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TSETSE AND TRYPANOSOMOSIS INFORMATION

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

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

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

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ABBREVIATIONS USED IN TTI

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

Organizations

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

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SECTION A – NEWS

PAN AFRICAN TSETSE AND TRYPANOSOMIASIS ERADICATION CAMPAIGN (PATTEC): 11TH COORDINATORS' MEETING 10-11 DECEMBER 2012, HAWASSA, ETHIOPIA

About 90 National PATTEC Coordinators and focal points from 29 African countries, representatives of international organizations, research institutions, private and public partners attended the opening ceremony of this meeting. Welcome remarks were made by Dr Thomas Cherenet, STEP Coordinator, who underscored the importance of livestock in agriculture. He urged participants to seriously discuss ways of addressing the T&T problem as it constitutes a major constraint to livestock production.

In his opening remarks, Dr Hassane, the AU-PATTEC Coordinator, noted that today, PATTEC is a household name because of the sustained commitment and support of Member States and partners. He then declared the year 2012 as the year when the AU-PATTEC Coordination Office has successfully consolidated and strengthened partnership with a number of partners in the T&T domain. He further announced that 2013 will be the year of resource mobilization and called for increased support from all partners to achieve this noble cause. He reminded participants that the PATTEC Coordination Office is committed to good governance, transparency and international cooperation and that is for this reason that the PATTEC Steering Committee has been constituted to give direction and provide checks and balances to the Coordination Office.

The representative of the AUC Commissioner for Rural Economy and Agriculture, Dr Karim Tounkara, also reiterated the important role that livestock play in contributing to agricultural GDP. He noted that livestock is part and parcel of the Comprehensive African Agriculture Development Programme agenda, and that T&T problems are amongst the major disease constraints to livestock production. He reminded participants that Agriculture is now at a crossroads and that livestock should assume its rightful place following the creation of tsetse free areas. He commended the commitment of those countries that took loans to combat the T&T problem adding that it was encouraging see increasing commitment from the Regional Economic Communities. Dr Tounkara also observed that it is encouraging to note that this is the first time in the history of the PATTEC initiative that the Coordinators' meeting is held back to back with the PATTEC Steering Committee meeting which is also being held for the first time followed by a field visit to witness SAT and SIT operations.

In his remarks, the Deputy Minister responsible for Livestock and Fisheries Development in Tanzania, Hon. Benedict Ole Nangoro, started by making reference to the Partners' Conference that was held in Nairobi, Kenya in December 2011. He noted that the conference was

result-oriented and urged all PATTEC stakeholders to follow up on the resolutions that were made. With specific reference to Tanzania, the Hon. Minister informed the meeting that T&T are still a big problem in the country and that despite the large number of cattle in Tanzania, productivity is not consistent with the population size largely due to the T&T constraint. He called for a holistic approach in dealing with the T&T problem in order to secure the livelihoods of those dependent on livestock. He urged the PATTEC Coordination offices at both continental and national levels to manage for results. He thanked partners who have been supporting the PATTEC initiative and called on participants to the meeting to go back home with concrete ideas on how to add value to the existing efforts in the fight against T&T.

The meeting was officially opened by a speech of the President of Regional Southern Nations, Nationalities and People's Government, H.E. Ato Shiferaw Shigute, read by Dr Teshome Assefa. The President extended a very warm welcome to participants to Hawassa and urged them to feel secure and comfortable as they deliberate the important issue of T&T. He reminded delegates about the socio-economic impacts of T&T problems and the ongoing efforts by many affected African countries to address them so as to contribute to the attainment of sustainable agriculture and rural development (SARD). He underscored Ethiopia's commitment to achieve tsetse freedom through the Southern Tsetse Eradication Project (STEP). He urged affected countries to harmonize efforts to address T&T problems in view of their trans-boundary nature in order to contribute effectively to the PATTEC initiative as enshrined in the Decision of the African Heads of State and Government made in Togo in July 2000. He thanked donor organizations and development partners for their support to the STEP. He thanked the African Union Commission for ably organizing the meeting in Ethiopia, specifically in Hawassa, the capital of the Regional Southern Nations, Nationalities and People's Government.

Following the opening ceremony, the new office bearers of the meeting were elected. Dr Thomas Cherenet, National Coordinator PATTEC Ethiopia was elected Chairman and will be assisted by Dr Hassane H. Mahamat, AU-PATTEC Coordinator. Mr Seth Onyango from Kenya, Mrs Joyce Daffa from Tanzania, Mr Christian Hazoume and Dr Gift Wanda from the PATTEC Coordination Office and Dr Berisha Kapitano for STEP Ethiopia were elected rapporteurs.

The agenda was adopted after clarifying that statements by partners would be combined with their presentations in the second day and that the presentation on the network of entomologists was reflected twice in the Programme by error.

Dr Martin Abavana, PATTEC Ghana, chaired this session on presentations by the PATTEC Coordination Office and from T&T affected countries. The AU-PATTEC Coordination Office was first to present its six-month progress report followed by the STEP project. Country progress reports were then received from Ghana, Kenya, Uganda, Burkina Faso, Mali Angola, Zambia, Gabon, Cameroon, Benin, Senegal, Equatorial Guinea and Malawi in that order. The second part of the presentations from countries affected by T&T was chaired by Dr Tadele Dessie, ILRI, with reports being received from Nigeria, Tanzania, Togo, Guinea, Sudan, Sudan South, Chad, Sierra Leone, Democratic Republic Congo and Congo.

The next session was chaired by Mr Stephen Sloan of Steve Sloan Consulting, and consisted of activity reports in support of the PATTEC initiative from various partners including GALVmed, FAO, IAEA, WHO, ILRI, CIRDES, IRED, IRD, Vestergaard and TTRI.

The country presentations revealed wide disparities with respect to the status of implementation of activities contributing to the PATTEC initiative. The disparities range from countries that have no T&T coordination and no project on the ground to those which have made

big gains and are in the process of undertaking monitoring and evaluation activities in areas where tsetse flies have either been suppressed to very low densities or completely eliminated. Some countries that have made big gains are planning to expand T&T activities to new areas. The notable examples of countries that have registered substantial gains include Kenya which has established a tsetse eradication council to oversee the tsetse operations; Ghana which is planning to undertake SAT operations in the north of the country; the Government of Zambia which has allocated US$ 3.0 million to tsetse eradication in the 2013 budget; and Ethiopia which is currently implementing SIT and SAT in Deme Valley and Arba Minch respectively. Others are Uganda which has developed a new proposal as a follow-up to the STATFA which drew to a close in December 2011, Burkina Faso which has two ongoing projects supported by IAEA and FAO, the Gabon national proposal which has just been funded, and Cameroon which has a vision of eradicating HAT by 2020. Nigeria is also actively undertaking T&T operations in two states while Tanzania has drawn up a new tsetse distribution map in preparation for large-scale tsetse operations.

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

The partners that were present at the meeting presented various activities being undertaken to contribute to the PATTEC initiative. It is evident that there is good will towards the PATTEC initiative and that most of the activities being undertaken are consistent with the ultimate objective of the PATTEC initiative. In a "nutshell", all partners urged the AU-PATTEC Coordination Office to continue taking a central role in ensuring that all activities are better coordinated so as to contribute effectively to the PATTEC initiative.

The final session on special presentations was chaired by Dr Pamela Olet, PATTEC Kenya. Here, participants received presentations on the Mara situation, guidelines on standards for entomological monitoring, on the AU-PATTEC Network of Entomologists and on a regional project for the control of tsetse and trypanosomosis in Central Africa.

The closing ceremony was chaired by Drs Karim Tounkara, Thomas Cherenet and Hassane Mahamat.

Resolutions/Recommendations

1. The meeting noted the commendable efforts by various partners in the animal and public health domains regarding T&T issues. However, the low level of partnership among service providers of tsetse, AAT and HAT control was observed. The meeting recommended the one health approach.

2. The meeting noted that inadequate resources were highlighted as a problem common to all national projects on T&T interventions. Further, the PATTEC Coordinator declared that 2013 is the year of resource mobilization in support of the PATTEC initiative. The

meeting urged national governments of affected Member States to increase budget allocations for sustainable T&T activities.

3. The meeting noted the disparities in reporting on T&T activities and interventions. The meeting recommended that a standardized way of reporting on entomological and parasitological findings should be adhered to.

4. The meeting noted that there are different management levels of HAT in many countries. The meeting further noted that in dealing with HAT, the public health sector has placed less emphasis on vector control and treatment of animal reservoirs. The meeting advised the Member States to follow WHO Standard Operating Procedures for HAT.

5. The meeting noted with satisfaction that an increasing number of countries are developing national proposals and strategies in support of the PATTEC initiative. The meeting urged the AU-PATTEC Coordination Office to expedite the process of validating the draft proposals/strategies by exploring possibilities of forming a think tank which can be assist in validating proposals.

6. The meeting noted with concern that 12 years after the inception of the PATTEC initiative, there are still a significant number of T&T affected countries that have not established national PATTEC coordination offices. The meeting recommended that the PATTEC Coordination Office should visit all such countries to assess their capacities and help them establish and work out mechanisms to operationalize national coordination offices.

7. The meeting recommended that the PATTEC Coordination Office works closely with all relevant partners in the development of new tools and technologies to enhance the capacity of affected countries to successfully implement the PATTEC initiative. In addition, the meeting recommended that the PATTEC Coordination Office coordinates and facilitates collaboration between all stakeholders to achieve the introduction of the final products for field use in tsetse and trypanosomosis eradication.

PATTEC 1ST STEERING COMMITTEE MEETING, 12 DECEMBER, AWASSA, ETHIOPIA

The first Steering Committee of the AU-PATTEC was held back to back with the 11th PATTEC Coordinators' meeting. The key PATTEC Steering Committee members and others were present and the agenda was fully discussed. The Steering Committee meeting was chaired by Dr Karim Tounkara, Director of AU-PANVAC, on behalf of the AUC Commissioner for Rural Economy and Agriculture, H.E. Mrs. Tumusiime Rhoda Peace. The meeting was attended by invited guests (Hon. Dessie Dalke, Minister of Science and Technology Ethiopia and Hon. Benedict N. Ole Nangoro (Deputy Minister of Livestock and Fisheries Development, Tanzania)); the Steering Committee members: Mr Pere Perez Simarro (WHO), Dr Daniel Bourzat (OIE), Prof. Oumar Alfaroukh Idriss (Chad), Prof. Valentine Yapi Gnaore (CIRDES), Dr Christopher Schofield

(London STM), Mr Steve Sloan, Prof. Mike Lehane (Vector Group, LSTMH), Dr Asefa Mebrate (Ethiopia), Dr Tadele Dessie (ILRI), and Dr Hassane Mahamat (PATTEC Coordinator); representatives of the Steering Committee members: Mr Grant Napier (GALVmed) and Dr Udo Feldmann (IAEA, FAO); other observers: Mr Ramon Mituy (PS, Ministry of Agriculture and Forestry Equatorial Guinea), Dr Jocelyne Bontoulougou (PS, Ministry of Livestock, Burkina Faso), Dr Diarra Abdoulaye Mamadou (Gabon), Dr Jackton Jalango (DVS, Kenya), Dr Thomas Cherenet (DG, STEP Ethiopia), Mr Akaki Ayumu Jovino (Chairman, Uganda Trypanosomiasis CC), and Mr Lawrence Semakula (Executive Director, COCTU, Uganda) and by staff of the PATTEC Coordination units.

Other members of the Steering Committee: Dr Modibo Traoré (FAO), Dr Qi Liang (Director, Joint FAO/IAEA Division), Prof. Christian Borgemeister (DG, ICIPE), Prof. Andy Peters (Int. CEO GALVmed), Prof. Albert Ilemobade (Nigeria) and Prof. Ahmed Elsawalhy (Director, AU-IBAR) who were not be able to attend the meeting because of prior engagements had sent their apologies.

The first AU- PATTEC Steering Committee recognized unanimously that significant progress had been achieved over the past 18 months.

The field visit comprised three demonstrations of field operations. The first took place in Arba Minch at the airport where participants witnessed the sequential aerosol technique (SAT) to eradicate tsetse flies. Participants were given briefings by Mike Sanders of Osmond Aviation, Patrick Kgori, the SAT operations manager, and Edwin Butler of AVIMA. The three experts briefed participants on the ground preparations for the SAT operations, the actual aerial spraying logistics, and on the properties of insecticide used in the spraying.

Secondly, the participants had a chance to witness the application of pour-on insecticides on live cattle as one method of controlling tsetse fly.

The final stage of the field visit was a demonstration of the sterile insect technique (SIT) in the Deme Valley. Participants witnessed how the flies are released by plane along the river which is a suitable habitat for the species of tsetse fly that is a major problem in the valley.

WHO MEETING ON ELIMINATION OF HUMAN AFRICAN TRYPANOSOMIASIS (*Trypanosoma brucei gambiense*), 3–5 DECEMBER 2012, GENEVA

A meeting on elimination of human African trypanosomiasis caused by infection with *Trypanosoma brucei gambiense* was held on 3–5 December 2012 at the headquarters of the World Health Organization (WHO) in Geneva, Switzerland. Focal points of national control programmes and experts from WHO collaborating centres convened to discuss the feasibility, strategies and criteria for eliminating the disease.

After three days of debate, the representatives of endemic countries agreed and committed to the goal of eliminating the disease according to the target set in the WHO roadmap on neglected tropical diseases, namely elimination of human African trypanosomiasis as a public-health problem by 2020.

However, the group considered a more adequate definition of elimination to be "the reduction to zero of the incidence of infection caused by a specific pathogen in a defined geographical area, as a result of deliberate efforts; continued actions to prevent re-establishment of transmission may be required" as recommended by WHO's Strategic and Advisory group for Neglected Tropical Diseases. The meeting therefore agreed to keep the goal of eliminating human African trypanosomiasis as a public-health problem by 2020, defined as the detection of less than one case per 10 000 inhabitants in at least 90 percent of endemic foci in 2020, with the total number of cases reported annually at continental level to below 2000. Efforts should nevertheless be maintained to achieve the total interruption of transmission and reaching zero cases.

Following the agreement on elimination principles, a strategic plan will be elaborated by WHO to conduct pilot testing in countries for planning and implementing national elimination strategies.

A biennial meeting with endemic countries to monitor progress in the elimination process is scheduled to begin from 2014.

NEW WHO PLAN TARGETS THE DEMISE OF SLEEPING SICKNESS

Published in The Lancet, 5 January 2013, written by John Maurice

Seventeen (17) so-called neglected tropical diseases have been sitting for decades on the to-do list of WHO. Together they bring physical, social, and economic suffering to more than one billion people, according to WHO. A decade or so ago, WHO and its partners decided to pull these diseases out of the closet and to find the resources needed to finish what has too long been unfinished business. "These diseases are now being brought to their knees with stunning speed", WHO Director-General Margaret Chan told an international meeting in London last year.

Bringing diseases "to their knees" can mean, in health-speak, either eradication (incidence is permanently reduced to zero cases worldwide and no further action is needed); or elimination (incidence is reduced to zero cases worldwide or in a defined geographical area but action might be needed to keep it that way); or elimination as a public health problem (incidence is reduced to a defined level). Until now, of the 17 neglected tropical diseases only two–guinea-worm disease and yaws–have been slated for eradication. Only three, blinding trachoma, leprosy, and lymphatic filariasis, are headed for worldwide elimination. Plans to eliminate a fourth disease, sleeping sickness (human African trypanosomiasis), have recently been agreed on by experts at a WHO meeting held in December, 2012.

Theoretically, sleeping sickness could be eradicated but the parasite that causes the disease (a subspecies of *Trypanosoma brucei*) is transmitted by a fly, the tsetse fly: eradicating the disease would call for eradication of the fly, a formidable task. The fact, too, that animals can be infected by the parasite and might constitute a reservoir of infection also casts doubt on eradicability of the disease.

Sleeping sickness, however, is certainly a strong candidate for elimination. "What we are aiming for", says Pere Simarro, head of WHO's human African trypanosomiasis programme, "is to bring the disease down to zero cases, to stop transmission, and to keep it that way indefinitely. It means using all the tools at our disposal to find infected people, to rid them of the infection, to stop the infection spreading, and to prevent it from resurging". There are, he believes, good reasons for making these efforts: "For one thing, it's a lethal disease. If you get infected and you don't get treatment, death is almost certain. For another, it's a disease that hits people at the most productive period of their lives and thus represents a serious economic burden on many African countries."

Achieving the zero-cases-zero-transmission target, the WHO meeting participants agreed, will not be easy. Hunting for cases is difficult in the remote jungle villages where most patients—and most tsetse flies—live. Screening for cases and diagnosis of the disease are hampered by the absence of specific signs or symptoms in the early months or years of the infection. During this period, the parasite is still in the patient's blood and lymph and hasn't yet passed into the central nervous system where it will produce a host of visible symptoms—behaviour changes, drowsiness, and other neurological changes. The available drugs are effective but notoriously toxic and difficult to administer. There is a lack of field-friendly diagnostic tests. Efforts to stop transmission by killing the fly vector by spraying its forest haunts and animal hosts with insecticide or by trapping methods have, up to now, been too costly to deploy on a large scale. A further complication is the fact that there are two distinct forms of sleeping sickness, a West African (*gambiense*) form, which accounts for about 96 percent of sleeping sickness cases, and an east African (*rhodesiense*) form, which is essentially a zoonotic disease involving cattle and wildlife. The two forms differ biologically, clinically, and epidemiologically and call for quite different control strategies.

