre-Moreira, M. S., Varela, J., Cerecetto, H., Gonzalez, M., Doriguetto, A. C., Landre, I. M., Barreiro, E. J. & Lima, L. M., 2015.** Design, synthesis and *in vitro* trypanocidal and leishmanicidal activities of novel semicarbazone derivatives. *European Journal of Medicinal Chemistry*, **100**: 24-33.

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17541. **Carrillo, A. K., Guiguemde, W. A. & Guy, R. K., 2015.** Evaluation of histone deacetylase inhibitors (HDACi) as therapeutic leads for human African trypanosomiasis (HAT). *Bioorganic & Medicinal Chemistry*, **23**(16): 5151-5155.

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17542. **Carvalho, L., Martinez-Garcia, M., Perez-Victoria, I., Manzano, J. I., Yardley, V., Gamarro, F. & Perez-Victoria, J. M., 2015.** The oral antimalarial drug tafenoquine shows activity against *Trypanosoma brucei*. *Antimicrobial Agents & Chemotherapy*. **E**

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The protozoan parasite *Trypanosoma brucei* causes human African trypanosomiasis, or sleeping sickness, a neglected tropical disease that requires new, safer, and more effective treatments. Repurposing oral drugs could reduce both the time and cost involved in sleeping sickness drug discovery. Tafenoquine (TFQ) is an oral antimalarial drug belonging to the 8-aminoquinoline family which is currently in clinical phase III. Here we show that TFQ efficiently kills different *T. brucei* spp. in the sub-micromolar concentration range. Our results suggest that TFQ accumulates into acidic compartments and induces a necrotic process involving cell membrane disintegration and loss of cytoplasmic content leading to parasite death. Cell lysis is preceded by a wide and multi-target drug action, affecting the lysosome, mitochondria and acidocalcisomes, and inducing a depolarization of the mitochondrial membrane potential, elevation of intracellular Ca^{2+} and production of reactive oxygen species. This is the first report of an 8-aminoquinoline demonstrating significant *in vitro* activity against *T. brucei*.

17543. **Cnops, J., Bockstal, V., De Trez, C., Clopes, M. M., Radwanska, M. & Magez, S., 2015.** Curative drug treatment of trypanosomosis leads to the restoration of B cell lymphopoiesis and splenic B cell compartments. *Parasite Immunology*, **37**(5): 489-491.

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African trypanosomosis is a parasitic disease affecting both humans (sleeping sickness) and animals (nagana). In murine trypanosomosis, the B cell compartment is rapidly destroyed after infection. In addition, B cell lymphopoiesis in the bone marrow is abrogated, B cell subsets in the spleen are irreversibly depleted and B cell memory is destroyed. Here we investigated the effect of cure of infection on the B cell compartment. Suramin and diminazene aceturate were used in this study as these drugs exhibit different modes of uptake and different mechanisms of trypanocidal action. Curative drug treatment of trypanosomosis led to the re-initiation of B cell lymphopoiesis in the bone marrow, and to the repopulation of splenic B cell subsets, independent of the drug used. Neither of these drugs by itself induced measurable effects on B cell lymphopoiesis in the bone marrow or B cell homeostasis in the spleen in healthy, naive animals.

17544. **Cretton, S., Breant, L., Pourrez, L., Ambuehl, C., Perozzo, R., Marcourt, L., Kaiser, M., Cuendet, M. & Christen, P., 2015.** Chemical constituents from *Waltheria indica* exert *in vitro* activity against *Trypanosoma brucei* and *T. cruzi*. *Fitoterapia*, **105**: 55-60.

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17545. **de Macedo, J. P., Schumann Burkard, G., Niemann, M., Barrett, M. P., Vial, H., Maser, P., Roditi, I., Schneider, A. & Butikofer, P., 2015.** An atypical mitochondrial carrier that mediates drug action in *Trypanosoma brucei*. *PLoS Pathogens*, **11** (5): e1004875.

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Elucidating the mechanism of action of trypanocidal compounds is an important step in the development of more efficient drugs against *Trypanosoma brucei*. In a screening approach using an RNAi library in *T. brucei* bloodstream forms, we identified a member of the mitochondrial carrier family, TbMCP14, as a prime candidate mediating the action of a group of anti-parasitic choline analogues. Depletion of TbMCP14 by inducible RNAi in both bloodstream and procyclic forms increased resistance of parasites towards the compounds by seven-fold and three-fold, respectively, compared with uninduced cells. In addition, down-regulation of TbMCP14 protected bloodstream form mitochondria from a drug-induced decrease in mitochondrial membrane potential. Conversely, over-expression of the carrier in procyclic forms increased parasite susceptibility more than 13-fold. Metabolomic analyses of parasites over-expressing TbMCP14 showed increased levels of the proline metabolite, pyrroline-5-carboxylate, suggesting a possible involvement of TbMCP14 in energy production. The generation of TbMCP14 knock-out parasites showed that the carrier is not essential for survival of *T. brucei* bloodstream forms, but reduced parasite proliferation under standard culture conditions. In contrast, depletion of TbMCP14 in procyclic forms resulted in growth arrest, followed by parasite death. The time point at which parasite proliferation stopped was dependent on the major energy source, i.e. glucose versus proline, in the culture medium. Together with our findings that proline-dependent ATP production in crude mitochondria from TbMCP14-depleted trypanosomes was reduced compared with control mitochondria, the study demonstrated that TbMCP14 is involved in energy production in *T. brucei*. Since TbMCP14 belongs to a trypanosomatid-specific clade of mitochondrial carrier family proteins showing very poor similarity to mitochondrial carriers of mammals, it may represent an interesting target for drug action or targeting.

17546. **De Mieri, M., Kaiser, M., Brun, R., Thormann, U., Imanidis, G. & Hamburger, M., 2015.** Anti-trypanosomal cadinanes synthesized by transannular cyclization of the natural sesquiterpene lactone nobilin. *Bioorganic & Medicinal Chemistry*, **23** (7): 1521-1529.

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17547. **Ellendorff, T., Brun, R., Kaiser, M., Sendker, J. & Schmidt, T. J., 2015.** PLS-prediction and confirmation of hydrojuglone glucoside as the antitrypanosomal constituent of *Juglans* spp. *Molecules*, **20** (6): 10082-10094.

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17548. **Ellis, S., Sexton, D. W. & Steverding, D., 2015.** Trypanotoxic activity of thiosemicarbazone iron chelators. *Experimental Parasitology*, **150**: 7-12.

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Only a few drugs are available for treating sleeping sickness and nagana disease, parasitic infections caused by protozoans of the genus *Trypanosoma* in sub-Saharan Africa. There is an urgent need for the development of new medicines for chemotherapy of these devastating diseases. In this study, three newly designed thiosemicarbazone iron chelators, TSC24, Dp44mT and 3-AP, were tested for *in vitro* activity against bloodstream forms of *Trypanosoma brucei* and human leukaemia HL-60 cells. In addition to their iron chelating properties, TSC24 and Dp44mT inhibit topoisomerase II alpha, while 3-AP inactivates ribonucleotide reductase. All three compounds exhibited anti-trypanosomal activity, with minimum inhibitory concentration (MIC) values ranging between 1 and 100 μM and 50 percent growth inhibition (GI_{50}) values of around 250 nM. Although the compounds did not kill HL-60 cells (MIC values >100 μM), TSC24 and Dp44mT displayed considerable cytotoxicity based on their GI_{50} values. Iron supplementation partly reversed the trypanotoxic and cytotoxic activity of TSC24 and Dp44mT but not of 3-AP. This finding suggests possible synergy between the iron chelating and topoisomerase II alpha inhibiting activity of the compounds. However, further investigation using separate agents, the iron chelator deferoxamine and the topoisomerase II inhibitor epirubicin, did not support any synergy for the interaction of iron chelation and topoisomerase II inhibition. Furthermore, TSC24 was shown to induce DNA degradation in bloodstream forms of *T. brucei* indicating that the mechanism of trypanotoxic activity of the compound is topoisomerase II independent. In conclusion, the data support further investigation of thiosemicarbazone iron chelators with dual activity as lead compounds for anti-trypanosomal drug development.