Yet, despite these difficulties, the meeting participants expressed few doubts about their ability to reach the zero-case target, at least for the West African form of the disease. One reason for optimism is that the target was almost reached in the past. Sleeping sickness first achieved recognition in the medical literature at the end of the 19th century, shortly before the "invasion" of Africa by European colonisers and the upheaval they caused over the next two or three decades in the lives of the indigenous populations. Many people were uprooted from their homes, conscripted into workforces, and obliged to move into the interior as new lands were opened up

to mineral mining and other industries. A good number of these new lands were infested with the tsetse fly. The result was a repeated onslaught of sleeping sickness epidemics that killed thousands of people, including those working for the colonisers. In 1906, Britain's Colonial Undersecretary, Winston Churchill, returned from a visit to Lake Victoria in Uganda to announce to the House of Commons that Uganda had lost a third of its population because of sleeping sickness.

By the early 1930s, annual reported cases in west and central Africa had reached a record 50 000. Two decades later, the number had decreased to about 3000, thanks to mass campaigns undertaken by the colonial administrations and involving systematic screening, treatment, and follow-up of millions of people throughout west and central Africa. By 1962, experts triumphantly declared the disease well and truly conquered. For the newly independent governments it was no longer a priority and sleeping sickness control programmes could be dismantled.

The triumph, however, was short-lived. Over the next four decades, with armed conflict preventing health workers from reaching endemic villages, with the drug supply unreliable, and with almost no support from the international health community, the disease resurged. By the turn of the century reported cases of sleeping sickness exceeded 30 000 annually (in 1995 a WHO expert committee estimated the true number to be more than 300 000).

Today, 70 million people are still living in areas where they risk being infected with sleeping sickness. Nearly two-thirds of these people are in the Democratic Republic of the Congo, which currently accounts for 85 percent of the total cases reported in Africa. However, new reported cases number less than 7000 (a 75 percent decrease over the past decade) while estimated cases are around 21 000 (a 90 percent decrease).

Jose Ramon Franco Minguell, medical officer with WHO's neglected tropical disease team, attributes success in slashing case numbers since 2000 to a combination of factors: “First and foremost, we launched a large-scale advocacy campaign that put sleeping sickness back on the international radar screen. Then WHO assembled a powerful coalition of charitable and humanitarian institutions, pharmaceutical companies, bilateral country partnerships, non-governmental organisations, and research centres to fight the disease. We also got strong commitment from the African countries where the disease is endemic. We now know more about the dynamics of the disease and where exactly we must look for cases, thanks to a huge mapping exercise we have done that covered 29 000 villages and unearthed 175 000 new cases in all countries reporting cases in the last decade. And, thankfully, there are now far fewer areas where conflict could prevent health workers from reaching patients. I think we can be optimistic about reaching our elimination target.”

Anne Moore, who chaired the WHO meeting and is a medical epidemiologist with the US Centers for Disease Control and Prevention, in Atlanta, GA, USA, agrees that “the plan to eliminate sleeping sickness is looking very good”.

“We have a really effective strategy for controlling the disease. We screen the whole population of a village, confirm diagnosis in suspected cases, hospitalise, and treat, and get them to come back every two years for a follow-up test. That strategy can reduce the prevalence of the disease by three-to-four fold with just one round. It worked for the colonial powers, although it took them 30 years to reach near-elimination of the disease. With the experience and better, if not perfect, tools we now have, and by adapting the strategy to different epidemiological contexts, it should take us less time.”

The new drugs and new diagnostic tests in the pipeline, she believes, are added reasons for optimism. One serious omission, though, that the colonial powers made and that the current sleeping sickness combatants want to avoid was the failure to put in place a surveillance programme to prevent resurgence of the disease. This measure, says Moore, "is going to be a key factor in reaching our targets".

Before the WHO meeting broke up, it unanimously gave the green light to bringing down the incidence of the disease, by 2020, to less than one case per 10 000 of the population in at least 90 percent of the areas where cases exist. Thereafter, efforts will continue to bring the incidence of the disease to zero and to ensure that sleeping sickness will never again awaken to plague the African continent.

PAAT SECRETARIAT MEETING. 25-27 SEPTEMBER 2012, VIENNA, AUSTRIA

PAAT Secretariat members (FAO, IAEA and WHO), AU-IBAR and AU-PATTEC agreed on a strategy for a renewed united fight against tsetse fly transmitted trypanosomoses.

Representatives of the Food and Agriculture Organisation of the United Nations (FAO), the World Health Organization (WHO), the International Atomic Energy Agency (IAEA) and the African Union Commission (AUC) met at IAEA Headquarters to discuss a united and streamlined strategy against tsetse fly transmitted trypanosomoses, known as sleeping sickness in humans and nagana in livestock.

In the past years the three UN organizations FAO, IAEA and WHO, provided technical assistance within the remit of their respective mandates and competencies to AU-PATTEC and to tsetse and trypanosomoses (T&T) affected Member States. The three UN agencies collaborate, exchange information and harmonize their relevant activities in the Programme Against African Trypanosomosis (PAAT) forum. All three specialized UN organizations also signed a memorandum of understanding with the AUC to formalize their collaboration and their support to PATTEC.

At the meeting the representatives of the participating organizations agreed that – besides participating and interacting in the Steering Committee of AU-PATTEC – they will continue to interact in the PAAT forum, in order to:

- Harmonize their policies and strategies for T&T interventions within their respective mandates in support of the implementation of the AU-PATTEC Plan of Action;
- Coordinate their efforts in a complementary way in support of the AU-PATTEC;
- Review, appraise and validate project proposals generated by T&T affected countries on request from the respective Member States or AU-PATTEC;
- Share knowledge, information and data on T&T for evidence-based decision making at international, regional and national levels;
- Convene meetings to exchange views and information on T&T intervention actions; and
- Harmonize efforts to strengthen national and regional capacities through the provision of training, technical assistance and decision support tools.

The PAAT Forum will continue to be open to other interested stakeholders (e.g. ILRI, ICIPE, OIE, CIRDES), whose objectives are contributing to the successful implementation of the PATTEC Initiative.

WORK SUPPORTED BY THE JOINT FAO/IAEA AND IAEA TECHNICAL CO-OPERATION PROGRAMMES

CREATING A TSETSE-FREE ZONE IN THE SOUTHERN RIFT VALLEY

IAEA DG Visits Ethiopia

During a visit to Ethiopia in conjunction with his participation in the Second Conference of States and Parties to the Treaty of Pelindaba, which established in 2009 the African continent as a nuclear-weapon-free zone, the IAEA Director General, Yukiya Amano, met Prime Minister Hailemariam Desalegn. In their meeting, the Prime Minister expressed his thanks for the IAEA's assistance in the areas of cancer treatment, water management and in particular the technical support received in relation to the Southern Tsetse Eradication Project (STEP), which intends to integrate the SIT on an area-wide basis to eventually eradicate tsetse flies from 25 000 km² of the Southern Rift valley.

Visit of DG Yukiya Amano to the STEP tsetse rearing facility. (Left to right, foreground: Dr. Thomas Cherenet, DG of STEP, IAEA DG Amano, HE Ato Desie Delkie, Minister of Science and Technology).

He stressed the importance of this project for agriculture and rural development in southern Ethiopia, where farming communities involving over 100 000 farmers and 2.5 million cattle are already benefitting from improved living conditions, including the availability of oxen to plough the land, donkeys to pull carts to transport agricultural products to market, and meat and milk to improve human nutrition. They both expressed their commitment to ensuring continued progress in this ambitious and complex project that is very demanding in terms of management and logistics.

Also during the visit to Ethiopia the IAEA Director General, Yukiya Amano, visited the tsetse rearing facility of the Southern Tsetse Eradication Project (STEP) in Addis Ababa on Tuesday 13 November. He was accompanied by HE Ato Desie Delkie, Minister of Science and Technology and HE Ms Tumusiime Rhoda Peace, African Union Commissioner for Rural Economy & Agriculture.

Mr Amano was greeted by the STEP Director General, Thomas Cherenet and senior staff of the project. After a short presentation on the history and progress of the project, the visitors were shown around the mass-rearing facility, including the blood preparation for feeding the flies, the old and new tsetse fly holding systems and the irradiator.

QUALITY CONTROL OF TRYPANOCIDAL DRUGS

Tsetse-transmitted African animal trypanosomosis is arguably the most important animal disease impairing livestock agricultural development in sub-Saharan Africa. Besides vector control, the use of trypanocidal drugs is the main method to control the impact of the disease on animal health and production in most sub-Saharan African countries. Presently only three compounds belonging to two chemical classes are widely available to treat trypanosomosis: diminazene aceturate (belonging to the class of aromatic diamidines); and isometamidium chloride hydrochloride and homidium (chloride and bromide salts) which belong to the phenanthridinium class of trypanocidal agents. Diminazene aceturate is indicated in the treatment of cattle trypanosomosis, the homidium salts are used both for treatment and prevention, and isometamidium chloride hydrochloride is mainly used as a prophylactic drug.
Studies and market surveys on the quality of the various trypanocidal pharmaceutical formulations sold in different markets in sub-Saharan Africa have shown that a substantial proportion of the products were of sub-standard quality or even fake, containing no active trypanocidal substance. The use of poor-quality and fake trypanocides has severe implications for animal health, public health and the local economy. Inappropriate treatment results in animal morbidity or mortality and increases the risk of the emergence of drug resistance in trypanosome populations; there are currently 17 African countries in which animal trypanocidal drug resistance has been reported. Moreover, food safety is compromised by allowing unspecified and potentially harmful chemicals to enter the food chain.

No internationally agreed standards for the quality control of these compounds, as either pharmacopoeia-type monographs or documented product specifications, are available. This means it is impossible to establish independent quality control and quality assurance standards for these agents. Quality control of veterinary trypanocides in sub-Saharan Africa is, therefore, difficult and compliance is impossible to achieve.

In line with the recommendation of the OIE Conference on Veterinary Medicinal Products in Africa, Dakar, Senegal, 2008, and in consultation with African institutions, an international alliance was created to help address this problem. The alliance includes FAO, IFAH, GALVmed, IAEA and the Universities of Strathclyde and Manchester in the UK. The OIE also collaborates in this initiative by providing institutional support and a framework for the promotion of the monographs of the trypanocidal drugs and their formulations as international reference points. The alliance has recently completed pharmacopoeia-type monographs for isometamidium chloride hydrochloride, diminazene aceturate, homidium chloride and homidium bromide and their formulations, and these have been submitted (annexed to a paper) for publication in the OIE Review. Standardized chemical analytical procedures have been elaborated and cross-validated in the University of Strathclyde and the Joint FAO/IAEA Division's Food and Environmental Protection Laboratory (FEPL) to assist in authenticating and/or evaluating the quality of the drugs.

Trainees at the FAO/IAEA Laboratory, Seibersdorf, Austria

The knowledge and analytical procedures developed for the quality control and quality assurance of trypanocidal drugs are being transferred to two laboratories, one in East Africa and a second in West Africa. These laboratories will form the basis of a system to enable reliable quality control, for use by drug registration authorities and to serve as an arbiter if there is a legal dispute regarding a counterfeit or fake product. Following a public tender for proposals, the Laboratoire de Contrôle des Médicaments Vétérinaires (LACOMEV), Dakar, Senegal and the Tanzania Food and Drugs Authority (TFDA) laboratory, Dar Es Salaam, were selected. Through the alliance, these laboratories are being provided with the necessary analytical equipment, and laboratory analysts were trained on the chromatographic analytical methods in the FEPL in January 2013. It is expected that the sustainability of the two selected laboratories will be enhanced by expanding their existing mandate for the quality control of veterinary drugs at a local and regional level, effectively ensuring the maintenance of the trypanocide quality control capability.

The monographs developed through this alliance and the supporting analytical methodology will ultimately allow laboratories in Africa, Asia and South America, as well as those of veterinary pharmaceutical companies to carry out the quality control of the described trypanocidal drugs on a common platform.

IAEA TECDOC ON QUALITY CONTROL FOR EXPANDED TSETSE PRODUCTION, STERILIZATION AND FIELD APPLICATION

An IAEA TECDOC with the results of a Coordinated Research Project (CRP) on Quality Control for Expanded Tsetse Production, Sterilization and Field Application was published. This publication is a report of the results and outputs of that CRP, including the new and revised quality control tests that resulted from this 6-year CRP.

The use of the sterile insect technique (SIT) for the control of pest insects as part of an integrated, area-wide approach is widely accepted. Its application for the eradication of different tsetse populations, the vectors of human sleeping sickness and African animal trypanosomosis, is attracting increasing interest.

This interest in the application of the SIT against different species of this pest is leading to increased demand for sterile flies, the current bottleneck for expanded SIT application in

sub-Saharan Africa. Little systematic work has been done specifically on the quality of mass reared and sterilized tsetse, and there is an urgent need to harmonize and improve the existing *ad hoc* measures. This CRP produced a quality control manual similar to the one already produced for tephritid fruit flies.

The development of large-scale rearing highlighted the need for improved quality control procedures and, with this in mind, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture established a CRP in 2003 entitled *Improved and Harmonized Quality Control for Expanded Tsetse Production, Sterilization and Field Application* with the objective of improving and expanding the quality control sections of the *FAO/IAEA Standard Operating Procedures for Mass Rearing Tsetse Flies*. Sixteen institutions from thirteen countries in Africa, Europe and Central America participated in the CRP.

MEMORANDUM OF UNDERSTANDING BETWEEN THE GLOBAL ALLIANCE FOR LIVESTOCK VETERINARY MEDICINE (GALVmed) AND THE FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO)

Below is the text of this Memorandum of Understanding which was signed by Dr B. Tekola, Director of FAO's Animal Production and Health Division, and Professor A.R. Peters, Interim CEO of GALVmed.

PREAMBLE

Considering that the Global Alliance for Livestock Veterinary Medicine (hereinafter referred as GALVmed) is a company limited by guarantee and incorporated in England & Wales under the Companies Acts (Registered Number 05393391) and having its registered office at c/o Maclay Murray & Spens LLP, One London Wall, London, EC2Y 5AB, and a registered charity in England & Wales with registered charity number 1115606, and that GALVmed is a not-for-profit company which seeks to protect livestock and save human lives by making livestock vaccines, diagnostics and medicines accessible and affordable to the millions for whom livestock is a lifeline;

Whereas the Food and Agriculture Organization of the United Nations (hereinafter referred to as FAO), carries out activities on sustainable animal health and contained animal-related human health risks, in support of the emerging One-Health strategy, to contribute to improve food security and food safety, livelihoods, alleviate poverty and improve socio-economic development;

Bearing in mind that FAO, over the years, has been involved in efforts to mobilize and coordinate activities aimed at increasing the control of animal African disease burden within the context of sustainable agriculture and rural development (SARD) and, in particular, to develop and promote strategies for poor livestock keepers and smallholders. In this commitment to implement responsible and sustainable improved animal health interventions, several sub-Saharan countries have initiated actions aiming at improving animal health services, including availability, accessibility, affordable and quality of veterinary medicines.

Whereas GALVmed promotes the effective use of resources for charitable purposes through the identification, management, funding and co-ordination of research into livestock vaccines, pharmaceuticals and diagnostic products and services; and the development and delivery of those products and services at affordable prices. It seeks to do this by (i) facilitating partnerships for livestock pharmaceutical research and development and

manufacturing; (ii) creating and supporting sustainable markets and access to products for poor livestock keepers; and (iii) by developing project design and project and portfolio management.

Whereas within the context of their respective mandates, roles and common objectives, and based on their shared interest to work more concertedly through consolidating, rationalizing and harmonizing their contribution in support of the efforts in affected Member States in their bid to reduce animal disease burden through improved animal health services and quality of veterinary drugs;

The GALVmed and FAO (hereinafter jointly referred to as "the Parties" and in the singular as "a Party"), in furtherance of their aims and objectives, wish to make an arrangement whereby they will work collaboratively in conceptualizing, planning and/or implementing research, development, validation and deployment of animal health products that benefit resource-poor livestock keepers in the developing world.

Therefore the Parties have agreed to enter into the following Memorandum of Understanding (hereinafter referred to as the "MOU"):

Article 1
Objectives

1. The overall objective of this Memorandum of Understanding is to formalize the collaboration between the GALVmed and FAO in the identification of the modalities for improving cooperation and coordination of activities in respect to matters of common interest in the Parties' efforts to address matters related to research into livestock vaccines, quality of pharmaceuticals and diagnostic products and services; and the development and delivery of those products and services.
2. The Parties agree that they will act in close cooperation and consult each other in regard to matters of common interest, aim at international harmonization and synergized contributions by relevant partners whenever this may be appropriate in light of their respective mandates.

Article 2
Institutional arrangements

1. The Parties shall establish full transparency and communication channels to facilitate and enhance cooperation between GALVmed and FAO while avoiding overlap or duplication in exercising their respective mandates.
2. The Parties shall appoint a Focal Point responsible for the coordination of activities as laid out in Articles 3 and 4 below of the present Memorandum of Understanding.

Article 3
Areas of cooperation

GALVmed and FAO shall cooperate at international, regional and national levels and shall, within their respective mandates, explore possibilities for effective joint action in conformity with their respective rules, regulations, procedures and administrative practices in the following areas:

(a) Preparation and presentation of documents, reports and proposals, as appropriate;

(b) Preparation of plans and project proposals aimed at promoting research into livestock vaccines, quality of pharmaceuticals and diagnostic products and services; and the development and delivery of those products and services;
(c) Involvement in activities prepared or being undertaken by the other Party, such as training courses, workshops, planning, monitoring and evaluation of projects;
(d) Contact and communication with countries and third parties on issues related to research into livestock vaccines, quality of pharmaceuticals and diagnostic products and services; and the development and delivery of those products and services;
(e) Dissemination of information about collaborative activities, goals and objectives;
(f) The Parties shall engage in regular consultations and shall actively participate in bilateral and other meetings and events related to the cooperation under this Memorandum of Understanding, subject to the respective Party's rules and practices with regard to meetings and events.

Article 4
Specific areas of cooperation

1. Each particular cooperation activity shall be agreed to by the Parties on a case-by-case basis. Specific agreements shall be concluded between the Parties whereby roles and obligations of the Parties for each particular cooperation activity shall be reflected.
2. Each Party shall implement its activities under its sole control and shall be responsible for the implementation of its own activities.
3. The Parties, subject to their respective mandates, financial regulations and rules, policies and procedures, agree to cooperate in specific areas including the following:
   (a) Assistance in training and capacity development activity;
   (b) Methods development and validation to address technical gaps and bottlenecks and to improve the efficiency and cost-effectiveness of livestock vaccines, quality of pharmaceuticals and diagnostic products and services; and the development and delivery of those products and services;
   (c) Mutual participation in relevant policy coordination, planning, research and other meetings and workshops and events;
   (d) Mutual continuous exchanging of data and information subject to their confidentiality obligations;
   (e) Assistance in the development of national and regional legislation and regulatory measures;
   (f) Sharing of reports and publications of mutual interest.

Article 5
Review of the cooperation

The Parties shall meet once a year at a mutually agreed date and location to discuss their collaboration under this Memorandum of Understanding.

Article 6
Financial arrangements

The Memorandum of Understanding does not create any legal or financial obligations between the Parties, notwithstanding anything to the contrary in this document.
Where measures taken to implement this Memorandum of Understanding may give rise to any financial or legal obligations, the Parties shall conclude a separate agreement, subject to the GALVmed and the FAO Financial Rules and Regulations, prior to such measures being undertaken.

Article 7
Personnel

Any personnel employed by the Parties shall remain subject to the rules and regulations of their respective institutions in all matters of employment, medical and life insurance and employee rights and benefits.

Article 8
Dissemination of information

GALVmed and FAO shall support the widest possible dissemination of unclassified information provided or exchanged under this Memorandum of Understanding, subject to the need to protect proprietary information. GALVmed and FAO shall ensure the confidentiality of information classified by the other party as restricted or confidential.

Article 9
Privileges and immunities

Nothing in this Memorandum of Understanding or in any document or arrangement relating thereto, shall be construed as constituting a waiver of privileges or immunities of the Parties, nor as conferring any privileges or immunities of one Party to the other Party or to its personnel.

Article 10
Intellectual property

Intellectual property rights, in particular copyright, of material such as information, software and designs, made available by the GALVmed and FAO to be used to carry out the activities under this Memorandum of Understanding shall remain with the originating Party.

1. Copyright of the information, as well as rights to any other intellectual property, developed jointly by GALVmed and FAO shall be jointly vested in the Parties.

Article 11
Use of name, emblem or official seal

1. FAO shall not use the name, emblem or official seal of GALVmed for any purpose other than expressly authorized in writing by GALVmed.

2. GALVmed shall not use the name, emblem or official seal of FAO for any purpose other than expressly authorized in writing by FAO.

Article 12
Dispute Settlement

Any dispute between the Parties arising out of or relating to the interpretation or implementation of the present Memorandum of Understanding shall be finally amicably settled through negotiations or by such means, as the Parties may mutually agree on.

Article 13
Amendment

The provisions of the present Memorandum of Understanding may be modified by written agreement between the Parties. Any such modification shall enter into force thirty (30) days from the date of such written agreement, or where such agreement is made by exchange of letters, from the date of the later letter.

Article 14
Termination

This Memorandum of Understanding may be terminated by either Party upon three (3)-months written notice given to the other Party. In that event, the Parties shall agree on measures required for the orderly conclusion of on-going activities. In the absence of written notice by one Party to the other of non-renewal, the present Memorandum of Understanding shall be automatically renewable for further three (3)-year periods.

Article 15
Entry into force

This Memorandum of Understanding shall enter into force upon signature by the Parties and shall be valid for three (3) years. Where signature takes place on two different dates, the present Memorandum of Understanding shall enter into force from the date of the second signature.

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

The major constraints African trypanosomoses pose to food security are well recognized by the New Partnership for Africa's Development (NEPAD) and its programme for agriculture, the Comprehensive Africa Agriculture Development Programme (CAADP). The Government of Italy is committed to supporting the objectives of NEPAD/CAADP, and it has therefore funded the FAO project "Improving food security in sub-Saharan Africa by supporting the progressive reduction of tsetse-transmitted trypanosomosis in the framework of the NEPAD" (GTFS/RAF/474/ITA).

The project hinges on four pillars: (i) technical assistance, (ii) capacity development, (iii) partnership, networking and knowledge sharing and (iv) streamlining of the gender dimension. The project has a regional dimension, with priority given to the six countries (i.e.

Burkina Faso, Ethiopia, Ghana, Kenya, Mali and Uganda) that first started implementing field interventions in the framework of the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), an initiative of the African Union. Other beneficiaries will be the PATTEC Coordination Unit, other key stakeholders such as the World Health Organization (WHO), the International Atomic Energy Agency (IAEA), and the African Union–Interafrican Bureau for Animal Resources (AU–IBAR).

The project will enable FAO to provide reinforced technical assistance to trypanosomosis-affected countries in the fields of project implementation, execution, monitoring and evaluation, as well as project identification, planning and formulation. Particular attention will be given to data management and analysis in a Geographic Information Systems (GIS) environment, as well as to innovative, integrated animal health packages.

Capacity development will be at the core of all project's activities. Training courses will be organized at the national and regional level, in close collaboration with the leading Africa-based training institutions. Capacity will also be developed through collaborative technical activities and on-the-job training. Special attention will be given to supporting staff from affected countries who have an interest in raising their level of expertise by seeking postgraduate academic degrees, so as to ensure that a new generation of professionals is available to address the present and future challenges. A range of publications will be developed, focusing on manuals, guidelines, as well as on technical and scientific papers.

GIS tools in general, and the Atlas of tsetse and African animal trypanosomosis (AAT), will be developed for improved technical and strategic decision making, awareness raising and advocacy. The Atlas and GIS technology will provide affected countries with a clear framework and readily usable tools for better managing epidemiological information, in a moment when GIS is increasingly recognized as a key component of any field intervention against tsetse and trypanosomosis. The Atlas, together with other dissemination tools (e.g. Programme against African Trypanosomosis - Information System (PAAT–IS) and web site, FAO GeoNetwork, etc.) will also promote data sharing for improved accessibility and visibility of information.

The project will enable to continue providing support to the Atlas of human African trypanosomiasis (HAT), a WHO initiative, jointly implemented with FAO in the framework of PAAT, for the benefit of the affected countries, policy makers, donors and scientists. FAO support will focus on methodological development, data harmonization and mapping, risk assessment, GIS training for endemic countries and production of technical and scientific documentation.

The principles of partnership (e.g. interagency collaboration within the UN system and other international and regional organizations) and ownership (by beneficiary countries and field beneficiaries/stakeholders) will guide the execution of all project components.

AUC AND GALVMED SIGN MOU ON CONTROL AND ERADICATION OF TRYPANOSOMIASIS, NOVEMBER 9, 2012

Trypanosomosis remains one of the most serious Livestock and Human diseases on the African continent. Affecting over half of the countries in Africa, the disease carried by the dreaded Tsetse fly, causes immeasurable suffering and economic losses to livestock farmers and country economies in addition to threatening food security.

GALVmed with support and funding from DFID, embarked upon a project seeking to develop new control tools for African trypanosomosis. This has seen our activities synergizing with those of the African Union PATTEC and others, in a continental approach

and collaborative partnership to try to strengthen the fight against this disease which has plagued Africa for many decades.

A recent development is the signing of an MoU between the African Union and GALVmed which took place on Friday 9th November at the African Union Commission HQ in Addis Ababa, Ethiopia.

Professor Andy Peters, GALVmed's Interim CEO and H.E. Mrs. Rhoda P. Tumusiime, Commissioner for Rural Economy & Agriculture – AUC signing the MOU between GALVmed and AUC

SECTION B – ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

16294 **Alviano, D. S., Barreto, A. L., Dias Fde, A., Rodrigues Ide, A., Rosa Mdo, S., Alviano, C. S. & Soares, R. M., 2012.** Conventional therapy and promising plant-derived compounds against trypanosomatid parasites. *Frontiers in Antimicrobials, Resistance & Chemotherapy*, **3**: 283.

Laboratorio de Estruturas de Superficie de Microrganismos, Instituto de Microbiologia Prof. Paulo de Goes, Universidade Federal do Rio de Janeiro Rio de Janeiro, Rio de Janeiro, Brazil. [rasoares@micro.ufrj.br].

Leishmaniasis and trypanosomiasis are two neglected and potentially lethal diseases that affect mostly the poor and marginal populations of developing countries around the world and consequently have an important impact on public health. Clinical manifestations such as cutaneous, mucocutaneous, and visceral disorders are the most frequent forms of leishmaniasis. American trypanosomiasis, or Chagas disease, is caused by *Trypanosoma cruzi*, a parasite that causes progressive damage to different organs, particularly the heart, oesophagus, and lower intestine. African trypanosomiasis, or sleeping sickness, is caused by *Trypanosoma brucei* and is characterized by first presenting as an acute form that affects blood clotting and then becoming a chronic meningoencephalitis. The limited number, low

efficacy, and side effects of conventional anti-leishmania and anti-trypanosomal drugs and the resistance developed by parasites are the major factors responsible for the growth in mortality rates. Recent research focused on plants has shown an ingenious way to obtain a solid and potentially rich source of drug candidates against various infectious diseases. Bioactive phytocompounds present in the crude extracts and essential oils of medicinal plants are components of an important strategy linked to the discovery of new medicines. These compounds have proven to be a good source of therapeutic agents for the treatment of leishmaniasis and trypanosomiasis. This work highlights some chemotherapeutic agents while emphasizing the importance of plants as a source of new and powerful drugs against these widespread diseases.

16295. **Amaral, I., 2012.** Bacteria or parasite?: the controversy over the aetiology of sleeping sickness and the Portuguese participation, 1898-1904. *História, Ciências, Saúde-Manguinhos,* **E Publication ahead of print, November 7.**

Departamento de Ciencias Sociais Aplicadas, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal, 2829-516. [ima@fct.unl.pt].

The aetiology of sleeping sickness was unknown until the early twentieth century. This African disease soon became the main obstacle to European colonization. Sending scientific missions to the colonies to monitor its progression *in loco* thus became inevitable. Portugal sent the first research mission to Angola in 1901, and the Royal Society of London sponsored two British missions to study the disease in Entebbe (1902 and 1903). Their results led to a controversy in which Portugal was involved from 1898 to 1904 on the national and international circuits, and which are analysed in this article.

16296. **Barrett, M. P. & Croft, S. L., 2012.** Management of trypanosomiasis and leishmaniasis. *British Medical Bulletin*, **104**: 175-196.

London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK. [simon.croft@lshtm.ac.uk].

The current treatments for human African trypanosomiasis (HAT), Chagas disease and leishmaniasis (collectively referred to as the kinetoplastid diseases) are far from ideal but, for some, there has been significant recent progress. For HAT the only advances in treatment over the past two decades have been the introduction of an eflornithine/nifurtimox co-administration and a shorter regime of the old standard melarsoprol. There is a need for new safe oral drugs for cost-effective treatment of patients and use in control programmes for all the trypanosomatid diseases. Cutaneous leishmaniasis is not on the agenda and treatments are lagging behind. There are three compounds in development for the treatment of the CNS stage of HAT: fexinidazole, currently due to enter phase II clinical studies, a benzoxaborole (SCYX-7158) in phase I trials and a diamidine derivative (CPD-0802), in advanced pre-clinical development. For Chagas disease, two anti-fungal triazoles are now in clinical trial. In addition, clinical studies with benznidazole, a drug previously recommended only for acute stage treatment, are close to completion to determine the effectiveness in the treatment of early chronic and indeterminate Chagas disease. For visceral leishmaniasis new formulations, therapeutic switching, in particular AmBisome, and the potential for combinations of established drugs have significantly improved the opportunities for treatment in the Indian

subcontinent, but not in East Africa. Improved diagnostic tools are needed to support treatment, for test of cure in clinical trials and for monitoring/surveillance of populations in control programmes.

16297. **Burri, C., 2012.** An alternative form of melarsoprol in sleeping sickness: is an old drug always the best basis for a new one? *Trends in Parasitology*, **28** (9): 354-355.

Swiss Tropical and Public Health Institute, Department of Medicines Research, Basel, Switzerland; and University of Basel, Department of Pharmaceutical Sciences, Basel, Switzerland. [christian.burri@unibas.ch].

The almost lifelong efforts of Professor Peter Kennedy to improve the situation of patients suffering from sleeping sickness must be lauded. He has published a number of important and innovative papers on the neurology and pathogenesis of human African trypanosomiasis (HAT). Recently, his group turned towards treatment and brought out a paper on an alternative formulation of melarsoprol for the treatment of second stage sleeping sickness. A recent Opinion article published in *Trends in Parasitology* is a follow-up of this publication, intended to support the development of the melarsoprol–cyclodextrin inclusion complexes. In my opinion, there are several factual issues with this publication: I feel that the statement: "the treatment situation for HAT was sobering and has not changed until recently since the times of Dr Livingstone"' is tendentious. Indeed, for over 50 years until the beginning of this century there was almost no progress in treatment of HAT. However, since then much clinical research has been carried out, large-scale clinical development projects were conducted for the first time, the political situation allowed patients access to free medicine, and research results were translated into action. As a result, (i) non-governmental organizations (NGOs) started treating patients with eflornithine about 15 years ago, (ii) the melarsoprol treatment regimen could be shortened from about 30 to 10 days, and (iii) the nifurtimox–eflornithine combination treatment (NECT) was developed and listed on the World Health Organization (WHO) essential drug list 2 years ago. It is true that the situation of the patients infected by *Trypanosoma brucei rhodesiense* is much less favourable, with the only improvement so far being the recent recommendation to switch melarsoprol treatment also to the abridged 10 - day treatment course. However, *T. b. gambiense* causes the form of the disease that affects over 98 percent of HAT patients, 88 percent of whom by now benefit from the ground-breaking NECT treatment and have a fundamentally lower risk during treatment.. To my eyes this progress is not sobering but rather refreshing. In Dr Kennedy's publication, a case is made for orally applicable drugs. Such a medication would clearly have a logistic and economic advantage, and patients who do not yet have access to the NECT treatment might profit. However, the development of new medicines is a very expensive enterprise. Funding is a particular issue for neglected diseases, and one must ask into which basket the eggs should be placed –into that of a new compound which is being developed from scratch according to current standards and regulations, and has the support of international enterprises, or into that of an old drug which has been modified to the extent that it must be considered a new chemical entity but still potentially bears the known risks? All decisions concerning new developments should be based on an assessment of risk and benefit for the patients, also weighing the investment against the likelihood of success.

I am not sure whether the authors are fully aware of the complexity of the undertaking they propose: from the regulatory point of view the melarsoprol–cyclodextrin complexes must be considered as new chemical entities, and consequently a complete drug development

programme will be required to develop and register the compounds. Before human trials may start a full preclinical programme with the tests in multiple animal species, as described in the pertinent regulations, would have to be performed. This programme would be a particular challenge because melarsoprol has a very poor safety profile. The claimed absence of encephalopathic syndromes would need verification, probably using the existing monkey model of *T. b. rhodesiense* infection. Performing a proof-of-concept trial in a limited population might be realizable, but owing to the relative infrequency of the encephalopathic syndromes (in the order of 10 percent) a small trial would be insufficiently powered to estimate the true frequency or to demonstrate their absence. At this point, the researchers would actually be confronted with another scientific and regulatory challenge: it is claimed that melarsoprol levels, particularly within the central nervous system, are very low. Therefore, not only the safety of the new combination, but also its efficacy, will need to be demonstrated. Once a proof-of-concept trial has been conducted, the programme would then enter a completely new phase, namely the necessary Phase III trials. Such a project cannot be financed and conducted without an industrial partner or a dedicated product development partnership. However, their interest would probably be lukewarm: 10 years ago the manufacturer of the arsenic-containing melarsoprol threatened to cease production, not only because of its limited use but also because of environmental considerations. Only through negotiations with the WHO and interested NGOs is production still maintained and the drug made available for treatment of *T. b. rhodesiense*. It is difficult to imagine that a pharmaceutical company will be interested in investing in the production of a new chemical entity containing arsenicals.