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Eskitis Institute for Drug Discovery, Griffith University, Queensland, Australia; Institute for Molecular Bioscience, The University of Queensland, Brisbane, Q4072, Australia. [k.andrews@griffith.edu.au]

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Department of Physical Chemistry, University of Valencia, Avenida V.A. Estelles s/n,

46100, Burjassot, Valencia, Spain. [ramon.garcia@uv.es].

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R&D Sigma-Tau Industrie Farmaceutiche Riunite S.p.A., Via Pontina Km 30 400, I-00040, Pomezia, Roma, Italy. [giuseppe.giannini@sigma-tau.it].

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Department of Chemistry, University of Basel, 4056 Basel, Switzerland. [thomas.pfohl@unibas.ch].

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Parasite Chemotherapy, Swiss Tropical and Public Health Institute, Basel, Switzerland; University of Basel, Basel, Switzerland; Laboratory of Microbiology, Parasitology and Hygiene, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Antwerp University, Antwerp, Belgium; Drugs for Neglected Diseases Initiative, Geneva, Switzerland; Medicines for Malaria Venture, Geneva, Switzerland; [jrioset@dndi.org].

Phenotypic screening has successfully been used for hit generation, especially in the field of neglected diseases, in which feeding the drug pipeline with new chemotypes remains a constant challenge. Here, we catalyse drug discovery research using a publicly available screening tool to boost drug discovery. The Malaria Box, assembled by the Medicines for Malaria Venture, is a structurally diverse set of 200 drug-like and 200 probe-like compounds distilled from more than 20 000 antimalarial hits from corporate and academic libraries. Repurposing such compounds has already identified new scaffolds against cryptosporidiosis and schistosomiasis. In addition to initiating new hit-to-lead activities, screening the Malaria Box against a plethora of other parasites would enable the community to better understand the similarities and differences between them. We describe the screening of the Malaria Box and triaging of the identified hits against kinetoplastids responsible for human African trypanosomiasis (*Trypanosoma brucei*), Chagas disease (*Trypanosoma cruzi*), and visceral leishmaniasis (*Leishmania donovani* and *Leishmania infantum*). The *in vitro* and *in vivo* profiling of the most promising active compounds with respect to efficacy, toxicity, pharmacokinetics, and complementary druggable properties are presented and a collaborative model used as a way to accelerate the discovery process discussed.

17554. **Konziase, B., 2015.** Biotinylated probes of artemisinin with labelling affinity toward *Trypanosoma brucei brucei* target proteins. *Annals of Biochemistry*, **482**: 25-31.

Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565-0871, Japan. [benetode@hotmail.com].

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Eskitis Institute for Drug Discovery, Griffith University, Brisbane, QLD 4111, Australia; Australian Prostate Cancer Research Centre-Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Princess Alexandra Hospital, Translational Research Institute, Brisbane, QLD 4102, Australia.

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Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA.

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Sir William Dunn School of Pathology, University of Oxford, Oxford, UK. [laura.makin@path.ox.ac.uk].

Despite recent research linking cAMP signalling to virulence in trypanosomatids, and detailed studies of trypanosomatid adenylyl cyclases (ACs) and phosphodiesterases (PDEs) since their discoveries 40 years ago, downstream components of the pathway and their biological functions have remained remarkably elusive. However, in recent years, significant discoveries have been made: a role for parasite ACs has been proposed in cytokinesis, evasion of the host immune response, and social motility. cAMP phosphodiesterases PDEB1 and PDEB2 were found to be essential for survival and virulence of *Trypanosoma brucei* and, in *Trypanosoma cruzi*, PDEC2 was shown to be required for normal osmoregulation. As we discuss here, these breakthroughs have led to an ongoing surge in the development of PDE inhibitors as lead compounds for trypanocidal drugs.

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Instituto de Quimica Medica, Consejo Superior de Investigaciones Cientificas (IQM-CSIC), Juan de la Cierva 3, E-28006 Madrid, Spain.

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Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8TA, UK; Wellcome Trust Centre for Molecular Parasitology, University of Glasgow, Glasgow, UK; Department of Laboratory Technology, University of Cape Coast, Cape Coast, Ghana; Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria; Department of Biology, College of Science, Aljouf University, Sakaka, Saudi Arabia; Faculty of Medical Sciences, Taif University, Taif, Saudi Arabia; Department of Chemistry and Chemical Biology, 417 Egan Research Center, Northeastern University, 360 Huntington Avenue, Boston, MA, 02115, USA. [Harry.de-Koning@glasgow.ac.uk].

The *Trypanosoma brucei* aminopurine transporter P2/TbAT1 has long been implicated in the transport of, and resistance to, the diamidine and melaminophenyl arsenical classes of drugs that form the backbone of the pharmacopoeia against African trypanosomiasis. Genetic alterations including deletions and single nucleotide polymorphisms (SNPs) have been observed in numerous strains and clinical isolates. Here, we systematically investigate each reported mutation and assess their effects on transporter function after expression in a tbat1 (-/-) *T. brucei* line. Out of a set of six reported SNPs from a reported "resistance allele", none significantly impaired sensitivity to pentamidine, diminazene or melarsoprol, relative to the TbAT1-WT allele, although several combinations, and the deletion of the codon for residue F316, resulted in highly significant impairment. These combinations of SNPs, and Delta F316, also strongly impaired the uptake of [^{3}H]-adenosine and [^{3}H]-diminazene, identical to the tbat1 (-/-) control. The TbAT1 protein model predicted that residues F19, D140 and F316 interact with the substrate of the transporter. Mutation of D140 to alanine resulted in an inactive transporter, whereas the mutation F19A produced a transporter with a slightly increased affinity for [^{3}H]-diminazene but reduced the uptake rate. The results presented here validate earlier hypotheses of drug binding motifs for TbAT1.

17561. **Nwodo, N. J., Ibezim, A., Ntie-Kang, F., Adikwu, M. U. & Mbah, C. J., 2015.** Anti-trypanosomal activity of Nigerian plants and their constituents. *Molecules*, **20** (5): 7750-7771.

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African trypanosomiasis is a vector-borne parasitic disease causing serious risks to the lives of about 60 million people and 48 million cattle globally. Nigerian medicinal plants are known to contain a large variety of chemical structures and some of the plant extracts have been screened for anti-trypanosomal activity, in the search for potential new drugs against the illness. We surveyed the literatures on plants and plant-derived products with anti-trypanosomal activity from Nigerian flora published from 1990 to 2014. About 90 plants were identified, with 54 compounds as potential active agents and presented by plant families in alphabetical order. This review indicates that the Nigerian flora may be suitable as a starting

point in searching for new and more efficient trypanocidal molecules.

17562. **Nwodo, N., Okoye, F., Lai, D., Debbab, A., Kaiser, M., Brun, R. & Proksch, P., 2015.** Evaluation of the *in vitro* trypanocidal activity of methylated flavonoid constituents of *Vitex simplicifolia* leaves. *BMC Complementary Alternative Medicine*, **15**: 82.

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Trypanosomiasis is a neglected tropical disease with complex clinical manifestations, tedious diagnosis, and difficult treatments. The drugs available for the treatment of this endemic disease are old, expensive, and associated with other problems including safety and drug resistant parasites. Therefore, there is an urgent need for the development of new, effective, cheap, and safe drugs for its treatment. Plants are potentially rich sources of leads for new drugs against trypanosomiasis. *Vitex simplicifolia* (*Verbenaceae*) is used traditionally for the treatment of toothache, oedema, skin diseases, gout and trypanosomiasis in Nigeria. In a preliminary study, the methanol extract of *Vitex simplicifolia* was shown to exhibit a pronounced trypanocidal activity against *T. b. rhodesiense*. The present study was undertaken to investigate the active component responsible for the acclaimed activity of the leaves of *Vitex simplicifolia* in the traditional treatment of trypanosomiasis in Nigeria and other African countries. Our investigations aimed at assessing the plant as a new source of potential trypanocidal compounds. The crude extracts were prepared from the dried leaves using methanol; successive extraction with hexane, dichloromethane, ethyl acetate and butanol was also done. The ethyl acetate fraction was further fractionated and compounds isolated using a preparative chromatographic technique and their structures were elucidated by NMR, mass spectrometry and comparison with literature data. Trypanocidal activities and cytotoxicity using rat skeletal myoblast (L6) cells were investigated and their selectivity indices were determined. The chromatographic separations of the methanol extracts gave rise to seven compounds. The isolated compounds 2, 3, 6 and 7 exhibited promising trypanocidal activity with IC_{50} values ranging from 4.7-12.3 µg/mL and cytotoxicity in the range of 1.58-46.20 µg/mL. Compound 6, however, showed the most selective trypanocidal activity with a selectivity index of 9.8. This is the first report of trypanocidal activity of the flavonoids from this plant genus. In conclusion, the isolated compounds from *Vitex simplicifolia* exhibited noteworthy trypanocidal activities and hence may provide a source of new anti-trypanosomal agents. These results also support the traditional use of *Vitex simplicifolia* in the treatment of trypanosomiasis. This is the first report of the trypanocidal effect of flavonoids from this plant genus.

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Departments of Veterinary Medicine and Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria.

17564. **O'Sullivan, M. C., Durham, T. B., Valdes, H. E., Dauer, K. L., Karney, N. J., Forrestel, A. C., Bacchi, C. J. & Baker, J. F., 2015.** Dibenzosuberyl substituted polyamines and analogues of clomipramine as effective inhibitors of trypanothione

reductase; molecular docking, and assessment of trypanocidal activities. *Bioorganic & Medicinal Chemistry*, **23** (5): 996-1010.

Department of Chemistry and Biochemistry, Canisius College, 2001 Main Street, Buffalo, NY 14208, USA. [osulliv1@canisius.edu].

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Molecular Discovery Research, Tres Cantos Medicines Development Campus, GlaxoSmithKline, Tres Cantos, Spain.

Using whole-cell phenotypic assays, the GlaxoSmithKline high throughput screening (HTS) diversity set of 1.8 million compounds was screened against the three kinetoplastids most relevant to human disease, i.e. *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosoma brucei*. Secondary confirmatory and orthogonal intracellular parasiticidal assays were conducted, and the potential for non-specific cytotoxicity determined. Hit compounds were chemically clustered and triaged for desirable physicochemical properties. The hypothetical biological target space covered by these diversity sets was investigated through bioinformatics methodologies. Consequently, three anti-kinetoplastid chemical boxes of ~200 compounds each were assembled. Functional analyses of these compounds suggest a wide array of potential modes of action against kinetoplastid kinases, proteases and cytochromes as well as potential host-pathogen targets. This is the first published parallel high throughput screening of a pharma compound collection against kinetoplastids. The compound sets are provided as an open resource for future lead discovery programmes, and to address important research questions.

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Université Lille 1, UGSF, UMR 8576 CNRS, 59650 Villeneuve d'Ascq, France. [christophe.biot@univ-lille1.fr].

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

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

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Laboratori de Quimica Farmaceutica (Unitat Associada al CSIC), Facultat de Farmacia, and Institut de Biomedicina (IBUB), Universitat de Barcelona, Av. Joan XXIII, 27-31, E-08028 Barcelona, Spain. [dmunoztorrero@ub.edu].

Dual sub-micromolar trypanocidal-antiplasmodial compounds have been identified by screening and chemical synthesis of 4-aminoquinoline-based heterodimeric compounds of three different structural classes. In *Trypanosoma brucei*, inhibition of the enzyme trypanothione reductase seems to be involved in the potent trypanocidal activity of these heterodimers, although it is probably not the main biological target. Regarding antiplasmodial activity, the heterodimers seem to share the mode of action of the antimalarial drug chloroquine, which involves inhibition of the haem detoxification process. Interestingly, all of these heterodimers display good brain permeabilities, thereby being potentially useful for late stage human African trypanosomiasis. Future optimization of these compounds should focus mainly on decreasing cytotoxicity and acetylcholinesterase inhibitory activity.

17570. **Steverding, D., 2015.** Evaluation of trypanocidal activity of combinations of anti-sleeping sickness drugs with cysteine protease inhibitors. *Experimental Parasitology*, **151-152**: 28-33.

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Chemotherapy of human African trypanosomiasis (HAT) is unsatisfactory because only a few drugs, with serious side effects and poor efficacy, are available. As drug combination regimes often achieve greater therapeutic efficacy than monotherapies, here the trypanocidal activity of the cysteine protease inhibitor K11777 in combination with current anti-HAT drugs using bloodstream forms of *Trypanosoma brucei* was investigated. Isobolographic analysis was used to determine the interaction between cysteine protease inhibitors (K11777, CA-074Me and CAA0225) and anti-HAT drugs (suramin, pentamidine, melarsoprol and eflornithine). Bloodstream forms of *T. brucei* were incubated in culture medium containing cysteine protease inhibitors or anti-HAT drugs alone or in combination at a 1:1 fixed-dose ratio. After 48 h incubation, live cells were counted, the 50 percent growth inhibition values determined and combination indices calculated. The general cytotoxicity of drug combinations was evaluated with human leukaemia HL-60 cells. Combinations of K11777 with suramin, pentamidine and melarsoprol showed antagonistic effects while with eflornithine a synergistic effect was observed. Whereas eflornithine antagonises with CA-074Me, an inhibitor inactivating the targeted TbCATL only under reducing conditions, it synergises with CAA0255, an inhibitor structurally related to CA-074Me which inactivates TbCATL independently of thiols. These findings indicate an essential role of thiols for the synergistic interaction between K11777 and eflornithine. Encouragingly, the K11777/eflornithine combination displayed higher

trypanocidal than cytotoxic activity. The results of this study suggest that the combination of the cysteine protease inhibitor K11777 and eflornithine displays promising synergistic trypanocidal activity that warrants further investigation of the drug combination as possible alternative treatment of HAT.

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Faculty of Pharmaceutical Sciences, Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan [shoyama@niu.ac.jp].

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College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea. [thaonp@imbc.vast.vn].

17573. **Thuita, J. K., Wolf, K. K., Murilla, G. A., Bridges, A. S., Boykin, D. W., Mutuku, J. N., Liu, Q., Jones, S. K., Gem, C. O., Ching, S., Tidwell, R. R., Wang, M. Z., Paine, M. F. & Brun, R., 2015.** Chemotherapy of second stage human African trypanosomiasis: comparison between the parenteral diamidine DB829 and its oral prodrug DB868 in vervet monkeys. *PLoS Neglected Tropical Diseases*, **9** (2): e0003409.

Trypanosomiasis Research Centre, Kenya Agricultural Research Institute (TRC-KARI), Kikuyu, Kenya; University of North Carolina Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; Department of Pathology and Laboratory Medicine, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; Department of Chemistry, Georgia State University, Atlanta, Georgia, USA; SVC Associates Inc., Apex, North Carolina, USA; Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, Kansas, USA; Swiss Tropical and Public Health Institute, Basel, Switzerland; University of Basel, Basel, Switzerland. [thuitajk@yahoo.com].