Currently clinical research is focusing on fexinidazole, which is about to enter Phase II field trials and can optimistically become registered in 5–7 years. A new and very promising class of oxaboroles is in late-stage (good laboratory practice) preclinical testing, and the programme to validate these drugs will not last much longer. Finally, promising derivatives of the class of diamidines are currently being pursued in the early preclinical phase, and serious lead-identification activities in several other drug classes are on-going. Even if the choice is still limited, we are looking at an unprecedented pipeline of new compounds for treating sleeping sickness. In contrast to the melarsoprol–cyclodextrin complexes, the development of these compounds is supported by potent international partners. With this in mind, it is doubtful that the development of melarsoprol–cyclodextrin complexes would be faster. In reality, a problematic competition may result for funding and for accessing patients for clinical research –between a new delivery form of a very old drug, associated with various problems, versus modern approaches including new chemical entities. In conclusion, the efforts of the authors to contribute to the development of new treatments for sleeping sickness are much appreciated, but in this very case the principle of Sir James Black that "the most fruitful basis for the discovery of a new drug is to start with an old drug" seems to be too far-fetched

16298. **Cestari, I., Evans-Osses, I., Schlapbach, L. J., de Messias-Reason, I. & Ramirez, M. I., 2012.** Mechanisms of complement lectin pathway activation and resistance by trypanosomatid parasites. *Molecular Immunology*, **53** (4): 328-334.

Seattle Biomedical Research Institute, Seattle, WA 98109, USA; Laboratorio de Biologia Molecular de Parasitas e Vetores, Instituto Oswaldo Cruz - Fiocruz, Rio de Janeiro 21040-900, Brazil. [marcelramirez4@hotmail.com].

Studies in the past decade have demonstrated a crucial role for the complement lectin pathway in host defence against protozoan microbes. Recognition of pathogen surface molecules by mannan-binding lectin and ficolins revealed new mechanisms of innate immune defence and a diversity of parasite strategies of immune evasion. In the present review, we discuss the current knowledge of: (i) the molecular mechanism of lectin pathway activation by trypanosomes; (ii) the mechanisms of complement evasion by trypanosomes; and (iii) host genetic deficiencies of complement lectin pathway factors that contribute to infection susceptibility and disease progression. This review focuses on trypanosomatids, the parasites that cause Chagas disease, leishmaniasis and sleeping sickness (African trypanosomiasis).

16299. **Cook, G. C., 2012.** Patrick Manson (1844-1922) FRS: *Filaria (Mansonella) perstans* and sleeping sickness (African trypanosomiasis). *Journal of Medical Biography*, **20** (2): 69.

University College London, 11 Old London Road, St Albans, Herts., UK.

Abstract not available.

16300. **Deribe, K., Meribo, K., Gebre, T., Hailu, A., Ali, A., Assefa, A. & Davey, G., 2012.** The burden of neglected tropical diseases in Ethiopia, and opportunities for integrated control and elimination. *Parasites & Vectors*, **5** (1): 240.

Brighton and Sussex Medical School, Falmer, Brighton, UK.; Federal Ministry of Health Ethiopia, Addis Ababa, Ethiopia; International Trachoma Initiative, The Task Force for Global Health, Addis Ababa, Ethiopia; Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia; School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia; and Armauer Hansen Research Institute/ALERT, Addis Ababa, Ethiopia. [kebededeka@yahoo.com].

Neglected tropical diseases (NTDs) are a group of chronic parasitic diseases and related conditions that are the most common diseases among the 2.7 billion people globally living on less than US$2 per day. In response to the growing challenge of NTDs, Ethiopia is preparing to launch a NTD Master Plan. The purpose of this review is to underscore the burden of NTDs in Ethiopia, highlight the state of current interventions, and suggest ways forward. This review indicates that NTDs are significant public health problems in Ethiopia. From the analysis reported here, Ethiopia stands out for having the largest number of NTD cases after Nigeria and the Democratic Republic of Congo. Ethiopia is estimated to have the highest burden of trachoma, podoconiosis and cutaneous leishmaniasis in sub-Saharan Africa (SSA), the second highest burden in terms of ascariasis, leprosy and visceral leishmaniasis, and the third highest burden of hookworm. Infections such as schistosomiasis, trichuriasis, lymphatic filariasis and rabies are also common. A third of Ethiopians are infected with ascariasis, one quarter is infected with trichuriasis and one in eight Ethiopians lives with hookworm or is infected with trachoma. However, despite these high burdens of infection, the control of most NTDs in Ethiopia is in its infancy. In terms of NTD control achievements, Ethiopia reached the leprosy elimination target of 1 case/10 000 population in 1999. No cases of human African trypanosomiasis have been reported since 1984. Guinea worm eradication is in its final phase. The Onchocerciasis Control Programme has been making steady progress since 2001. A national blindness survey was conducted in 2006 and the trachoma programme has

kicked off in some regions. Lymphatic filariasis, podoconiosis and rabies mapping are underway. In conclusion, Ethiopia bears a significant burden of NTDs compared with other SSA countries. To achieve success in integrated control of NTDs, integrated mapping, rapid scale-up of interventions and operational research into co-implementation of intervention packages will be crucial.

16301. **Espuelas, S., Plano, D., Nguewa, P., Font, M., Palop, J. A., Irache, J. M. & Sanmartin, C., 2012.** Innovative lead compounds and formulation strategies as newer kinetoplastid therapies. *Current Medicinal Chemistry*, **19** (25): 4259-4288.

Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Navarra, Irunlarrea 1, E-31008, Pamplona, Spain.

The protozoan diseases leishmaniasis, human African trypanosomiasis (HAT) and Chagas disease (CD) are responsible for substantial global morbidity and mortality in tropical and subtropical regions. Environmental changes, drug resistance and immunosuppression are contributing to the emergence and spread of these diseases. In the absence of safe and efficient vaccines, chemotherapy, together with vector control, remains the most important measure to control kinetoplastid diseases. Nevertheless, the current chemotherapeutic treatments are clearly inadequate because of their toxic effects, generation of resistances as well as route and schedules of administration not adapted to the field conditions. This review examines the strategies that can be addressed to meet immediately the patient needs such as the reconsideration of current regimens of administration and the rational combination of drugs in use. In the medium-long term, due to new methodologies of medicinal chemistry, the screening of natural products and the identification of new therapeutic targets, new lead compounds have a great chance to advance through the drug development pipeline to the clinic. Modern pharmaceutical formulation strategies and nanomedicines also merit a place in view of the benefits of a single dose of liposomal Amphotericin B (AmBisome®) against visceral leishmaniasis. Blood-brain barrier (BBB)-targeted nanodevices could be suited for selective delivery of drugs against HAT encephalitic phase. Bioadhesive nanoparticles can be proposed to enhance the bioavailability of drugs after oral administration by reason of improving the drug solubility and permeability across the intestinal epithelia.

16302. **Finsterer, J. & Auer, H., 2012.** Parasitoses of the human central nervous system. *Journal of Helminthology*: 1-14. **E Publication ahead of print, October 10.**

Krankenanstalt Rudolfstiftung, Vienna, Austria.

Cerebral involvement in parasitoses is an important clinical manifestation of most of the human parasitoses. Parasites that have been described to affect the central nervous system (CNS), either as the dominant or as a collateral feature, include cestodes (*Taenia solium* (neurocysticerciasis), *Echinococcus granulosus* (cerebral cystic echinococcosis), E. *multilocularis* (cerebral alveolar echinococcosis), *Spirometra mansoni* (neurosparganosis)), nematodes (*Toxocara canis* and *T. cati* (neurotoxocariasis), *Trichinella spiralis* (neurotrichinelliasis), *Angiostrongylus cantonensis* and *A. costaricensis* (neuroangiostrongyliasis), *Gnathostoma spinigerum* (gnathostomiasis)), trematodes (*Schistosoma mansoni* (cerebral bilharziosis), *Paragonimus westermani* (neuroparagonimiasis)), or protozoa (*Toxoplasma gondii* (neurotoxoplasmosis),

Acanthamoeba spp. or *Balamuthia mandrillaris* (granulomatous amoebic encephalitis), *Naegleria* (primary amoebic meningo-encephalitis), *Entamoeba histolytica* (brain abscess), *Plasmodium falciparum* (cerebral malaria), *Trypanosoma brucei gambiense/rhodesiense* (sleeping sickness) or *Trypanosoma cruzi* (cerebral Chagas disease)). Adults or larvae of helminths or protozoa enter the CNS and cause meningitis, encephalitis, ventriculitis, myelitis, ischaemic stroke, bleeding, venous thrombosis or cerebral abscess, clinically manifesting as headache, epilepsy, weakness, cognitive decline, impaired consciousness, confusion, coma or focal neurological deficits. Diagnosis of cerebral parasitoses is dependent on the causative agent. Available diagnostic tools include clinical presentation, blood tests (eosinophilia, *Plasmodia* in blood smear, antibodies against the parasite), cerebrospinal fluid (CSF) investigations, imaging findings and occasionally cerebral biopsy. Treatment relies on drugs and sometimes surgery. Outcome of cerebral parasitoses is highly variable, depending on the effect of drugs, whether they are self-limiting or whether they remain undetected or asymptomatic, like 25 percent of neurocysticerciasis cases.

16303. **Goldston, A. M., Powell, R. R. & Temesvari, L. A., 2012.** Sink or swim: lipid rafts in parasite pathogenesis. *Trends in Parasitology*, **28** (10): 417-426.

Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634, USA. [ltemesv@clemson.edu].

Lipid rafts, sterol- and sphingolipid-rich membrane microdomains have been extensively studied in mammalian cells. Recently, lipid rafts have been shown to control virulence in a variety of parasites including *Entamoeba histolytica, Giardia intestinalis, Leishmania* spp., *Plasmodium* spp., *Toxoplasma gondii*, and *Trypanosoma* spp. Parasite rafts regulate adhesion to host and invasion, and parasite adhesion molecules often localize to rafts. Parasite rafts also control vesicle trafficking, motility, and cell signalling. Parasites disrupt host cell rafts; the deregulation of host membrane function facilitates the establishment of infection and evasion of the host immune system. Discerning the mechanism by which lipid rafts regulate parasite pathogenesis is essential to our understanding of virulence. Such insight may guide the development of new drugs for disease management.

16304. **Kappagoda, S. & Ioannidis, J. P., 2012.** Neglected tropical diseases: survey and geometry of randomised evidence. *British Medical Journal*, **345**: e6512.

Center for Primary Care and Outcomes Research and Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA, USA. [jioannid@stanford.edu].

This study had the objective of assessing the quantity and distribution of evidence from randomised controlled trials for the treatment of the major neglected tropical diseases and to identify gaps in the evidence with network analysis through a systematic review and network analysis. Data sources included the Cochrane Central Register of Controlled Trials and PubMed from inception to 31 August 2011, while study selection used randomised controlled trials that examined treatment of 16 neglected tropical diseases or complications thereof published in English, French, Spanish, Portuguese, German, or Dutch. We identified 971 eligible randomised trials. Leishmaniasis (184 trials, 23 039 participants) and geohelminth infections (160 trials, 46 887 participants) were the most studied, while dracunculiasis (nine

trials, 798 participants) and Buruli ulcer (five trials, 337 participants) were least studied. Relative to its global burden of disease, lymphatic filariasis had the fewest trials and participants. Only 11 percent of trials were industry funded. Either a single trial or trials with fewer than 100 participants comprised the randomised evidence for first or second line treatments for Buruli ulcer, human African trypanosomiasis, American trypanosomiasis, cysticercosis, rabies, echinococcosis, New World cutaneous leishmaniasis, and each of the foodborne trematode infections. Among the 10 disease categories with more than 40 trials, five lacked sufficient head-to-head comparisons between first or second line treatments. In conclusion, there is considerable variation in the amount of evidence from randomised controlled trials for each of the 16 major neglected tropical diseases. Even in diseases with substantial evidence such as leishmaniasis and geohelminth infections, some recommended treatments have limited supporting data and lack head-to-head comparisons.

16305. **Kennedy, P. G., 2012.** Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness). *Lancet Neurology*. **E Publication, ahead of print, December 21.**

Department of Neurology, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Institute of Neurological Sciences, Southern General Hospital, Glasgow, UK. [peter.kennedy@glasgow.ac.uk].

Human African trypanosomiasis, or sleeping sickness, is caused by infection with parasites of the genus *Trypanosoma*, transmitted by the tsetse fly. The disease has two causative agents, *Trypanosoma brucei* (*T. b.*) *rhodesiense* and *T. b. gambiense*; and is almost always fatal if untreated. Despite a recent reduction in the number of reported cases, patients with African trypanosomiasis continue to present major challenges to clinicians. Because treatment for CNS-stage disease can be very toxic, diagnostic staging to distinguish early-stage from late-stage disease when the CNS in invaded is crucial but remains problematic. Melarsoprol is the only available treatment for late-stage *T. b. rhodesiense* infection, but can be lethal to 5 percent of patients owing to post-treatment reactive encephalopathy. Eflornithine combined with nifurtimox is the first-line treatment for late-stage *T. b. gambiense*. New drugs are in the pipeline for treatment of CNS human African trypanosomiasis, giving rise to cautious optimism.

16306. **Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K. Aboyans, V., et al., 2013.** Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, **380** (9859): 2095-2128.

Institute for Health Metrics and Evaluation, Seattle, WA, USA. [cjlm@uw.edu].

Reliable and timely information on the leading causes of death in populations, and how these are changing, is a crucial input into health policy debates. In the Global Burden of Diseases, Injuries, and Risk Factors Study 2010 (GBD 2010), we aimed to estimate annual deaths for the world and 21 regions between 1980 and 2010 for 235 causes, with uncertainty intervals (UIs), separately by age and sex. We attempted to identify all available data on causes of death for 187 countries from 1980 to 2010 from vital registration, verbal autopsy, mortality surveillance, censuses, surveys, hospitals, police records, and mortuaries. We

assessed data quality for completeness, diagnostic accuracy, missing data, stochastic variations, and probable causes of death. We applied six different modelling strategies to estimate cause-specific mortality trends depending on the strength of the data. For 133 causes and three special aggregates we used the Cause of Death Ensemble model (CODEm) approach, which uses four families of statistical models testing a large set of different models using different permutations of covariates. Model ensembles were developed from these component models. We assessed model performance with rigorous out-of-sample testing of prediction error and the validity of 95 percent UIs. For 13 causes with low observed numbers of deaths, we developed negative binomial models with plausible covariates. For 27 causes for which death is rare, we modelled the higher level cause in the cause hierarchy of the GBD 2010 and then allocated deaths across component causes proportionately, estimated from all available data in the database. For selected causes (African trypanosomiasis, congenital syphilis, whooping cough, measles, typhoid and parathyroid, leishmaniasis, acute hepatitis E, and HIV/AIDS), we used natural history models based on information on incidence, prevalence, and case-fatality. We separately estimated cause fractions by aetiology for diarrhoea, lower respiratory tract infections, and meningitis, as well as disaggregations by subcause for chronic kidney disease, maternal disorders, cirrhosis, and liver cancer. For deaths due to collective violence and natural disasters, we used mortality shock regressions. For every cause, we estimated 95 percent UIs that captured both parameter estimation uncertainty and uncertainty due to model specification where CODEm was used. We constrained cause-specific fractions within every age-sex group to sum to total mortality based on draws from the uncertainty distributions. In 2010, there were 52.8 million deaths globally. At the most aggregate level, communicable, maternal, neonatal, and nutritional causes were 24.9 percent of deaths worldwide in 2010, down from 15.9 million (34.1 percent) of 46.5 million in 1990. This decrease was largely due to decreases in mortality from diarrhoeal disease (from 2.5 to 1.4 million), lower respiratory infections (from 3.4 to 2.8 million), neonatal disorders (from 3.1 to 2.2 million), measles (from 0.63 to 0.13 million), and tetanus (from 0.27 to 0.06 million). Deaths from HIV/AIDS increased from 0.30 million in 1990 to 1.5 million in 2010, reaching a peak of 1.7 million in 2006. Malaria mortality also rose by an estimated 19.9 percent since 1990 to 1.17 million deaths in 2010. Tuberculosis killed 1.2 million people in 2010. Deaths from non-communicable diseases rose by just under 8 million between 1990 and 2010, accounting for two of every three deaths (34.5 million) worldwide by 2010. 8 million people died from cancer in 2010, 38 percent more than two decades ago; of these, 1.5 million (19 percent) were from trachea, bronchus, and lung cancer. Ischaemic heart disease and stroke collectively killed 12.9 million people in 2010, or one in four deaths worldwide, compared with one in five in 1990; 1.3 million deaths were due to diabetes, twice as many as in 1990. The fraction of global deaths due to injuries (5.1 million deaths) was marginally higher in 2010 (9.6 percent) compared with two decades earlier (8.8 percent). This was driven by a 46 percent rise in deaths worldwide due to road traffic accidents (1.3 million in 2010) and a rise in deaths from falls. Ischaemic heart disease, stroke, chronic obstructive pulmonary disease (COPD), lower respiratory infections, lung cancer, and HIV/AIDS were the leading causes of death in 2010. Ischaemic heart disease, lower respiratory infections, stroke, diarrhoeal disease, malaria, and HIV/AIDS were the leading causes of years of life lost due to premature mortality (YLLs) in 2010, similar to what was estimated for 1990, except for HIV/AIDS and preterm birth complications. YLLs from lower respiratory infections and diarrhoea decreased by 45-54 percent since 1990; ischaemic heart disease and stroke YLLs increased by 17-28 percent. Regional variations in leading causes of death were substantial. Communicable, maternal, neonatal, and nutritional causes still

accounted for 76 percent of premature mortality in sub-Saharan Africa in 2010. Age standardised death rates from some key disorders rose (HIV/AIDS, Alzheimer's disease, diabetes mellitus, and chronic kidney disease in particular), but for most diseases, death rates fell in the past two decades; including major vascular diseases, COPD, most forms of cancer, liver cirrhosis, and maternal disorders. For other conditions, notably malaria, prostate cancer, and injuries, little change was noted. These results indicate that population growth, increased average age of the world's population, and largely decreasing age-specific, sex-specific, and cause-specific death rates combine to drive a broad shift from communicable, maternal, neonatal, and nutritional causes towards non-communicable diseases. Nevertheless, communicable, maternal, neonatal, and nutritional causes remain the dominant causes of YLLs in sub-Saharan Africa. Overlaid on this general pattern of the epidemiological transition, marked regional variation exists in many causes, such as interpersonal violence, suicide, liver cancer, diabetes, cirrhosis, Chagas disease, African trypanosomiasis, melanoma, and others. Regional heterogeneity highlights the importance of sound epidemiological assessments of the causes of death on a regular basis.

16307. **Rochani, A. K., Singh, M. & Tatu, U., 2012.** Heat shock protein 90 inhibitors as broad spectrum anti-infectives. *Current Pharmaceutical Design*. **E Publication ahead of print, August 16.**

Department of Biochemistry, Indian Institute of Science, Bangalore-12, India. [tatu@biochem.iisc.ernet.in].

Combating stress is one of the prime requirements for any organism. For parasitic microbes, stress levels are highest during the growth inside the host. Their survival depends on their ability to acclimatize and adapt to new environmental conditions. Robust cellular machinery for stress response is, therefore, both critical and essential especially for pathogenic microorganisms. Microbes have cleverly exploited stress proteins as virulence factors for pathogenesis in their hosts. Owing to its ability to sense and respond to the stress conditions, heat shock protein 90 (Hsp90) is one of the key stress proteins utilized by parasitic microbes. There is growing evidence for the critical role played by Hsp90 in the growth of pathogenic organisms like *Candida, Giardia, Plasmodium*, *Trypanosoma*, and others. This review, therefore, explores potential of exploiting Hsp90 as a target for the treatment of infectious diseases. This molecular chaperone has already gained attention as an effective anti-cancer drug target. As a result, a lot of research has been done at laboratory, preclinical and clinical levels for several Hsp90 inhibitors as potential anti-cancer drugs. In addition, a great deal of data pertaining to toxicity studies, pharmacokinetics and pharmacodynamics studies, dosage regime, drug related toxicities, dose limiting toxicities as well as adverse drug reactions are available for Hsp90 inhibitors. Therefore, repurposing/repositioning strategies are also being explored for these compounds which have gone through advanced stage clinical trials. This review presents a comprehensive summary of the current status of development of Hsp90 as a drug target and its inhibitors as candidate anti-infectives. Particular emphasis is laid on the possibility of repositioning strategies coupled with pharmaceutical solutions required for fulfilling needs for the ever-growing pharmaceutical infectious disease market.

16308. **Rudenko, G., 2012.** High-throughput whole genome analysis provides insight into how the major drugs against African sleeping sickness operate. *Pathogens & Global*

Health, **106** (2): 79.

Imperial College, London, UK. [gloria.rudenko@imperial.ac.uk].

Abstract not available

16309. **Vreysen, M. J., Seck, M. T., Sall, B. & Bouyer, J., 2012.** Tsetse flies: their biology and control using area-wide integrated pest management approaches. *Journal of Invertebrate Pathology*. **E Publication ahead of print, 2 August 2012.**

Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria. [M.Vreysen@iaea.org].

Tsetse flies are the cyclical vectors of trypanosomes, the causative agents of "sleeping sickness" or human African trypanosomosis (HAT) in humans and "nagana" or African animal trypanosomosis (AAT) in livestock in sub-Saharan Africa. Many consider HAT as one of the major neglected tropical diseases and AAT as the single greatest health constraint to increased livestock production. This review provides some background information on the taxonomy of tsetse flies, their unique way of reproduction (adenotrophic viviparity) making the adult stage the only one easily accessible for control, and how their ecological affinities, their distribution and population dynamics influence and dictate control efforts. The paper likewise reviews four control tactics (sequential aerosol technique, stationary attractive devices, live bait technique and the sterile insect technique) that are currently accepted as friendly to the environment, and describes their limitations and advantages and how they can best be put to practise in an IPM context. The paper discusses the different strategies for tsetse control i.e. localised versus area-wide and focusses thereafter on the principles of area-wide integrated pest management (AW-IPM) and the phased-conditional approach with the tsetse project in Senegal as a recent example. We argue that sustainable tsetse-free zones can be created on the African mainland provided certain managerial and technical prerequisites are in place.

16310. **Wink, M., 2012.** Medicinal plants: a source of anti-parasitic secondary metabolites. *Molecules*, **17** (11): 12771-12791.

Institute of Pharmacy and Molecular Biotechnology, INF 364, Heidelberg University, D-69120 Heidelberg, Germany. [wink@uni-hd.de].

This review summarizes human infections caused by endoparasites, including protozoa, nematodes, trematodes, and cestodes, which affect more than 30 percent of the human population, and medicinal plants of potential use in their treatment. Because vaccinations do not work in most instances and the parasites have sometimes become resistant to the available synthetic therapeutics, it is important to search for alternative sources of anti-parasitic drugs. Plants produce a high diversity of secondary metabolites with interesting biological activities, such as cytotoxic, anti-parasitic and anti-microbial properties. These drugs often interfere with central targets in parasites, such as DNA (intercalation, alkylation), membrane integrity, microtubules and neuronal signal transduction. Plant extracts and isolated secondary metabolites which can inhibit protozoan parasites, such as *Plasmodium, Trypanosoma,*

Leishmania, Trichomonas and intestinal worms are discussed. The identified plants and compounds offer a chance to develop new drugs against parasitic diseases. Most of them need to be tested in more detail, especially in animal models and if successful, in clinical trials.

16311**. Witschel, M., Rottmann, M., Kaiser, M. & Brun, R., 2012.** Agrochemicals against malaria, sleeping sickness, leishmaniasis and Chagas disease. *PLoS Neglected Tropical Diseases*, **6** (10): e1805.

BASF SE, Global Research Herbicides, Ludwigshafen, Germany. [Reto.Brun@unibas.ch].

In tropical regions, protozoan parasites can cause severe diseases with malaria, leishmaniasis, sleeping sickness, and Chagas disease standing in the forefront. Many of the drugs currently being used to treat these diseases have been developed more than 50 years ago and can cause severe adverse effects. Above all, resistance to existing drugs is widespread and has become a serious problem threatening the success of control measures. In order to identify new antiprotozoal agents, more than 600 commercial agrochemicals have been tested on the pathogens causing the above mentioned diseases. For all of the pathogens, compounds were identified with similar or even higher activities than the currently used drugs in applied *in vitro* assays. Furthermore, *in vivo* activity was observed for the fungicide/oomyceticide azoxystrobin, and the insecticide hydramethylnon in the *Plasmodium berghei* mouse model, and for the oomyceticide zoxamide in the *Trypanosoma brucei rhodesiense* STIB900 mouse model, respectively.

16312. **Zamora-Vilchis, I., Williams, S. E. & Johnson, C. N., 2012.** Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: implications for disease in a warming climate. *PLoS One*, **7** (6): e39208.

Centre for Tropical Biodiversity and Climate Change, School of Marine and Tropical Biology, James Cook University, Townsville, Queensland, Australia. [Itzel.ZamoraVilchis@my.jcu.edu.au].

The rising global temperature is predicted to expand the distribution of vector-borne diseases both in latitude and altitude. Many host communities could be affected by increased prevalence of disease, heightening the risk of extinction for many already threatened species. To understand how host communities could be affected by changing parasite distributions, we need information on the distribution of parasites in relation to variables like temperature and rainfall that are predicted to be affected by climate change. We determined relations between prevalence of blood parasites, temperature, and seasonal rainfall in a bird community of the Australian Wet Tropics along an elevation gradient. We used PCR screening to investigate the prevalence and lineage diversity of four genera of blood parasites (*Plasmodium, Haemoproteus, Leucocytozoon* and *Trypanosoma*) in 403 birds. The overall prevalence of the four genera of blood parasites was 32.3 percent, with *Haemoproteus* the predominant genus. A total of 48 unique lineages were detected. Independent of elevation, parasite prevalence was positively and strongly associated with annual temperature. Parasite prevalence was elevated during the dry season. It is concluded that low temperatures of the higher elevations can help to reduce both the development of avian haematozoa and the abundance of parasite vectors, and hence parasite prevalence. In contrast, high temperatures of the lowland areas provide an

excellent environment for the development and transmission of haematozoa. We showed that rising temperatures are likely to lead to increased prevalence of parasites in birds, and may force shifts of bird distribution to higher elevations. We found that upland tropical areas are currently a low-disease habitat and their conservation should be given high priority in management plans under climate change.

16313. **Zheng, W., 2012.** Sirtuins as emerging anti-parasitic targets. *European Journal of Medicinal Chemistry*, **59C**: 132-140.

School of Pharmacy, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, PR China. [wzheng@ujs.edu.cn].

Silent information regulator 2 (Sir2) enzymes or sirtuins are a family of NAD^+-dependent protein N (epsilon)-acetyl-lysine (AcK) deacetylases. Sirtuins are also evolutionarily conserved proteins that are present in all kingdoms of life ranging from bacteria to humans. Interestingly, it was recently found that the sirtuins found in various human parasites (especially *Plasmodium*, *Trypanosoma*, and *Leishmania* species) were pro-survival for the parasites under various conditions. Therefore, these parasitic sirtuins have emerged as novel anti-parasitic therapeutic targets. This article reviews the currently available structural, biochemical, pharmacological, and medicinal chemistry studies on these enzymes, and discusses the perspectives of selectively targeting the parasitic sirtuins as a novel therapeutic strategy for the human parasitic diseases.

16314. **Zucca, M., Scutera, S. & Savoia, D., 2012.** New chemotherapeutic strategies against malaria, leishmaniasis, and trypanosomiases. *Current Medicinal Chemistry*. **E Publication ahead of print, December 3.**

Dept. of Clinical and Biological Sciences, S. Luigi Gonzaga Hospital, University of Torino, Regione Gonzole 10, 10043 Orbassano (To), Italy. [dianella.savoia@unito.it].

Due to the persistent lack of suitable vaccines, chemotherapy remains the only option for the treatment of patients infected by protozoan parasites. However, most available antiparasitic drugs have serious disadvantages, ranging from high cost and poor compliance of patients to high toxicity and rapid induction of resistance. In recent decades basic research laboratories identified a considerable number of promising new molecules, but their development has not been pursued in depth by pharmaceutical firms because of poor prospects of economic return. The establishment of adequately funded public-private partnerships is currently reversing the trend. This review deals with new drugs against *Plasmodium, Leishmania* and *Trypanosoma* parasites, focusing on the molecules that are in the most advanced stage of development. The purpose of this article is to provide the reader with a panoramic view of the updated literature on the challenges and strategies of contemporary antiprotozoal drug research, paying due attention to already published reviews.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

16315. **Abd-Alla, A. M., Bergoin, M., Parker, A. G., Maniania, N. K., Vlak, J. M., Bourtzis, K., Boucias, D. G. & Aksoy, S., 2012.** Improving sterile insect technique (SIT) for tsetse flies through research on their symbionts and pathogens. *Journal of Invertebrate Pathology*. **E Publication ahead of print**, **July 24.**

Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria; Laboratoire de Pathologie Comparée, Université Montpellier 2, Montpellier, France; International Centre of Insect Physiology and Ecology (ICIPE), PO Box 30772, Nairobi, Kenya; Laboratory of Virology, Wageningen University, Wageningen, The Netherlands; Department of Environmental and Natural Resources, University of Western Greece, 2 Seferi St., 30100 Agrinio, Greece; Biomedical Sciences Research Center Al. Fleming, 16672 Vari, Greece; Department of Entomology and Nematology, PO Box 110620, University of Florida, Gainesville, FL 32611-0620, USA; and Yale University School of Public Health, 60 College St., 626 LEPH, New Haven, CT 06520, USA. [a.m.m.abd-alla@iaea.org].

Tsetse flies (Diptera: Glossinidae) are the cyclical vectors of the trypanosomes, which cause human African trypanosomosis (HAT) or sleeping sickness in humans and African animal trypanosomosis (AAT) or nagana in animals. Due to the lack of effective vaccines and inexpensive drugs for HAT and the development of resistance of the trypanosomes against the available trypanocidal drugs, vector control remains the most efficient strategy for sustainable management of these diseases. Among the control methods used for tsetse flies, the sterile insect technique (SIT), in the frame of area-wide integrated pest management (AW-IPM), represents an effective tactic to suppress and/or eradicate tsetse flies. One constraint in implementing SIT is the mass production of target species. Tsetse flies harbour obligate bacterial symbionts and salivary gland hypertrophy virus which modulate the fecundity of the infected flies. In support of the future expansion of the SIT for tsetse fly control, the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture implemented a five year Coordinated Research Project (CRP) entitled "Improving SIT for Tsetse Flies through Research on their Symbionts and Pathogens". The consortium focused on the prevalence and the interaction between the bacterial symbionts and the virus, the development of strategies to manage virus infections in tsetse colonies, the use of entomopathogenic fungi to control tsetse flies in combination with SIT, and the development of symbiont-based strategies to control tsetse flies and trypanosomosis. The results of the CRP and the solutions envisaged to alleviate the constraints of the mass rearing of tsetse flies for SIT are presented in this special issue.

16316. **Malele, II, Manangwa, O., Nyingilili, H. H., Kitwika, W. A., Lyaruu, E. A., Msangi, A. R., Ouma, J. O., Nkwangulila, G. & Abd-Alla, A. M., 2012.** Prevalence of SGHV among tsetse species of economic importance in Tanzania and their implication for SIT application. *Journal of Invertebrate Pathology*. **E Publication ahead of print, July 26.**

Tsetse & Trypanosomiasis Research Institute, Box 1026, Tanga, Tanzania. [malele2i@yahoo.com].

The sterile insect technique is an important component in area-wide integrated tsetse

control. The presence of the salivary gland hypertrophy virus (SGHV) in wild tsetse, which are the seeds for colony adaptations in the laboratory has become a stumbling block in establishing and maintaining colonies in the laboratory. The virus is transmitted both vertically (in the wild) and horizontally (in the laboratory). However, its prevalence is magnified in the laboratory as a result of the use of an *in vitro* membrane feeding regimen. Fly species of *Glossina fuscipes fuscipes*, *G. pallidipes*, *G. morsitans* and *G. swynnertoni* were collected from the coastal and inland areas of Tanzania and virus infection rates were assessed microscopically and by PCR. The data showed that in a period of 4 years, the virus was present in all species tested irrespective of their age, sex, and season of the year. However, infection levels differed among species and from one location to another. Symptomatic infection determined by dissection was 1.2 percent (25/2 164) from the coast as compared with0.4 percent (6/1 725) for inland collected flies. PCR analysis indicated a higher infection rate of 19.81 percent (104/525) in asymptomatic flies. From these observations, we conclude that care should be taken when planning to initiate tsetse laboratory colonies for use in SIT eradication programmes. All efforts should be made to select non-infected flies when initiating laboratory colonies and to try to minimize the infection with SGHV. Also management of SGHV infection in the established colony should be applied.

16317. **Mutika, G. N., Marin, C., Parker, A. G., Boucias, D. G., Vreysen, M. J. & Abd-Alla, A. M., 2012.** Impact of salivary gland hypertrophy virus infection on the mating success of male *Glossina pallidipes*: consequences for the sterile insect technique. *PLoS One*, 7 (8): e42188.

Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria. [a.m.m.abd-alla@iaea.org]

Many species of tsetse flies are infected by a virus (GpSGHV) that causes salivary gland hypertrophy (SGH). Female *Glossina pallidipes* (Austen) with SGH symptoms (SGH+) have reduced fecundity and SGH+ male *G. pallidipes* are unable to inseminate female flies. Consequently, *G. pallidipes* laboratory colonies with a high prevalence of SGH have been difficult to maintain and have collapsed on several occasions. To assess the potential impact of the release of SGH+ sterile male *G. pallidipes* on the efficacy of an integrated control programme with a sterile insect technique (SIT) component, we examined the mating efficiency and behaviour of male *G. pallidipes* in field cages in relation to SGH prevalence. The results showed in a field cage setting a significantly reduced mating frequency of 19 percent for a male *G. pallidipes* population with a high prevalence of SGH (83 percent) compared with 38 percent for a male population with a low prevalence of SGH (7 percent). Premating period and mating duration did not vary significantly with SGH status. A high percentage (>80 percent) of females that had mated with SGH+ males had empty spermathecae. The remating frequency of female *G. pallidipes* was very low irrespective of the SGH status of the males in the first mating. These results indicate that a high prevalence of SGH+ in *G. pallidipes* not only affects colony stability and performance but, in view of their reduced mating propensity and competitiveness, releasing SGH+ sterile male *G. pallidipes* will reduce the efficiency of a sterile male release programme.

16318. **Van Den Abbeele, J., Bourtzis, K., Weiss, B., Cordon-Rosales, C., Miller, W., Abd-Alla, A. M. & Parker, A., 2012.** Enhancing tsetse fly refractoriness to trypanosome infection - a new FAO/IAEA coordinated research project. *Journal of*

Invertebrate Pathology. **E Publication ahead of print, July 25.**

Department of Biomedical Sciences, Unit of Veterinary Protozoology, Institute of Tropical Medicine Antwerp, Antwerp, Belgium. [jvdabbeele@itg.be].

To date, FAO/IAEA-supported sterile insect technique (SIT) projects for tsetse and trypanosomiasis control have been in areas without human sleeping sickness, but future projects could include areas of actual or potential human disease transmission. In this context it would be imperative that released sterile tsetse flies are incompetent to transmit the disease-causing trypanosome parasite. Therefore, development of tsetse fly strains refractory to trypanosome infection is highly desirable as a simple and effective method of ensuring vector incompetence of the released flies. This new FAO/IAEA Coordinated Research Project (CRP) focuses on gaining a deeper knowledge of the tripartite interactions between the tsetse fly vectors, their symbionts and trypanosome parasites. The objective of this CRP is to acquire a better understanding of mechanisms that limit the development of trypanosome infections in tsetse and how these may be enhanced.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

16319. **Kariithi, H. M., van Lent, J., van Oers, M. M., Abd-Alla, A. M. & Vlak, J. M., 2012.** Proteomic footprints of a member of *Glossina* virus (Hytrosaviridae): an expeditious approach to virus control strategies in tsetse factories. *Journal of Invertebrate Pathology*. **E Publication ahead of print, July 25.**

Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria; and Laboratory of Virology, Wageningen University, The Netherlands. [henry.kariithi@wur.nl].

The *Glossina* virus (*Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV)) is a rod-shaped enveloped insect virus containing a 190 032 bp-long, circular dsDNA genome. The virus is pathogenic for the tsetse fly *Glossina pallidipes* and has been associated with the collapse of selected mass-reared colonies. Maintenance of productive fly colonies is critical to tsetse and trypanosomiasis eradication in sub-Saharan Africa using the sterile insect technique. Proteomics, an approach to define the expressed protein complement of a genome, was used to further our understanding of the protein composition, morphology, morphogenesis and pathology of GpSGHV. Additionally, this approach provides potential targets for novel and sustainable molecular-based antiviral strategies to control viral infections in tsetse colonies. To achieve this goal, identification of key protein partners involved in virus transmission is required. In this review, we integrate the available data on GpSGHV proteomics to assess the impact of viral infections on host metabolism and to understand the contributions of such perturbations to viral pathogenesis. The relevance of the proteome findings to tsetse and trypanosomiasis management in sub-Sahara Africa is also considered.

16320. **Kohl, K. P., Jones, C. D. & Sekelsky, J., 2012.** Evolution of an MCM complex in flies that promotes meiotic crossovers by blocking BLM helicase. *Science*, **338** (6112): 1363-1365.

Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC 27599, USA. [sekelsky@unc.edu].

Generation of meiotic crossovers in many eukaryotes requires the elimination of anti-crossover activities by using the Msh4-Msh5 heterodimer to block helicases. Msh4 and Msh5 have been lost from the flies *Drosophila* and *Glossina*, but we identified a complex of minichromosome maintenance (MCM) proteins that functionally replace Msh4-Msh5. We found that REC, an orthologue of MCM8 that evolved under strong positive selection in flies, interacts with MEI-217 and MEI-218, which arose from a previously undescribed metazoan-specific MCM protein. Meiotic crossovers were reduced in *Drosophila* rec, mei-217, and mei-218 mutants; however, removal of the Bloom syndrome helicase (BLM) orthologue restored crossovers. Thus, MCMs were co-opted into a novel complex that replaced the meiotic pro-crossover function of Msh4-Msh5 in flies.

16321. **Snyder, A. K., McLain, C. & Rio, R. V., 2012.** The tsetse fly obligate mutualist *Wigglesworthia morsitans* alters gene expression and population density via exogenous nutrient provisioning. *Applied & Environmental Microbiology*, **78** (21): 7792-7797.

Department of Biology, West Virginia University, Morgantown, West Virginia, USA. [rita.rio@mail.wvu.edu].

The obligate mutualist *Wigglesworthia morsitans* provisions nutrients to tsetse flies. The symbiont's response to thiamine (B_1) supplementation of blood meals specifically towards the regulation of thiamine biosynthesis and population density is described. Despite an ancient symbiosis and associated genome tailoring, *Wigglesworthia* responds to nutrient availability, potentially accommodating a decreased need.

16322. **Wang, J., Brelsfoard, C., Wu, Y. & Aksoy, S., 2012.** Intercommunity effects on microbiome and GpSGHV density regulation in tsetse flies. *Journal of Invertebrate Pathology*. **E Publication ahead of print**, **April 18.**

Division of Epidemiology of Microbial Disease, Yale School of Public Health, Yale University, New Haven, CT, USA. [serap.aksoy@yale.edu].

Tsetse flies have a highly regulated and defined microbial fauna made of 3 bacterial symbionts (obligate *Wigglesworthia glossinidia*, commensal *Sodalis glossinidius* and parasitic *Wolbachia pipientis*) in addition to a DNA virus (*Glossina pallidipes* salivary gland hypertrophy virus, GpSGHV). It has been possible to rear flies in the absence of either *Wigglesworthia* or in totally aposymbiotic state by dietary supplementation of tsetse's blood meal. In the absence of *Wigglesworthia*, tsetse females are sterile, and adult progeny are immune compromised. The functional contributions for *Sodalis* are less known, while *Wolbachia* cause reproductive manipulations known as cytoplasmic incompatibility (CI). High GpSGHV virus titres result in reduced fecundity and lifespan, and have compromised efforts to colonize flies in the insectary for large rearing purposes. Here we investigated the within-community effects on the density regulation of the individual microbiome partners in tsetse lines with different symbiotic compositions. We show that absence of *Wigglesworthia*

results in loss of *Sodalis* in subsequent generations possibly due to nutritional dependencies between the symbiotic partners. While an initial decrease in *Wolbachia* and GpSGHV levels is also noted in the absence of *Wigglesworthia*, these infections eventually reach homeostatic levels indicating adaptations to the new host immune environment or nutritional ecology. Absence of all bacterial symbionts also results in an initial reduction of viral titres, which recover in the second generation. Our findings suggest that in addition to the host immune system, interdependencies between symbiotic partners result in a highly tuned density regulation for tsetse's microbiome.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

16323. **Chrudimsky, T., Husnik, F., Novakova, E. & Hypsa, V., 2012.** *Candidatus Sodalis melophagi* sp. *nov*.: phylogenetically independent comparative model to the tsetse fly symbiont *Sodalis glossinidius*. *PLoS One*, **7** (7): e40354.

Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic. [tomas.chrudimsky@gmail.com].

Bacteria of the genus *Sodalis* live in symbiosis with various groups of insects. The best known member of this group, a secondary symbiont of tsetse flies *Sodalis glossinidius*, has become one of the most important models in investigating establishment and evolution of insect-bacteria symbiosis. It represents a bacterium in the early/intermediate state of the transition towards symbiosis, which allows for exploring such interesting topics as: usage of secretory systems for entering the host cell, tempo of the genome modification, and metabolic interaction with a coexisting primary symbiont. In this study, we describe a new *Sodalis* species which could provide a useful comparative model to the tsetse symbiont. It lives in association with *Melophagus ovinus*, an insect related to tsetse flies, and resembles *S. glossinidius* in several important traits. Similar to *S. glossinidius*, it cohabits the host with another symbiotic bacterium, the bacteriome-harboured primary symbiont of the genus *Arsenophonus.* As a typical secondary symbiont, *Candidatus Sodalis melophagi* infects various host tissues, including bacteriome. We provide basic morphological and molecular characteristics of the symbiont and show that these traits also correspond to the early/intermediate state of the evolution towards symbiosis. Particularly, we demonstrate the ability of the bacterium to live in insect cell culture as well as in cell-free medium. We also provide basic characteristics of a type three secretion system and using three reference sequences (16 S rDNA, groEL and spaPQR region) we show that the bacterium branched within the genus *Sodalis*, but originated independently of the two previously described symbionts of hippoboscoids. We propose the name *Candidatus Sodalis melophagi* for this new bacterium.

16324. **Doudoumis, V., Alam, U., Aksoy, E., Abd-Alla, A. M., Tsiamis, G., Brelsfoard, C., Aksoy, S. & Bourtzis, K., 2012.** Tsetse-*Wolbachia* symbiosis: comes of age and has great potential for pest and disease control. *Journal of Invertebrate Pathology*. **In press, available online July 23.**

Department of Environmental and Natural Resources Management, University of Ioannina, 2 Seferi St., 30100 Agrinio, Greece. [edoudoum@cc.uoi.gr].

Tsetse flies (Diptera: Glossinidae) are the sole vectors of African trypanosomes, the causative agent of sleeping sickness in human and nagana in animals. Like most eukaryotic organisms, *Glossina* species have established symbiotic associations with bacteria. Three main symbiotic bacteria have been found in tsetse flies: *Wigglesworthia glossinidia*, an obligate symbiotic bacterium, the secondary endosymbiont *Sodalis glossinidius* and the reproductive symbiont *Wolbachia pipientis*. In the present review, we discuss recent studies on the detection and characterization of *Wolbachia* infections in *Glossina* species, the horizontal transfer of *Wolbachia* genes to tsetse chromosomes, the ability of this symbiont to induce cytoplasmic incompatibility in *Glossina morsitans morsitans* and also how new environment-friendly tools for disease control could be developed by harnessing *Wolbachia* symbiosis.

16325. **Hamidou Soumana, I., Berthier, D., Tchicaya, B., Thevenon, S., Njiokou, F., Cuny, G. & Geiger, A., 2012.** Population dynamics of *Glossina palpalis gambiensis* symbionts, *Sodalis glossinidius* and *Wigglesworthia glossinidia*, throughout host-fly development. *Infection, Genetics & Evolution*, **13**: 41-48.

UMR 177, IRD-CIRAD, CIRAD TA A-17/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France. [anne.geiger@ird.fr].

The tsetse fly (Diptera: Glossinidae), the vector of trypanosomes causing human and animal trypanosomiasis, harbours symbiotic microorganisms including the primary symbiont *Wigglesworthia glossinidia*, involved in the fly's nutrition and fertility, and the secondary symbiont *Sodalis glossinidius*, involved in the trypanosome establishment in the fly's midgut. Both symbionts are maternally transmitted to the intrauterine progeny through the fly's milk gland secretions. In this study, we investigated the population dynamics of these symbionts during fly development. *Wigglesworthia* and *Sodalis* densities were estimated using quantitative PCR performed on *Glossina palpalis gambiensis* at different developmental stages. The results showed that the density of the primary *Wigglesworthia* symbiont was higher than that of *Sodalis* for all host developmental stages. *Sodalis* densities remained constant in pupae, but increased significantly in adult flies. The opposite situation was observed for *Wigglesworthia*, whose density increased in pupae and remained constant during the female adult stage. Moreover, *Wigglesworthia* density increased significantly during the transition from the pupal to the teneral stage, while mating had a contradictory effect depending on the age of the fly. Finally, tsetse fly colonization by both symbionts appears as a continuous and adaptive process throughout the insect's development. Last, the study demonstrated both symbionts of *G. p. gambiensis*, the vector of the chronic form of human African trypanosomiasis, to be permanent inhabitants of the colony flies throughout their life span. This was expected for the primary symbiont, *Wigglesworthia*, but not necessarily for the secondary symbiont, *S. glossinidius*, whose permanent presence is not required for the fly's survival. This result is of importance as *Sodalis* could be involved in the tsetse fly vector competence and may constitute a target in the frame of sleeping sickness fighting strategies.

16326. **Hamidou Soumana, I., Simo, G., Njiokou, F., Tchicaya, B., Abd-Alla, A. M., Cuny, G. & Geiger, A., 2012.** The bacterial flora of tsetse fly midgut and its effect on trypanosome transmission. *Journal of Invertebrate Pathology***. In press, available online July 25**.

UMR 177, IRD-CIRAD, CIRAD TA A-17/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France. [anne.geiger@ird.fr]

The tsetse fly, *Glossina palpalis* is a vector of the trypanosome that causes sleeping sickness in humans and nagana in cattle along with associated human health problems and massive economic losses. The insect is also known to carry a number of symbionts such as *Sodalis*, *Wigglesworthia* and *Wolbachia* whose effects on the physiology of the insect have been studied in depth. However, effects of other bacterial flora on the physiology of the host and vector competence have received little attention. Epidemiological studies on tsetse fly populations from different geographic sites revealed the presence of a variety of bacteria in the midgut. The most common of the flora belong to the genera *Entrobacter* (most common), *Enterococcus*, and *Acinetobacter.* It was a little surprising to find such diversity in the tsetse midgut since the insect is monophagous consuming vertebrate blood only. Diversity of bacteria is normally associated with polyphagous insects. In contrast to the symbionts, the role of resident midgut bacterial flora on the physiology of the fly and vector competence remains to be elucidated. With regard to *Sodalis glossinidius*, our data showed that flies harbouring this symbiont have three times greater probability of being infected by trypanosomes than flies without the symbiont. The data delineated in these studies underscore the need to carry out detailed investigations on the role of resident bacteria on the physiology of the fly and vector competence.

16327. **Hyseni, C., Kato, A. B., Okedi, L. M., Masembe, C., Ouma, J. O., Aksoy, S. & Caccone, A., 2012.** The population structure of *Glossina fuscipes fuscipes* in the Lake Victoria basin in Uganda: implications for vector control. *Parasites & Vectors*, **5**: 222.

Department of Ecology and Evolutionary Biology, Yale University, 21 Sachem Street, New Haven, CT, USA. [chaz.hyseni@aya.yale.edu].

Glossina fuscipes fuscipes is the primary vector of trypanosomiasis in humans and livestock in Uganda. The Lake Victoria basin has been targeted for tsetse eradication using a rolling carpet initiative, from west to east, with four operational blocks (three in Uganda and one in Kenya), under the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). We screened tsetse flies from the three Ugandan PATTEC blocks for genetic diversity at 15 microsatellite loci from continental and offshore populations to provide empirical data to support this initiative. We collected tsetse samples from 11 sites across the Lake Victoria basin in Uganda. We performed genetic analyses on 409 of the collected tsetse flies and added data collected for 278 individuals in a previous study. The flies were screened across 15 microsatellite loci and the resulting data were used to assess the temporal stability of populations, to analyse patterns of genetic exchange and structuring, to estimate dispersal rates and evaluate the sex bias in dispersal, as well as to estimate demographic parameters (NE and NC). We found that tsetse populations in this region were stable over 4-16 generations and belong to four genetic clusters. Two genetic clusters (1 and 2) corresponded approximately to PATTEC blocks 1 and 2, while the other two (3 and 4) fell within PATTEC block 3. Island populations grouped into the same genetic clusters as neighbouring mainland sites, suggested gene flow between these sites. There was no evidence of the stretch of water separating islands from the mainland forming a significant barrier to dispersal. Dispersal rates ranged from 2.5 km per generation in cluster 1 to 14 km per generation in clusters 3 and 4. We found evidence of male-biased dispersal. Few breeders are successfully dispersing over

large distances. Effective population size estimates were low (33-310 individuals), while census size estimates ranged from 1 200 (cluster 1) to 4 100 (clusters 3 and 4). We present here a novel technique that adapts an existing census size estimation method to sampling without replacement, the scheme used in sampling tsetse flies. Our study suggests that different control strategies should be implemented for the three PATTEC blocks and that, given the high potential for re-invasion from island sites, mainland and offshore sites in each block should be targeted at the same time.