Human African trypanosomiasis (HAT, sleeping sickness) ranks among the most neglected tropical diseases based on limited availability of drugs that are safe and efficacious, particularly against the second stage (central nervous system [CNS]) of infection. In response to this largely unmet need for new treatments, the Consortium for Parasitic Drug Development developed novel parenteral diamidines and corresponding oral prodrugs that have shown cure of a murine model of second stage HAT. As a rationale for selection of one of these compounds for further development, the pharmacokinetics and efficacy of intramuscular (IM) active diamidine 2,5-bis(5-amidino-2-pyridyl)furan (DB829; CPD-0802) and oral prodrug 2,5-bis[5-(N-methoxyamidino)-2-pyridyl]furan (DB868) were compared in the vervet monkey model of

second stage HAT. Treatment was initiated 28 days post-infection of monkeys with *T. b. rhodesiense* KETRI 2537. Results showed that IM DB829 at 5 mg/kg/day for five consecutive days, 5 mg/kg/day every other day for five doses, or 2.5 mg/kg/day for five consecutive days cured all monkeys (5/5). Oral DB868 was less successful, with no cures (0/2) at 3 mg/kg/day for 10 days and cure rates of 1/4 at 10 mg/kg/day for 10 days and 20 mg/kg/day for 10 days; in total, only 2/10 monkeys were cured with DB868 dose regimens. The geometric mean plasma C_{max} of IM DB829 at 5 mg/kg following the last of five doses was 25-fold greater than that after 10 daily oral doses of DB868 at 20 mg/kg. These data suggest that the active diamidine DB829, administered IM, should be considered for further development as a potential new treatment for second stage HAT.

17574. **Unciti-Broceta, J. D., Arias, J. L., Maceira, J., Soriano, M., Ortiz-Gonzalez, M., Hernandez-Quero, J., Munoz-Torres, M., de Koning, H. P., Magez, S. & Garcia-Salcedo, J. A., 2015.** Specific cell targeting therapy bypasses drug resistance mechanisms in African trypanosomiasis. *PLoS Pathogens*, **11** (6): e1004942.

Unidad de Enfermedades Infecciosas y Microbiologia, Instituto de Investigacion Biosanitaria ibs. Granada, Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain. [jags@genyo.es].

African trypanosomiasis is a deadly neglected disease caused by the extracellular parasite *Trypanosoma brucei*. Current therapies are characterized by high drug toxicity and increasing drug resistance mainly associated with loss-of-function mutations in the transporters involved in drug import. The introduction of new antiparasitic drugs into therapeutic use is a slow and expensive process. In contrast, specific targeting of existing drugs could represent a more rapid and cost-effective approach for neglected disease treatment, impacting through reduced systemic toxicity and circumventing resistance acquired through impaired compound uptake. We have generated nanoparticles of chitosan loaded with the trypanocidal drug pentamidine and coated by a single domain nanobody that specifically targets the surface of African trypanosomes. Once loaded into this nanocarrier, pentamidine enters trypanosomes through endocytosis instead of via classical cell surface transporters. The curative dose of pentamidine-loaded nanobody-chitosan nanoparticles was 100-fold lower than pentamidine alone in a murine model of acute African trypanosomiasis. Crucially, this new formulation displayed undiminished *in vitro* and *in vivo* activity against a trypanosome cell line resistant to pentamidine as a result of mutations in the surface transporter aquaglyceroporin 2. We conclude that this new drug delivery system increases drug efficacy and has the ability to overcome resistance to some anti-protozoal drugs.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

17575. **Ajoko, C. & Steverding, D., 2015.** A cultivation method for growing bloodstream forms of *Trypanosoma brucei* to higher cell density and for longer time. *Parasitology Research*, **114** (4): 1611-1612.

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A novel cultivation technique using transwells allowing the growth of bloodstream forms of *Trypanosoma brucei* for longer periods of time and to higher cell densities is described. Trypanosomes cultured in transwells placed in cups of tissue culture plates containing six-fold excess of medium grew within four days to maximum cell densities of 2 x 10^7 parasites per mL. Compared with control cultures, the exponential growth of trypanosomes was one day longer and the maximum cell concentration was increased four-fold. The new culture system may be useful in drug screening assays and analysing the *T. brucei* secretome.

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

[See also **38**: 17491, 17514].

17576. **Dagnachew, S., Terefe, G., Abebe, G., Barry, D., McCulloch, R. & Goddeeris, B., 2015.** *In vivo* experimental drug resistance study in *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of northwest Ethiopia. *Acta Tropica*, **146**: 95-100.

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Ethiopia, particularly in the northwest region, is affected by both tsetse fly and non-tsetse fly transmitted trypanosomosis with a significant impact on livestock productivity. The control of trypanosomosis in Ethiopia relies on either curative or prophylactic treatment of animals with diminazene aceturate (DA) or isometamidium chloride (ISM), respectively. However, since these two trypanocides have been on the market for more than 40 years, this may have resulted in drug resistance. Therefore, *in vivo* drug resistance tests on two Ethiopian isolates of *Trypanosoma vivax* were completed, one from an area where tsetse flies are present and one from an area where tsetse flies are not present. Twenty four cattle (*Bos indicus*) aged between 6 and 12 months, purchased from a trypanosome-free area (Debre Brehan, northcentral Ethiopia) and confirmed to be trypanosome-negative, were randomly assigned into four groups of six animals, which were infected with *T. vivax* isolated from a tsetse-infested or non-tsetse infested area, and in each case treated with curative doses of DA or ISM. Each animal was inoculated intravenously 3x10(6) trypanosomes from donor animals. Parasitaemia became patent earlier in infections with non-tsetse *T. vivax* (approximately seven days post-infection) than tsetse (approximately 14 days post-infection). Both groups were treated at the highest peak parasitaemia with DA or ISM and nine cattle, four with non-tsetse *T. vivax* (two ISM- and two DA-treated) and five with tsetse *T. vivax* (three ISM- and two DA-treated) showed relapses of parasitaemia. Moreover, treatment did not improve diagnostic host markers of trypanosome infections in these animals. In conclusion, *in vivo* drug tests indicated the presence of resistant parasites (> 20 percent of treated animals in each group relapsed) against recommended doses of both available trypanocidal drugs.

17577. **Graf, F. E., Baker, N., Munday, J. C., de Koning, H. P., Horn, D. & Maser, P., 2015.** Chimerization at the AQP2-AQP3 locus is the genetic basis of melarsoprol-pentamidine cross-resistance in clinical *Trypanosoma brucei gambiense* isolates. *International Journal of Parasitology: Drugs & Drug Resistance*, **5** (2): 65-68.

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Aquaglyceroporin-2 is a known determinant of melarsoprol-pentamidine cross-resistance in *Trypanosoma brucei brucei* laboratory strains. Recently, chimerization at the AQP2-AQP3 tandem locus was described from melarsoprol-pentamidine cross-resistant *Trypanosoma brucei gambiense* isolates from sleeping sickness patients in the Democratic Republic of the Congo. Here, we demonstrate that reintroduction of wild-type AQP2 into one of these isolates fully restores drug susceptibility, while expression of the chimeric AQP2/3 gene in aqp2-aqp3 null *T. b. brucei* does not. This proves that AQP2-AQP3 chimerization is the cause of melarsoprol-pentamidine cross-resistance in the *T. b. gambiense* isolates.

17578. **Lun, Z. R., Wen, Y. Z., Uzureau, P., Lecordier, L., Lai, D. H., Lan, Y. G., Desquesnes, M., Geng, G. Q., Yang, T. B., Zhou, W. L., Jannin, J. G., Simarro, P. P., Truc, P., Vincendeau, P. & Pays, E., 2015.** Resistance to normal human serum reveals *Trypanosoma lewisi* as an underestimated human pathogen. *Molecular & Biochemical Parasitology*, **199** (1-2): 58-61.

Center for Parasitic Organisms, State Key Laboratory of Biocontrol, School of Life Sciences, and Key Laboratory of Tropical Disease and Control of the Ministry of Education, Sun Yat-Sen University, Guangzhou, PR China; Laboratoire de Médecine Expérimentale, Université Libre de Bruxelles, CHU Charleroi, 706 Route de Gozee, B-6110 Montigny-le-Tilleuil, Belgium [epays@ulb.ac.be].

Human-infectious trypanosomes such as *Trypanosoma cruzi, T. brucei rhodesiense*, and *T. b. gambiense* can be discriminated from those only infecting animals by their resistance to normal human serum (NHS). These parasites are naturally resistant to trypanolysis induced by the human-specific pore-forming serum protein apolipoprotein L1 (ApoL-1). *T. lewisi*, a worldwide distributed parasite, has been considered as rat-specific and non-pathogenic to the natural hosts. Here we provide evidence that 19 tested *T. lewisi* isolates from Thailand and China share resistance to NHS. Further investigation on one selected isolate CPO02 showed that it could resist at least 90 percent NHS or 30 µg/mL recombinant human ApoL-1 (rhApoL-1) *in vitro*, in contrast to *T. b. brucei* which could not survive in 0.0001 percent NHS and 0.1 µg/mL rhApoL-1. *In vivo* tests in rats also demonstrated that this parasite is fully resistant to lysis by NHS. Together with recent reports of atypical human infection by *T. lewisi*, these data allow the conclusion that *T. lewisi* is potentially an underestimated and thus a neglected human pathogen.

17579. **Motloang, M. Y., Masumu, J., Mans, B. J. & Latif, A. A., 2014.** Virulence of *Trypanosoma congolense* strains isolated from cattle and African buffaloes (*Syncerus caffer*) in KwaZulu-Natal, South Africa. *Onderstepoort Journal of Veterinary Research*, **81** (1). Art. 679.

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Trypanosoma congolense and *Trypanosoma vivax* are major species that infect cattle in north-eastern KwaZulu-Natal (KZN), South Africa. Of the two genetically distinct types of *T.*

congolense, savannah and Kilifi sub-groups, isolated from cattle and tsetse flies in KZN, the former is more prevalent and thought to be responsible for trypanosomosis outbreaks in cattle. Furthermore, variation in pathogenicity within the savannah sub-group is ascribed to strain differences and seems to be related to geographical locations. The objective of the present study was to compare the virulence of *T. congolense* strains isolated from African buffaloes (*Syncerus caffer*) inside Hluhluwe-iMfolozi Park, and from cattle on farms near wildlife parks (< 5 km), to isolates from cattle kept away (> 10 km) from parks. To obtain *T. congolense* isolates, blood of known parasitologically positive cattle or cattle symptomatically suspect with trypanosomosis, as well as isolates from buffaloes kept inside Hluhluwe-iMfolozi Park were passaged in inbred BALB/c mice. A total of 26 *T. congolense* isolates were obtained: five from buffaloes, 13 from cattle kept near parks and eight from cattle distant from parks. Molecular characterisation revealed 80 percent and 20 percent of isolates to belong to *T. congolense* savannah and Kilifi, respectively. To compare virulence, each isolate was inoculated into a group of six mice. No statistical differences were observed in the mean pre-patent period, maximum parasitaemia or drop in packed cell volume (PCV). Significant differences were found in days after infection for the drop in PCV, the patent period and the survival time. These differences were used to categorise the isolates as being of high, moderate or low virulence. Based on the virulence, 12 of 26 (46 percent) isolates were classified as highly virulent and 27 percent each as either of moderate or of low virulence. Whilst 11 of 12 high virulent strains were from buffaloes or cattle near the park, only 1 of 7 low virulent strains was from these animals. All the Kilifi *T. congolense* types were less virulent than the savannah types. These results confirm the higher virulence of *T. congolense* savannah type compared with Kilifi type and indicate the prevalence of highly virulent strains to be greater in wildlife parks and in cattle near the parks than on farms further away. The geographical location of these strains in relation to the wildlife parks in the area is discussed.

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17580. **Albisetti, A., Wiese, S., Schneider, A. & Niemann, M., 2015.** A component of the mitochondrial outer membrane proteome of *T. brucei* probably contains covalent bound fatty acids. *Experimental Parasitology*, **155**: 49-57.

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, Bern 3012, Switzerland. [niemann@dcb.unibe.ch].

17581. **Alizadehrad, D., Kruger, T., Engstler, M. & Stark, H., 2015.** Simulating the complex cell design of *Trypanosoma brucei* and its motility. *PLoS Computational Biology*, **11** (1): e1003967.

Institute of Theoretical Physics, Technische Universitat Berlin, Berlin, Germany. [davod.alizadehrad@tu-berlin.de].

17582. **Allison, H., O'Reilly, A. J., Sternberg, J. & Field, M. C., 2014.** An extensive endoplasmic reticulum-localised glycoprotein family in trypanosomatids. *Microbial Cell*, **1** (10): 325-345.

Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee, Scotland, DD1 5EH, UK. [mfield@mac.com].

17583. **Amata, E., Bland, N. D., Campbell, R. K. & Pollastri, M. P., 2015.** Evaluation of pyrrolidine and pyrazolone derivatives as inhibitors of trypanosomal phosphodiesterase B1 (TbrPDEB1). *Tetrahedron Letters*, **56** (21): 2832-2835.

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17584. **Baker, N., Hamilton, G., Wilkes, J. M., Hutchinson, S., Barrett, M. P. & Horn, D., 2015.** Vacuolar ATPase depletion affects mitochondrial ATPase function, kinetoplast dependency, and drug sensitivity in trypanosomes. *Proceedings of the National Academy of Sciences USA*, **112** (29): 9112-9117.

Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK; Wellcome Trust Centre of Molecular Parasitology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK. [d.horn@dundee.ac.uk].

Kinetoplastid parasites cause lethal diseases in humans and animals. The kinetoplast itself contains the mitochondrial genome, comprising a huge, complex DNA network that is also an important drug target. Isometamidium, for example, is a key veterinary drug that accumulates in the kinetoplast in African trypanosomes. Kinetoplast independence and isometamidium resistance are observed where certain mutations in the F1-gamma-subunit of the two-sector F1Fo-ATP synthase allow for Fo-independent generation of a mitochondrial membrane potential. To further explore kinetoplast biology and drug resistance, we screened a genome-scale RNA interference library in African trypanosomes for isometamidium resistance mechanisms. Our screen identified 14 V-ATPase subunits and all four adaptin-3 subunits, implicating acidic compartment defects in resistance; V-ATPase acidifies lysosomes and related organelles, whereas adaptin-3 is responsible for trafficking among these organelles. Independent strains with depleted V-ATPase or adaptin-3 subunits were isometamidium resistant, and chemical inhibition of the V-ATPase phenocopied this effect. While drug accumulation in the kinetoplast continued after V-ATPase subunit depletion, acriflavine-induced kinetoplast loss was specifically tolerated in these cells and in cells depleted for adaptin-3 or endoplasmic reticulum membrane complex subunits, also identified in our screen. Consistent with kinetoplast dispensability, V-ATPase defective cells were oligomycin resistant, suggesting ATP synthase uncoupling and bypass of the normal Fo-A6-subunit requirement; this subunit is the only kinetoplast-encoded product ultimately required for viability in bloodstream-form trypanosomes. Thus, we describe 30 genes and three protein complexes associated with kinetoplast-dependent growth. Mutations affecting these genes could explain natural cases of dyskinetoplasty and multidrug resistance. Our results also reveal potentially conserved communication between the compartmentalized two-sector rotary ATPases.

17585**. Bart, J. M., Cordon-Obras, C., Vidal, I., Reed, J., Perez-Pastrana, E., Cuevas, L., Field, M. C., Carrington, M. & Navarro, M., 2015.** Localization of serum resistance-associated protein in *Trypanosoma brucei rhodesiense* and transgenic *Trypanosoma brucei brucei*. *Cellular Microbiology*. **E Publication ahead of print, 26 June.**

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African trypanosomes infect a broad range of mammals, but humans and some higher primates are protected by serum trypanosome lytic factors that contain apolipoprotein L1 (ApoL1). In the human-infective subspecies of *Trypanosoma brucei*, *Trypanosoma brucei rhodesiense*, a gene product derived from the variant surface glycoprotein gene family member, serum resistance-associated protein (SRA protein), protects against ApoL1-mediated lysis. Protection against trypanosome lytic factor requires the direct interaction between SRA protein and ApoL1 within the endocytic apparatus of the trypanosome, but some uncertainty remains as to the precise mechanism and location of this interaction. In order to provide more insight into the mechanism of SRA-mediated resistance to trypanosome lytic factor, we assessed the localization of SRA in *T. b. rhodesiense* EATRO3 using a novel monoclonal antibody raised against SRA together with a set of well-characterized endosomal markers. By three-dimensional deconvolved immunofluorescence single-cell analysis, combined with double-labelling immunoelectron microscopy, we found that approximately 50 percent of SRA protein localized to the lysosome, with the remaining population being distributed through the endocytic pathway, but apparently absent from the flagellar pocket membrane. These data suggest that the SRA/trypanolytic factor interaction is intracellular, with the concentration within the endosomes potentially crucial for ensuring a high efficiency.