16328. **Kaba, D., Ravel, S., Acapovi-Yao, G., Solano, P., Allou, K., Bosson-Vanga, H., Gardes, L., N'Goran E, K., Schofield, C. J., Kone, M. & Dujardin, J. P., 2012.** Phenetic and genetic structure of tsetse fly populations (*Glossina palpalis palpalis*) in southern Ivory Coast. *Parasites & Vectors*, **5**: 153.

IRD, UMR 5090 MIVEGEC, Avenue Agropolis, IRD, Montpellier, France. [dujardinbe@gmail.com].

Sleeping sickness, transmitted by *G. p. palpalis*, is known to be present in the Ivory Coast. *G. p. palpalis* has recently been reported to occur in several places within the town of Abidjan, including: (i) the Banco forest, (ii) the Abobo Adjame University campus and (iii) the zoological park. Could these three places be treated sequentially, as separate tsetse populations, or should they be taken as one area comprising a single, panmictic population? The amount of gene flow between these places provides strategic information for vector control. It was estimated by the use of both genetic (microsatellite DNA) and morphometric markers. The idea was to assess the interest of the faster and much less expensive morphometric approach in providing relevant information about population structure. Thus, to detect possible lack of insect exchange between these neighbouring areas of Abidjan, we used both genetic (microsatellite DNA) and phenetic (geometric morphometrics) markers on the same specimens. Using these same markers, we also compared these samples with specimens from a more distant area of south Ivory Coast, the region of Aniassue (186 km north from Abidjan). Neither genetic nor phenetic markers detected significant differentiation between the three Abidjan *G. p. palpalis* samples. Thus, the null hypothesis of a single panmictic population within the city of Abidjan could not be rejected, suggesting the control strategy should not consider them separately. The markers were also in agreement when comparing *G. p. palpalis* from Abidjan with those of Aniassue, showing significant divergence between the two sites. In conclusion, both markers suggested that a successful control of tsetse in Abidjan would require the three Abidjan sites to be considered together, either by deploying control measures simultaneously in all three sites, or by a continuous progression of interventions following for instance the "rolling carpet" principle. To compare the geometry of wing venation of tsetse flies is a cheap and fast technique. Agreement with the microsatellite approach highlights its potential for rapid assessment of population structure.

16329. **Maltz, M. A., Weiss, B. L., O'Neill, M., Wu, Y. & Aksoy, S., 2012.** OmpA-mediated biofilm formation is essential for the commensal bacterium *Sodalis glossinidius* to colonize the tsetse fly gut. *Applied & Environmental Microbiology*, **78** (21): 7760-7768.

Yale School of Public Health, Department of Epidemiology of Microbial Diseases, New Haven, Connecticut, USA. [serap.aksoy@yale.edu].

Many bacteria successfully colonize animals by forming protective biofilms. Molecular processes that underlie the formation and function of biofilms in pathogenic bacteria are well characterized. In contrast, the relationship between biofilms and host colonization by symbiotic bacteria is less well understood. Tsetse flies (*Glossina* spp.) house three maternally transmitted symbionts, one of which is a commensal (*Sodalis glossinidius*) found in several host tissues, including the gut. We determined that *Sodalis* forms biofilms in the tsetse gut and that this process is influenced by the *Sodalis* outer membrane protein A (OmpA). Mutant *Sodalis* strains that do not produce OmpA (*Sodalis* DeltaOmpA mutants) fail to form biofilms *in vitro* and are unable to colonize the tsetse gut unless endogenous symbiotic bacteria are present. Our data indicate that in the absence of biofilms, *Sodalis* DeltaOmpA mutant cells are exposed to and eliminated by tsetse's innate immune system, suggesting that biofilms help *Sodalis* evade the host immune system. Tsetse is the sole vector of pathogenic African trypanosomes, which also reside in the fly gut. Acquiring a better understanding of the dynamics that promote *Sodalis* colonization of the tsetse gut may enhance the development of novel disease control strategies.

16330. **Mediannikov, O., Audoly, G., Diatta, G., Trape, J. F. & Raoult, D., 2012.** New *Rickettsia* sp. in tsetse flies from Senegal. *Comparative Immunology, Microbiology & Infectious Diseases*, **35** (2): 145-150.

Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, Université de la Méditerranée, Marseille, France. [didier.raoult@gmail.com]

Tsetse flies are blood-sucking insects transmitting African trypanosomiasis. They are known to harbour also three intracellular bacteria that play important role in their lifecycle: *Wigglesworthia glossinidia*, *Sodalis glossinidius* and *Wolbachia* sp. We have studied 78 *Glossina morsitans submorsitans* collected in Senegal. In all studied flies we amplified genes of bacterium phylogenetically close to obligate intracellular pathogen *Rickettsia felis*, the agent of spotted fever in humans. We also visualized this rickettsia in the cells of tsetse flies by fluorescence *in situ* hybridization. The role of this probable fourth endosymbiotic bacterium of tsetse flies in *Glossina* lifecycle and possible pathogenicity for humans should be further investigated.

16331. **Salou, E., Rayaisse, J. B., Laveissiere, C., Sanon, A. & Solano, P., 2012.** Behavioural interactions and rhythms of activity of *Glossina palpalis gambiensis* and *G. tachinoides* (Diptera: Glossinidae) in forest gallery in the Burkina Faso. *Parasite*, **19** (3): 217-225.

CIRDES URBIO, LAMIVECT, 01 BP 454 Bobo-Dioulasso 01, Burkina Faso.

Glossina palpalis gambiensis and *G. tachinoides* are the main vectors of human and animal trypanosomoses in West Africa. In some parts of their distribution area, they co-exist in sympatry, but little is known about their interactions. This study aimed to explore their respective flight height and daily activity when co-existing or alone. Attractive targets were used, made of a black/blue/black cloth covered with adhesive film, so that all tsetse that landed were caught. The study was conducted in two areas in south Burkina Faso: Kartasso, upstream the Mouhoun river, where *G. p. gambiensis* is the only tsetse occurring; and

Folonzo, on the Comoe river, where both species occur. Out of more than 3 800 tsetse flies caught in total, in Folonzo, *G. tachinoides* occurred at higher densities than *G. p. gambiensis* (84 percent vs 16 percent of the total densities). The mean height of capture was 55 cm for *G. tachinoides*, and 65 cm for *G. p. gambiensis*. As a comparison, in Kartasso where *G. p. gambiensis* is alone, the mean height of capture was 46 cm, these differences being statistically significant. On average, females were caught higher in altitude than males, and the two species showed a similar activity profile in the day. These results are discussed in the light of differences in the nature of the forest gallery, or possible interspecies competition behaviour in relation with their limited energy metabolism and flight capacities, or also in relation with species differences in landing behaviour, linked to host feeding detection. These observations have consequences on control tools releasing attractive odours, which may have contrasted efficacy depending on the flight height of the species.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also **35:** 16309, 16315, 16316, 16317, 16324, 16327, 16341]

16332. **Childs, S. J., 2012.** A set of discrete formulae for the performance of a tsetse population during aerial spraying. *Acta Tropica,* **125** (2): 202-213.

Department of Mathematics and Applied Mathematics, University of the Free State, P.O. Box 339, Bloemfontein, 9300, South Africa. [simonjohnchilds@gmail.com].

A set of discrete formulae that calculates the hypothetical impact of aerial spraying on a tsetse population is derived and the work is thought to be novel. Both the original population and the subsequent generations which survive the aerial spraying may ultimately be thought of as deriving from two, distinct sources. These origins are, however, neither distinct, nor relevant by the third generation. It is for this reason that the female population is considered to be composed of the following four categories for the purposes of derivation: Original flies which existed as such at the commencement of spraying; original pupae which existed as such at the commencement of spraying; the immediate descendants of both the aforementioned categories, during spraying; third and higher generation descendants. In theory, the latter category is a recurrence relation. In practice, the third generation's pupal stage has hardly come into existence, even by the end of a completed operation. Implicit in the formulae is the assumption of one, temperature-dependent mortality rate for the entire pupal stage, a second for the period between eclosion and ovulation and yet a third for the entire, adult life-span. Gravid female resistance to the insecticide is assumed to be inconsequential. A further assumption of the formulae is that at least one male is always available (degree of sterility variable).

16333. **Maniania, N. K. & Ekesi, S., 2012.** The use of entomopathogenic fungi in the control of tsetse flies. *Journal of Invertebrate Pathology*. **E Publication ahead of print, July 19.**

International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772, Nairobi, Kenya. [nmaniania@icipe.org].

Tsetse flies harbour a number of pathogens in nature; but their potential as biological control agents has not been fully exploited, especially due to the difficulty of their application in the field. Since entomopathogenic fungi infect their target organisms through the cuticle, it has been possible to develop a device that delivers and autodisseminates inoculum among tsetse in the field, resulting in population reduction, comparable to mass-trapping technology. However, the success of this technology depends on the effective horizontal transmission of the inoculum between insects. We present an overview of the prospects of entomopathogenic fungi for the control of tsetse flies and highlight the challenges.

16334. **Peck, S. L. & Bouyer, J., 2012.** Mathematical modelling, spatial complexity, and critical decisions in tsetse control. *Journal of Economic Entomology*, **105** (5): 1477-1486.

Biology Department, Brigham Young University, Provo, UT 84602, USA. [slp3141@gmail.com]

The tsetse fly complex (*Glossina* spp.) is widely recognized as a key contributor to the African continent's continuing struggle to emerge from deep economic, social, and political problems. Vector control, the backbone of intensive efforts to remove the human and livestock trypanosomosis problem, has been typified by spectacular successes and failures. There is widespread agreement that integrated vector control, combined with direct disease treatment and prevention, has to play a major role in alleviating the tsetse burden in Africa. Mathematical and computer-based simulation models have been extensively used to try to understand how best to manage these control efforts. Such models in ecology have been helpful in giving broad generalizations about population dynamics and control. Unfortunately, in many ways they have inadequately addressed key aspects of the fly's biology and ecology, particularly the spatio-temporal variability of its habitats. These too must factor in any control efforts. Mathematical models have inherent limitations that must be considered in their use for control programs. In this review, we consider some of the controversies being debated within the field of ecology and evolution about the use of mathematical models and critically review several models that have been influential in structuring tsetse control efforts. We also make recommendations on the appropriate role that mathematical and simulation models should play when used for these purposes. Management programmes are often vulnerable to naively using these models inappropriately. The questions raised in this review will apply broadly to many conservation and area-wide pest control programmes with an ecological component relying on mathematical and computer simulation models to inform their decisions.

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

[See also **35:** 16389]

16335. **Auty, H., Anderson, N. E., Picozzi, K., Lembo, T., Mubanga, J., Hoare, R., Fyumagwa, R. D., Mable, B., Hamill, L., Cleaveland, S. & Welburn, S. C., 2012.** Trypanosome diversity in wildlife species from the Serengeti and Luangwa Valley ecosystems. *PLoS Neglected Tropical Diseases*, **6** (10): e1828.

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

The importance of wildlife as reservoirs of African trypanosomes pathogenic to man and livestock is well recognised. While new species of trypanosomes and their variants have been identified in tsetse populations, our knowledge of trypanosome species that are circulating in wildlife populations and their genetic diversity is limited. Molecular phylogenetic methods were used to examine the genetic diversity and species composition of trypanosomes circulating in wildlife from two ecosystems that exhibit high host species diversity: the Serengeti in Tanzania and the Luangwa Valley in Zambia. Phylogenetic relationships were assessed by alignment of partial 18S, 5.8S and 28S trypanosomal nuclear ribosomal DNA array sequences within the Trypanosomatidae and using ITS1, 5.8S and ITS2 for more detailed analysis of the *T. vivax* clade. In addition to *Trypanosoma brucei*, *T. congolense*, *T. simiae*, *T. simiae* (Tsavo), *T. godfreyi* and *T. theileri*, three variants of *T. vivax* were identified from three different wildlife species within one ecosystem, including sequences from trypanosomes from a giraffe and a waterbuck that differed from all published sequences and from each other, and did not amplify with conventional primers for *T. vivax*. Wildlife carries a wide range of trypanosome species. The failure of the diverse *T. vivax* in this study to amplify with conventional primers suggests that *T. vivax* may have been under-diagnosed in Tanzania. Since conventional species-specific primers may not amplify all trypanosomes of interest, the use of ITS PCR primers followed by sequencing is a valuable approach to investigate diversity of trypanosome infections in wildlife; amplification of sequences outside the *T. brucei* clade raises concerns regarding ITS primer specificity for wildlife samples if sequence confirmation is not also undertaken.

16336. **Chitanga, S., Namangala, B., De Deken, R. & Marcotty, T., 2013.** Shifting from wild to domestic hosts: the effect on the transmission of *Trypanosoma congolense* to tsetse flies. *Acta Tropica*, **125** (1): 32-36.

Institute of Tropical Medicine Antwerp, Department of Biomedical Sciences, Nationalestraat 155, B-2000 Antwerp, Belgium; and Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, Great East Road Campus, Lusaka, Zambia. [tmarcotty@itg.be].

The epidemiology and impact of animal African trypanosomosis are influenced by the transmissibility and the pathogenicity of the circulating trypanosome strains in a particular biotope. The transmissibility of 22 *Trypanosoma congolense* strains isolated from domestic and wild animals was evaluated in a total of 1 213 flies. Multivariate mixed models were used to compare infection and maturation rates in function of trypanosome origin (domestic or sylvatic) and pathogenicity. Both trypanosome pathogenicity and origin significantly affected the ability to establish a midgut infection in tsetse flies but not the maturation rates. The interaction between pathogenicity and origin was not significant. Since being pathogenic and having a domestic origin both increased transmissibility, dominant lowly pathogenic trypanosomes from domestic environments and highly pathogenic trypanosomes from sylvatic environments presented similar levels of transmissibility: 12 percent and 15 percent, respectively. Blood meals with parasite concentration ranging from 0.05 to 50 trypanosomes/µL blood for three strains of *T. congolense* were provided to different batches of tsetse flies to evaluate the relationship between the parasite load in blood meals and the

likelihood for a fly to become infected. A linear relationship between parasite load and transmissibility was observed at low parasitaemia and a plateau was observed for meals containing more than five trypanosomes/µL. Maximum transmission was reached with 12.5 trypanosomes/µL blood. About 50 percent of the flies were refractory to *T. congolense*, whatever their concentration in the blood meal. The results suggest that the dose-transmissibility relationship presents a similar profile for different *T. congolense* isolates.

16337. **Funk, S., Nishiura, H., Heesterbeek, H., Edmunds, W. J. & Checchi, F. 2013.** Identifying transmission cycles at the human-animal interface: the role of animal reservoirs in maintaining *gambiense* human African trypanosomiasis. *PloS Computational Biology*, **9(1):** e1002855.

Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey, USA [sf7@princeton.edu].

Many infections can be transmitted between animals and humans. The epidemiological roles of different species can vary from important reservoirs to dead-end hosts. Here, we present a method to identify transmission cycles in different combinations of species from field data. We used this method to synthesise epidemiological and ecological data from Bipindi, Cameroon, a historical focus of *gambiense* human African trypanosomiasis (HAT, sleeping sickness), a disease that has often been considered to be maintained mainly by humans. We estimated the basic reproduction number R_0 of *gambiense* HAT in Bipindi and evaluated the potential for transmission in the absence of human cases. We found that that under the assumption of random mixing between vectors and hosts, *gambiense* HAT could not be maintained in this focus without the contribution of animals. This result remains robust under extensive sensitivity analysis. When using the distributions of species among habitats to estimate the amount of mixing between those species, we found indications for an independent transmission cycle in wild animals. Stochastic simulation of the system confirmed that unless vectors moved between species very rarely, reintroduction would usually occur shortly after elimination of the infection from human populations. This suggests that elimination strategies may have to be reconsidered as targeting human cases alone would be insufficient for control, and reintroduction from animal reservoirs would remain a threat. Our approach is broadly applicable and could reveal animal reservoirs critical to the control of other infectious diseases.

16338. **Hoppenheit, A., Bauer, B., Steuber, S., Terhalle, W., Diall, O., Zessin, K. H. & Clausen, P. H., 2012.** Multiple host feeding in *Glossina palpalis gambiensis* and *Glossina tachinoides* in southeast Mali. *Medical & Veterinary Entomology.* **Published online September 25.**

Department of Veterinary Medicine, Institute for Parasitology and Tropical Veterinary Medicine, Free University of Berlin, Berlin, Germany; Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany; Food and Agriculture Organization of the United Nations (FAO), Accra, Ghana; and Department of Veterinary Medicine, International Animal Health, Free University of Berlin, Berlin, Germany. [antje_hoppenheit@yahoo.de].

Changes in agricultural practices and the resulting extinction of wildlife have led to the

reduction or disappearance of savannah tsetse species. Riparian tsetse such as *Glossina palpalis gambiensis* Vanderplank 1949 and *Glossina tachinoides* Westwood 1850 (Diptera: Glossinidae) continue to persist in peridomestic sites, transmitting trypanosomiasis. At present, little is known about interspecies differences in feeding behaviour in these two species in southeast Mali, or of the phenomenon of multiple blood meals. To study these topics, 279 samples of *G. p. gambiensis* and *G. tachinoides* containing host DNA, caught in the Sikasso region between November 2008 and April 2009, were analysed by applying host species-specific primers and sequencing. Humans accounted for > 66 percent of *G. p. gambiensis* blood meals, whereas *G. tachinoides* contained equal parts DNA of human, cattle or both, showing a significantly higher proportion of multiple host use. Further, the trypanosome infection rate was found to be three-fold higher in *G. tachinoides*. Logistic regression analysis revealed double-feeding and infection to be independent of one another, but showed infection to be correlated with engorgement in *G. p. gambiensis* and female sex in *G. tachinoides*. Enhanced host-seeking activities paired with the high trypanosome infection rate found in *G. tachinoides* would indicate that this species has a higher vectorial capacity than *G. p. gambiensis*.