17586. **Brennand, A., Rico, E., Rigden, D. J., Van Der Smissen, P., Courtoy, P. J. & Michels, P. A., 2015.** ATG24 represses autophagy and differentiation and is essential for homeostasy of the flagellar pocket in *Trypanosoma brucei*. *PLoS One*, **10** (6): e0130365.

Research Unit for Tropical Diseases, de Duve Institute, and Laboratory of Biochemistry, Université catholique de Louvain, Brussels, Belgium; Departamento de Bioquimica y Biologia Molecular, Campus Universitario, Universidad de Alcala, 28871 Alcala de Henares, Madrid, Spain; Institute of Integrative Biology, Biosciences Building, University of Liverpool, Liverpool, UK; Cell Biology Unit, de Duve Institute, Université catholique de Louvain, Brussels, Belgium. [paul.michels@ed.ac.uk].

17587. **Bringaud, F., Biran, M., Millerioux, Y., Wargnies, M., Allmann, S. & Mazet, M., 2015.** Combining reverse genetics and nuclear magnetic resonance-based metabolomics unravels trypanosome-specific metabolic pathways. *Molecular Microbiology*, **96** (5): 917-926.

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Numerous eukaryotes have developed specific metabolic traits that are not present in extensively studied model organisms. For instance, the procyclic insect form of *Trypanosoma brucei*, a parasite responsible for sleeping sickness in its mammalian-specific bloodstream form, metabolizes glucose into excreted succinate and acetate through pathways with unique features. Succinate is primarily produced from glucose-derived phosphoenolpyruvate in peroxisome-like organelles, also known as glycosomes, by a soluble NADH-dependent fumarate reductase only described in trypanosomes so far. Acetate is produced in the mitochondrion of the parasite from acetyl-CoA by a CoA-transferase, which forms an ATP-

producing cycle with succinyl-CoA synthetase. The role of this cycle in ATP production was recently demonstrated in procyclic trypanosomes and has only been proposed so far for anaerobic organisms, in addition to trypanosomatids. We review how nuclear magnetic resonance spectrometry can be used to analyse the metabolic network perturbed by deletion (knockout) or downregulation (RNAi) of the candidate genes involved in these two particular metabolic pathways of procyclic trypanosomes. The role of succinate and acetate production in trypanosomes is discussed, as well as the connections between the succinate and acetate branches, which increase the metabolic flexibility probably required by the parasite to deal with environmental changes such as oxidative stress.

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Departamento de Parasitologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Sao Paulo, SP, 05508-900, Brazil. [zcaballero1178@yahoo.com].

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Laboratorio Especial de Ciclo Celular, Instituto Butantan, Sao Paulo, SP 05503-900, Brazil Center of Toxins, Immune Response and Cell Signalling - CeTICS, Instituto Butantan, Sao Paulo, SP 05503-900, Brazil. [carol@butantan.gov.br].

17590. **Carnes, J., Anupama, A., Balmer, O., Jackson, A., Lewis, M., Brown, R., Cestari, I., Desquesnes, M., Gendrin, C., Hertz-Fowler, C., Imamura, H., Ivens, A., Koreny, L., Lai, D. H., MacLeod, A., McDermott, S. M., Merritt, C., Monnerat, S., Moon, W., Myler, P., Phan, I., Ramasamy, G., Sivam, D., Lun, Z. R., Lukes, J., Stuart, K. & Schnaufer, A., 2015.** Genome and phylogenetic analyses of *Trypanosoma evansi* reveal extensive similarity to *T. brucei* and multiple independent origins for dyskinetoplasty. *PLoS Neglected Tropical Diseases*, **9** (1): e3404.

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Two key biological features distinguish *Trypanosoma evansi* from the *T. brucei* group: independence from the tsetse fly as obligatory vector, and independence from the need for functional mitochondrial DNA (kinetoplast or kDNA). In an effort to better understand the molecular causes and consequences of these differences, we sequenced the genome of an akinetoplastic *T. evansi* strain from China and compared it to the *T. b. brucei* reference strain. The annotated *T. evansi* genome shows extensive similarity to the reference, with 94.9 percent of the predicted *T. b. brucei* coding sequences (CDS) having an orthologue in *T. evansi*, and 94.6 percent of the non-repetitive orthologues having a nucleotide identity of 95 percent or greater. Interestingly, several procyclin-associated genes (PAGs) were disrupted or not found in this *T. evansi* strain, suggesting a selective loss of function in the absence of the insect life-cycle stage. Surprisingly, orthologous sequences were found in *T. evansi* for all 978 nuclear CDS predicted to represent the mitochondrial proteome in *T. brucei*, although a small number

of these may have lost functionality. Consistent with previous results, the F1FO-ATP synthase gamma subunit was found to have an A281 deletion, which is involved in generation of a mitochondrial membrane potential in the absence of kDNA. Candidates for CDS that are absent from the reference genome were identified in supplementary *de novo* assemblies of *T. evansi* reads. Phylogenetic analyses show that the sequenced strain belongs to a dominant group of clonal *T. evansi* strains with worldwide distribution that also includes isolates classified as *T. equiperdum*. At least three other types of *T. evansi* or *T. equiperdum* have emerged independently. Overall, the elucidation of the *T. evansi* genome sequence reveals extensive similarity to *T. brucei* and supports the contention that *T. evansi* should be classified as a subspecies of *T. brucei*.

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School of Medicine of Botucatu, UNESP - University Estadual Paulista, Department of Pathology, Botucatu, State of Sao Paulo, Brazil. [castelli@fmb.unesp.br].

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African trypanosomes evade clearance by host antibodies by periodically changing their variant surface glycoprotein (VSG) coat. They transcribe only one VSG gene at a time from one of about 20 telomeric expression sites (ESs). They undergo antigenic variation by switching transcription between telomeric ESs or by recombination of the VSG gene expressed. We show that the inositol phosphate (IP) pathway controls transcription of telomeric ESs and VSG antigenic switching in *Trypanosoma brucei*. Conditional knockdown of phosphatidylinositol 5-kinase (TbPIP5K) or phosphatidylinositol 5-phosphatase (TbPIP5Pase) or overexpression of phospholipase C (TbPLC) derepresses numerous silent ESs in *T. brucei* bloodstream forms. The derepression is specific to telomeric ESs, and it coincides with an increase in the number of colocalizing telomeric and RNA polymerase I foci in the nucleus. Monoallelic VSG transcription resumes after re-expression of TbPIP5K; however, most of the resultant cells switched the VSG gene expressed. TbPIP5K, TbPLC, their substrates, and products localize to the plasma membrane, whereas TbPIP5Pase localizes to the nucleus proximal to telomeres. TbPIP5Pase associates with repressor/activator protein 1 (TbRAP1), and their telomeric silencing function is altered by TbPIP5K knockdown. These results show that specific steps in the IP pathway control ES transcription and antigenic switching in *T. brucei* by epigenetic regulation of telomere silencing.

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Metabolomics coupled with heavy-atom isotope-labelled glucose has been used to probe the metabolic pathways active in cultured bloodstream form trypomastigotes of *Trypanosoma brucei*, a parasite responsible for human African trypanosomiasis. Glucose enters many branches of metabolism beyond glycolysis, which has been widely held to be the sole route of glucose metabolism. Whilst pyruvate is the major end-product of glucose catabolism, its transamination product, alanine, is also produced in significant quantities. The oxidative branch of the pentose phosphate pathway is operative, although the non-oxidative branch is not. Ribose 5-phosphate generated through this pathway distributes widely into nucleotide synthesis and other branches of metabolism. Acetate, derived from glucose, is found associated with a range of acetylated amino acids and, to a lesser extent, fatty acids; while labelled glycerol is found in many glycerophospholipids. Glucose also enters inositol and several sugar nucleotides that serve as precursors to macromolecule biosynthesis. Although a Krebs cycle is not operative, malate, fumarate and succinate, primarily labelled in three carbons, were present, indicating an origin from phosphoenolpyruvate via oxaloacetate. Interestingly, the enzyme responsible for conversion of phosphoenolpyruvate to oxaloacetate, phosphoenolpyruvate carboxykinase, was shown to be essential to the bloodstream form trypanosomes, as demonstrated by the lethal phenotype induced by RNAi-mediated downregulation of its expression. In addition, glucose derivatives enter pyrimidine biosynthesis via oxaloacetate as a precursor to aspartate and orotate.

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Instituto de Parasitologia y Biomedicina "Lopez-Neyra", IPBLN-CSIC. Parque Tecnologico de Ciencias de la Salud, Avda. del Conocimiento, s/n. 18016, Armilla, Granada, Spain. [aestevez@ipb.csic.es].

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One of the first steps in understanding a protein's function is to determine its localization; however, the methods for localizing proteins in some systems have not kept pace with the developments in other fields, creating a bottleneck in the analysis of the large datasets that are generated in the post-genomic era. To address this, we developed tools for tagging proteins in trypanosomatids. We made a plasmid that, when coupled with long primer PCR, can be used to produce transgenes at their endogenous loci encoding proteins tagged at either terminus or within the protein coding sequence. This system can also be used to generate deletion mutants

to investigate the function of different protein domains. We show that the length of homology required for successful integration precluded long primer PCR tagging in *Leishmania mexicana*. Hence, we developed plasmids and a fusion PCR approach to create gene tagging amplicons with sufficiently long homologous regions for targeted integration, suitable for use in trypanosomatids with less efficient homologous recombination than *Trypanosoma brucei*. Importantly, we have automated the primer design, developed universal PCR conditions and optimized the workflow to make this system reliable, efficient and scalable such that whole genome tagging is now an achievable goal.

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University of Bordeaux, Microbiologie Fondamentale et Pathogénicité, Bordeaux, France; Institut Polytechnique de Bordeaux, Microbiologie Fondamentale et Pathogénicité, UMR-CNRS 5234, Bordeaux, France. [derrick-roy.robinson@u-bordeaux.fr].

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School of Life Sciences, University of Nottingham, Nottingham NG2 7UH, UK; [catarina.gadelha@nottingham.ac.uk].

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Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK. [svaughan@brookes.ac.uk].

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DRBD13 RNA-binding protein (RBP) regulates the abundance of AU-rich element (ARE)-containing transcripts in trypanosomes. Here we show that DRBD13 regulates RBP6, the developmentally critical protein in trypanosomatids. We also show DRBD13-specific regulation of transcripts encoding cell surface coat proteins including GPEET2, variable surface glycoprotein (VSG) and invariant surface glycoprotein (ISG). Accordingly, alteration in DRBD13 levels leads to changes in the target mRNA abundance and parasite morphology. The high consistency of the observed phenotype with known cell membrane exchanges that occur during progression of *T. brucei* through the insect stage of its life cycle suggests that DRBD13 is an important regulator in this largely unknown developmental process.

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Human apolipoprotein L1 (APOL1) kills African trypanosomes except *Trypanosoma rhodesiense* and *Trypanosoma gambiense*, the parasites causing sleeping sickness. APOL1 uptake into trypanosomes is favoured by its association with the haptoglobin-related protein-haemoglobin complex, which binds to the parasite surface receptor for haptoglobin-haemoglobin. As haptoglobin-haemoglobin can saturate the receptor, APOL1 uptake is increased in haptoglobin-poor (hypohaptoglobinaemic) serum (HyHS). While *T. rhodesiense* resists APOL1 by RNA polymerase I (pol-I)-mediated expression of the serum resistance-associated (SRA) protein, *T. gambiense* resists by pol-II-mediated expression of the *T. gambiense*-specific glycoprotein (TgsGP). Moreover, in *T. gambiense*, resistance to HyHS is linked to haptoglobin-haemoglobin receptor inactivation by mutation. We report that unlike *T. gambiense*, *T. rhodesiense* possesses a functional haptoglobin-haemoglobin receptor, and that like *T. gambiense* experimentally provided with active receptor, this parasite is killed in HyHS because of receptor-mediated APOL1 uptake. However, *T. rhodesiense* could adapt to low haptoglobin by increasing transcription of SRA. When assayed in *Trypanosoma brucei*, resistance to HyHS occurred with pol-I-, but not with pol-II-mediated SRA expression. Similarly, *T. gambiense* provided with active receptor acquired resistance to HyHS only when TgsGP was moved to a pol-I locus. Thus, transcription by pol-I favours adaptive gene regulation, explaining the presence of SRA in a pol-I locus.

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Human-infecting microbial pathogens all face a serious problem of elimination by the host immune response. Antigenic variation is an effective immune evasion mechanism where the pathogen regularly switches its major surface antigen. In many cases, the major surface antigen is encoded by genes from the same gene family, and its expression is strictly monoallelic. Among pathogens that undergo antigenic variation, *Trypanosoma brucei* (a kinetoplastid), which causes human African trypanosomiasis, *Plasmodium falciparum* (an apicomplexan), which causes malaria, *Pneumocystis jirovecii* (a fungus), which causes

pneumonia, and *Borrelia burgdorferi* (a bacterium), which causes Lyme disease, also express their major surface antigens from loci next to the telomere. Except for *Plasmodium*, DNA recombination-mediated gene conversion is a major pathway for surface antigen switching in these pathogens. In the last decade, more sophisticated molecular and genetic tools have been developed in *T. brucei*, and our knowledge of functions of DNA recombination in antigenic variation has been greatly advanced. VSG is the major surface antigen in *T. brucei*. In sub-telomeric VSG expression sites (ESs), VSG genes invariably are flanked by a long stretch of upstream 70-bp repeats. Recent studies have shown that DNA double-strand breaks (DSBs), particularly those in 70-bp repeats in the active ES, are a natural potent trigger for antigenic variation in *T. brucei*. In addition, telomere proteins can influence VSG switching by reducing the DSB amount at sub-telomeric regions. These findings are summarized and their implications discussed in this review.

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The causative agent of human African trypanosomiasis, *Trypanosoma brucei*, lacks *de novo* purine biosynthesis and depends on purine salvage from the host. The purine salvage pathway is redundant and contains two routes to guanosine-5'-monophosphate (GMP) formation: conversion from xanthosine-5'-monophosphate (XMP) by GMP synthase (GMPS) or direct salvage of guanine by hypoxanthine-guanine phosphoribosyl transferase (HGPRT). We show recombinant *T. brucei* GMPS efficiently catalyses GMP formation. Genetic knockout of GMPS in bloodstream parasites led to depletion of guanine nucleotide pools and was lethal. Growth of gmps null cells was only rescued by supra-physiological guanine concentrations (100 μM) or by expression of an extrachromosomal copy of GMPS. Hypoxanthine was a competitive inhibitor of guanine rescue, consistent with a common uptake/metabolic conversion mechanism. In mice, gmps null parasites were unable to establish an infection demonstrating that GMPS is essential for virulence and that plasma guanine is insufficient to support parasite purine requirements. These data validate GMPS as a potential therapeutic target for treatment of human African trypanosomiasis. The ability to strategically inhibit key metabolic enzymes in the purine pathway unexpectedly bypasses its functional redundancy by exploiting both the nature of pathway flux and the limited nutrient environment of the parasite's extracellular niche.

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Ribose 5-phosphate isomerase is an enzyme involved in the non-oxidative branch of the pentose phosphate pathway, and catalyses the inter-conversion of D-ribose 5-phosphate and D-ribulose 5-phosphate. Trypanosomatids, including the agent of African sleeping sickness namely *Trypanosoma brucei*, have a type B ribose-5-phosphate isomerase. This enzyme is absent from humans, which have a structurally unrelated ribose 5-phosphate isomerase type A, and therefore has been proposed as an attractive drug target waiting further characterization. In this study, *Trypanosoma brucei* ribose 5-phosphate isomerase B showed *in vitro* isomerase activity. RNAi against this enzyme reduced parasites' *in vitro* growth, and more importantly, bloodstream forms infectivity. Mice infected with induced RNAi clones exhibited lower parasitaemia and a prolonged survival compared to control mice. Phenotypic reversion was achieved by complementing induced RNAi clones with an ectopic copy of the *Trypanosoma cruzi* gene. Our results present the first functional characterization of *Trypanosoma brucei* ribose 5-phosphate isomerase B, and show the relevance of an enzyme belonging to the non-oxidative branch of the pentose phosphate pathway in the context of *Trypanosoma brucei* infection.

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Drug resistance in pathogenic protozoa is very often caused by changes to the "transportome" of the parasites. In *Trypanosoma brucei*, several transporters have been implicated in uptake of the main classes of drugs, diamidines and melaminophenyl arsenicals. The resistance mechanism had been thought to be due to loss of a transporter known to carry both types of agents: the aminopurine transporter P2, encoded by the gene TbAT1. However, although loss of P2 activity is well-documented as the cause of resistance to the veterinary diamidine diminazene aceturate (DA; Berenil®), cross-resistance between the human-use arsenical melarsoprol and the diamidine pentamidine (melarsoprol/pentamidine cross resistance, MPXR) is the result of loss of a separate high affinity pentamidine transporter (HAPT1). A genome-wide RNAi library screen for resistance to pentamidine, published in 2012, gave the key to the genetic identity of HAPT1 by linking the phenomenon to a locus that contains the closely related *T. brucei* aquaglyceroporin genes TbAQP2 and TbAQP3. Further analysis determined that knockdown of only one pore, TbAQP2, produced the MPXR phenotype. TbAQP2 is an unconventional aquaglyceroporin with unique residues in the "selectivity region" of the pore, and it was found that in several MPXR lab strains the WT gene was either absent or replaced by a chimeric protein, recombined with parts of TbAQP3. Importantly, wild-type AQP2 was also absent in field isolates of *T. b. gambiense*, correlating with the outcome of melarsoprol treatment. Expression of a wild-type copy of TbAQP2 in even the most resistant strain completely reversed MPXR and re-introduced HAPT1 function and transport kinetics. Expression of TbAQP2 in *Leishmania mexicana* introduced a pentamidine transport activity indistinguishable from HAPT1. Although TbAQP2 has been shown to function as a classical aquaglyceroporin it is now clear that it is also a high affinity drug transporter, HAPT1. We discuss here a possible structural rationale for this remarkable ability.

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The protozoan parasite *Trypanosoma brucei* engages in surface-induced social behaviour, termed social motility, characterized by single cells assembling into multicellular groups that coordinate their movements in response to extracellular signals. Social motility requires sensing and responding to extracellular signals, but the underlying mechanisms are unknown. Here we report that *T. brucei* social motility depends on cyclic AMP (cAMP) signalling systems in the parasite's flagellum (synonymous with cilium). Pharmacological inhibition of cAMP-specific phosphodiesterase (PDE) completely blocks social motility without impacting the viability or motility of individual cells. Using a fluorescence resonance energy transfer (FRET)-based sensor to monitor cAMP dynamics in live cells, we demonstrate that this block in social motility correlates with an increase in intracellular cAMP levels. RNA interference (RNAi) knockdown of the flagellar PDEB1 phenocopies pharmacological PDE inhibition, demonstrating that PDEB1 is required for social motility. Using parasites expressing distinct fluorescent proteins to monitor individuals in a genetically heterogeneous community, we found that the social motility defect of PDEB1 knockdowns is complemented by wild-type parasites. Therefore, PDEB1 knockdown cells are competent for social motility but appear to lack a necessary factor that can be provided by wild-type cells. The combined data demonstrate that the role of cyclic nucleotides in regulating microbial social behaviour extends to African trypanosomes and provide an example of transcomplementation in parasitic protozoa.

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One distinctive feature of the *Trypanosoma brucei* life cycle is the presence of two discrete populations that are based on differential expression of variant surface glycoproteins (VSGs). Both are adapted to the environmental pressures they face and more importantly, both contribute directly to transmission. Metacyclics in the tsetse fly enable transmission to a new mammalian host, whereas bloodstream trypanosomes must avoid immune destruction to the extent that sufficient numbers are available for transmission, when the insect vector takes a blood meal. At present, there are few investigations on the molecular aspects of parasite biology in the tsetse vector and specifically about the activation of metacyclic VSG gene expression. Here we used an established *in vitro* differentiation system based on the overexpression of the RNA-binding protein 6 (RBP6), to monitor two metacyclic VSGs (VSG 397 and VSG 653) during development from procyclics to infectious metacyclic forms. We observed that activation of these two mVSGs was simultaneous both at the transcript and protein level, and manifested by the appearance of only one of the mVSGs in individual cells.

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To identify miRNAs whose expression are differentially regulated during trypanosome infections a microarray targeting more than 600 rat miRNA was used to analyse the miRNA expression profiles between uninfected rats and animals infected by *Trypanosoma congolense* and *Trypanosoma brucei* s.l. The potential targets of dysregulated miRNAs as well as their biological pathways and functions were predicted using several bioinformatics software tools. Irrespective of the infecting trypanosome species, eight miRNAs (seven up- and one down-regulated) were dysregulated during infections. Moreover, other miRNAs were differentially regulated in rats infected by specific trypanosome species. Functional analyses of differentially regulated miRNAs indicated their involvement in diverse biological processes. Among these, transcription repressor activity, gene expression control as well as protein transporter activity were predominant. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis of dysregulated miRNAs revealed their involvement in several biological pathways and disease conditions. This suggests possible modulation of such pathways following trypanosome infection; for example, the MAPK signalling pathway which is known to play vital roles in apoptosis, innate immune response and response to viral infections was highly affected. Axon guidance was equally highly impacted and may indicate a cross reactivity between pathogen proteins and guidance molecules representing one pathological mechanism as it has been observed with influenza HA (hemagglutinin). Furthermore, Ingenuity pathway analyses of dysregulated miRNAs and potential targets indicated strong association with inflammatory responses, cell death and survival as well as infectious diseases. The data generated here provide valuable information to understand the regulatory function of miRNAs during trypanosome infections. They improve our knowledge on host-parasite cross-talks and provide

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Apolipoprotein L-1 (APOL1), the trypanolytic factor of human serum, can lyse several African trypanosome species including *Trypanosoma brucei brucei*, but not the human-infective pathogens *T. brucei rhodesiense* and *T. brucei gambiense*, which are resistant to lysis by human serum. Lysis follows the uptake of APOL1 into acidic endosomes and is apparently caused by colloid-osmotic swelling due to an increased ion permeability of the plasma membrane. Here we demonstrate that nanogram quantities of full-length recombinant APOL1 induce ideally cation-selective macroscopic conductances in planar lipid bilayers. The conductances were highly sensitive to pH: their induction required acidic pH (pH 5.3), but their magnitude could be increased 3 000-fold upon alkalinisation of the milieu (pK_a = 7.1). We show that this phenomenon can be attributed to the association of APOL1 with the bilayer at acidic pH, followed by the opening of APOL1-induced cation-selective channels upon pH neutralization. Furthermore, the conductance increase at neutral pH (but not membrane association at acidic pH) was prevented by the interaction of APOL1 with the serum resistance-associated protein, which is produced by *T. brucei rhodesiense* and prevents trypanosome lysis by APOL1. These data are consistent with a model of lysis that involves endocytic recycling of APOL1 and the formation of cation-selective channels at neutral pH in the parasite plasma membrane.

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