16339. **Kolev, N. G., Ramey-Butler, K., Cross, G. A., Ullu, E. & Tschudi, C., 2012.** Developmental progression to infectivity in *Trypanosoma brucei* triggered by an RNA-binding protein. *Science*, **338** (6112): 1352-1353.

Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT 06536, USA. [elisabetta.ullu@yale.edu].

Unravelling the intricate interactions between *Trypanosoma brucei*, the protozoan parasite causing African trypanosomiasis, and the tsetse (*Glossina*) vector remains a challenge. Metacyclic trypanosomes, which inhabit the tsetse salivary glands, transmit the disease and are produced through a complex differentiation and unknown programme. By overexpressing a single RNA-binding protein TbRBP6 in cultured noninfectious trypanosomes, we recapitulated the developmental stages that have been observed in tsetse, including the generation of infective metacyclic forms expressing the variant surface glycoprotein. Thus, events leading to acquisition of infectivity in the insect vector are now accessible to laboratory investigation, providing an opening for new intervention strategies.

16340. **Morand, S., Renggli, C. K., Roditi, I. & Vassella, E., 2012.** MAP kinase kinase 1 (MKK1) is essential for transmission of *Trypanosoma brucei* by *Glossina morsitans*. *Molecular & Biochemical Parasitology*, **186** (1): 73-76.

Institut fur Zellbiologie, Universitat Bern, Switzerland. [isabel.roditi@izb.unibe.ch].

MAP kinase kinase 1 (MKK1) is encoded by a single copy gene in *Trypanosoma brucei*. It has been shown recently that MKK1 is not essential for bloodstream forms. To investigate the requirement for MKK1 in other life-cycle stages we generated null mutants in procyclic forms of a fly-transmissible strain. These grew normally in culture and were able to establish midgut infections in tsetse at normal rates and intensities, but were incapable of colonising the salivary glands. Transformation of null mutants with an ectopic copy of MKK1 enabled parasites to complete the life cycle in tsetse and infect mice. This is the first example of a gene that is indispensable for transmission of *T. brucei*. It also raises the possibility that

activating the MKK1 signalling cascade *in vitro* might trigger the differentiation and proliferation of life-cycle stages of *T. brucei* that are currently refractory to culture.

16341. **Munang'andu, H. M., Siamudaala, V., Munyeme, M. & Nalubamba, K. S., 2012.** A review of ecological factors associated with the epidemiology of wildlife trypanosomiasis in the Luangwa and Zambezi valley ecosystems of Zambia. *Interdisciplinary Perspectives on Infectious Diseases*, **2012**: 372523.

Section of Aquatic Medicine and Nutrition, Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Sciences, Ullevalsveien 72, P.O. Box 8146 Dep, 0033 Oslo, Norway; Kavango Zambezi Transfrontier Conservation Area Secretariat, Kasane 821, Gaborone, Botswana; Department of Disease Control, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka 10101, Zambia; and Department of Clinical Studies, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka 10101, Zambia. [hetron2002@yahoo.co.uk].

Trypanosomiasis has been endemic in wildlife in Zambia for more than a century. The disease has been associated with neurological disorders in humans. Current conservation strategies by the Zambian government of turning all game reserves into state-protected National Parks (NPs) and game management areas (GMAs) have led to the expansion of the wildlife and tsetse population in the Luangwa and Zambezi valley ecosystem. This ecological niche lies in the common tsetse fly belt that harbours the highest tsetse population density in Southern Africa. Ecological factors such as climate, vegetation and rainfall found in this niche allow for a favourable interplay between wild reservoir hosts and vector tsetse flies. These ecological factors that influence the survival of a wide range of wildlife species provide adequate habitat for tsetse flies thereby supporting the coexistence of disease reservoir hosts and vector tsetse flies leading to prolonged persistence of trypanosomiasis in the area. On the other hand, increase in anthropogenic activities poses a significant threat of reducing the tsetse and wildlife habitat in the area. Here, we demonstrate that while conservation of wildlife and biodiversity is an important preservation strategy of natural resources, it could serve as a long-term reservoir of wildlife trypanosomiasis.

16342. **Nakayima, J., Nakao, R., Alhassan, A., Mahama, C., Afakye, K. & Sugimoto, C., 2012.** Molecular epidemiological studies on animal trypanosomiases in Ghana. *Parasites & Vectors*, **5**: 217.

Division of Collaboration and Education, Research Center for Zoonosis Control, Hokkaido University, Kita 20, Nishi 10, Kita-ku, Sapporo, Hokkaido 001-0020, Japan. [sugimoto@czc.hokudai.ac.jp].

African trypanosomes are extracellular protozoan parasites that are transmitted between mammalian hosts by the bite of an infected tsetse fly. Human African trypanosomiasis (HAT) or sleeping sickness is caused by *Trypanosoma brucei rhodesiense* or *T. brucei gambiense*, while African animal trypanosomiasis (AAT) is caused mainly by *T. vivax*, *T. congolense*, *T. simiae, T. evansi* and *T. brucei brucei*. Trypanosomiasis is of public health importance in humans and is also the major constraint for livestock productivity in sub-Saharan African countries. Scanty information exists about the trypanosomiasis status in Ghana especially

regarding molecular epidemiology. Therefore, this study intended to apply molecular tools to identify and characterize trypanosomes in Ghana. A total of 219 tsetse flies, 248 pigs and 146 cattle blood samples were collected from Adidome and Koforidua regions in Ghana in 2010. Initial PCR assays were conducted using the internal transcribed spacer one (ITS1) of ribosomal DNA (rDNA) primers, which can detect most of the pathogenic trypanosome species and *T. vivax*-specific cathepsin L-like gene primers. In addition, species- or subgroup-specific PCRs were performed for *T. b. rhodesiense*, *T. b. gambiense*, *T. evansi* and three subgroups of *T. congolense*. The overall prevalences of trypanosomes were 17.4 percent (38/219), 57.5 percent (84/146) and 28.6 percent (71/248) in tsetse flies, cattle and pigs, respectively. *T. congolense* subgroup-specific PCR revealed that *T. congolense* savannah (52.6 percent) and *T. congolense* forest (66.0 percent) were the endemic subgroups in Ghana with 18.6 percent being mixed infections. *T. evansi* was detected in a single tsetse fly. Human infective trypanosomes were not detected in the tested samples. Our results showed that there is a high prevalence of parasites in both tsetse flies and livestock in the study areas in Ghana. This enhances the need to strengthen control policies and institute measures that help prevent the spread of the parasites.

16343. **Pagabeleguem, S., Sangare, M., Bengaly, Z., Akoudjin, M., Belem, A. M. & Bouyer, J., 2012.** Climate, cattle rearing systems and African animal trypanosomosis risk in Burkina Faso. *PLoS One*, **7** (11): e49762.

Centre International de Recherche-Développement sur l'Elevage en Zone subhumide (CIRDES), Bobo-Dioulasso, Burkina Faso. [bouyer@cirad.fr].

In sub-Saharan countries infested by tsetse flies, African animal trypanosomosis (AAT) is considered as the main pathological constraint to cattle breeding. Africa has known a strong climatic change and its population increased four-fold during the last half century. The aim of this study was to characterize the impact of production practices and climate on tsetse occurrence and abundance, and the associated prevalence of AAT in Burkina Faso. Four sites were selected along a south-north transect of increasing aridity. The study combined parasitological and entomological surveys. For the parasitological aspect, blood samples were collected from 1 041 cattle selected through a stratified sampling procedure including location and livestock management system (long transhumance, short transhumance, sedentary). Parasitological and serological prevalences specific to livestock management systems showed a gradual increase from the Sahelian to the Sudano-Guinean area ($p<0.05$). Livestock management system had also a significant impact on parasitological prevalence ($p<0.05$). Tsetse diversity, apparent densities and their infection rates overall decreased with aridity, from four species, an apparent density of 53.1 flies/trap/day and an infection rate of 13.7 percent to an absence at the northern edge of the transect, where the density and diversity of other biting flies were on the contrary highest ($p<0.001$). Climatic pressure clearly had a negative impact on tsetse abundance and AAT risk. However, the persistence of tsetse habitats along the Mouhoun river loop maintains a high risk of cyclical transmission of *T. vivax*. Moreover, an "epidemic mechanical livestock trypanosomosis" cycle is likely to occur in the northern site, where trypanosomes are brought in by cattle moving from the tsetse infested area and are locally transmitted by mechanical vectors. In Burkina Faso, the impact of tsetse thus extends to a buffer area around their distribution belt, corresponding to the herd transhumance radius.

16344. **Simo, G., Silatsa, B., Flobert, N., Lutumba, P., Mansinsa, P., Madinga, J., Manzambi, E., De Deken, R. & Asonganyi, T., 2012.** Identification of different trypanosome species in the mid-guts of tsetse flies of the Malanga (Kimpese) sleeping sickness focus of the Democratic Republic of Congo. *Parasites & Vectors*, **5**: 201.

Department of Biochemistry, University of Dschang, Dschang, Cameroon. [gsimoca@yahoo.fr].

The Malanga sleeping sickness focus of the Democratic Republic of Congo has shown an epidemic evolution of disease during the last century. However, following case detection and treatment, the prevalence of the disease decreased considerably. No active survey has been undertaken in this focus for a couple of years. To understand the current epidemiological status of sleeping sickness as well as of animal African trypanosomiasis in the Malanga focus, we undertook the identification of tsetse blood meals as well as different trypanosome species in flies trapped in this focus. Pyramidal traps were used to trap tsetse flies. All flies caught were identified and live flies were dissected and their mid-guts collected. Fly mid-gut was used for the molecular identification of the blood meal source, as well as for the presence of different trypanosome species. About 949 *Glossina palpalis palpalis* were trapped; 296 (31.2 percent) of which were dissected, 60 (20.3 percent) blood meals were collected and 57 (19.3 percent) trypanosome infections identified. The infection rates were 13.4 percent, 5.1 percent, 3.5 percent and 0.4 percent for *Trypanosoma congolense* savannah type, *Trypanosoma brucei* s.l., *Trypanosoma congolense* forest type and *Trypanosoma vivax*, respectively. Three mixed infections including *Trypanosoma brucei* s.l. and *Trypanosoma congolense* savannah type, and one mixed infection of *Trypanosoma vivax* and *Trypanosoma congolense* savannah type were identified. Eleven *Trypanosoma brucei gambiense* infections were identified; indicating an active circulation of this trypanosome subspecies. Of all the identified blood meals, about 58.3 percent were identified as being taken on pigs, while 33.3 percent and 8.3 percent were from man and other mammals, respectively. The presence of *Trypanosoma brucei* in tsetse mid-guts associated with human blood meals is indicative of an active transmission of this parasite between tsetse and man. The considerable number of pig blood meals combined with the circulation of *Trypanosoma brucei gambiense* in this focus suggests a transmission cycle involving humans and domestic animals and could hamper eradication strategies. The various species of trypanosomes identified in the Malanga sleeping sickness focus indicate the coexistence of animal and human African trypanosomiasis. The development of new strategies integrating control measures for human and animal trypanosomiasis may enable the reduction of control costs in this locality.

16345. **Torr, S. J., Chamisa, A., Mangwiro, T. N. & Vale, G. A., 2012.** Where, when and why do tsetse contact humans? Answers from studies in a national park of Zimbabwe. *PLoS Neglected Tropical Diseases*, **6** (8): e1791.

Natural Resources Institute, University of Greenwich, Chatham, UK. [s.torr@greenwich.ac.uk].

Sleeping sickness, also called human African trypanosomiasis, is transmitted by the tsetse, a blood-sucking fly confined to sub-Saharan Africa. The form of the disease in West and Central Africa is carried mainly by species of tsetse that inhabit riverine woodland and feed avidly on humans. In contrast, the vectors for the East and Southern African form of the

disease are usually savannah species that feed mostly on wild and domestic animals and bite humans infrequently, mainly because the odours produced by humans can be repellent. Hence, it takes a long time to catch many savannah tsetse from people, which in turn means that studies of the nature of contact between savannah tsetse and humans, and the ways of minimizing it, have been largely neglected. The savannah tsetse, *Glossina morsitans morsitans* and *G. pallidipes*, were caught from men in the Mana Pools National park of Zimbabwe. Mostly the catch consisted of young *G. m. morsitans*, with little food reserve. Catches were increased by 4-8 times if the men were walking, not stationary, and increased about ten times more if they rode on a truck at 10 km/h. Catches were unaffected if the men used deodorant or were baited with artificial ox odour, but declined by about 95 percent if the men were with an ox. Surprisingly, men pursuing their normal daily activities were bitten about as much when in or near buildings as when in woodland. Catches from oxen and a standard ox-like trap were poor indices of the number and physiological state of tsetse attacking men. The search for new strategies to minimize the contact between humans and savannah tsetse should focus on that occurring in buildings and vehicles. There is a need to design a man-like trap to help to provide an index of sleeping sickness risk.

16346. **Wardrop, N. A., Fevre, E. M., Atkinson, P. M., Kakembo, A. S. & Welburn, S. C., 2012.** An exploratory GIS-based method to identify and characterise landscapes with an elevated epidemiological risk of Rhodesian human African trypanosomiasis. *BMC Infectious Diseases*, **12**: 316.

Geography and Environment, University of Southampton, Highfield Campus, Southampton, SO17 1BJ, UK. [Nicola.Wardrop@soton.ac.uk].

Specific land cover types and activities have been correlated with *Trypanosoma brucei rhodesiense* distributions, indicating the importance of landscape for epidemiological risk. However, methods proposed to identify specific areas with elevated epidemiological risk (i.e. where transmission is more likely to occur) tend to be costly and time consuming. This paper proposes an exploratory spatial analysis using geo-referenced human African trypanosomiasis (HAT) cases and matched controls from Serere hospital, Uganda (December 1998 to November 2002) to identify areas with an elevated epidemiological risk of HAT. Buffers 3 km from each case and control were used to represent areas in which village inhabitants would carry out their daily activities. It was hypothesised that the selection of areas where several case village buffers overlapped would enable the identification of locations with increased risk of HAT transmission, as these areas were more likely to be frequented by HAT cases in several surrounding villages. The landscape within these overlap areas should more closely relate to the environment in which transmission occurs as opposed to using the full buffer areas. The analysis was carried out for each of four annual periods, for both cases and controls, using a series of threshold values (number of overlapping buffers), including a threshold of one, which represented the benchmark (e.g. use of the full buffer area as opposed to the overlap areas). A greater proportion of the overlap areas for cases consisted of seasonally flooding grassland and lake fringe swamp than the control overlap areas, correlating well with the preferred habitat of the predominant tsetse species within the study area (*Glossina fuscipes fuscipes*). The use of overlap areas also resulted in a greater difference between case and control landscapes, when compared with the benchmark (using the full buffer area). These results indicate that the overlap analysis has enabled the selection of areas more likely to represent epidemiological risk zones than similar analyses using full buffer

areas. The identification of potential epidemiological risk zones using this method requires fewer data than other proposed methods and further development may provide vital information for the targeting of control measures.

5. HUMAN TRYPANOSOMOSIS

(a) SURVEILLANCE

[See also **35**: 16337, 16345, 16346, 16388]

16347. **Checchi, F., Cox, A. P., Chappuis, F., Priotto, G., Chandramohan, D. & Haydon, D. T., 2012.** Prevalence and under-detection of *gambiense* human African trypanosomiasis during mass screening sessions in Uganda and Sudan. *Parasites & Vectors*, **5**: 157.

Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK. [francesco.checchi@lshtm.ac.uk].

Active case detection through mass community screening is a major control strategy against human African trypanosomiasis (HAT, sleeping sickness) caused by *T. brucei gambiense*. However, its impact can be limited by incomplete attendance at screening sessions (screening coverage) and diagnostic inaccuracy. We developed a model-based approach to estimate the true prevalence and the fraction of cases detected during mass screening based on observed prevalence, and adjusting for incomplete screening coverage and inaccuracy of diagnostic algorithms for screening, confirmation and HAT stage classification. We applied the model to data from three Médecins Sans Frontières projects in Uganda (Adjumani, Arua-Yumbe) and Southern Sudan (Kiri). We analysed 604 screening sessions, targeting about 710 000 people. Cases were about twice as likely to attend screening as non-cases, with no apparent difference by stage. Past incidence, population size and repeat screening rounds were strongly associated with observed prevalence. The estimated true prevalence was 0.46 percent to 0.90 percent in Kiri depending on the analysis approach, compared to an observed prevalence of 0.45 percent; 0.59 percent to 0.87 percent in Adjumani, compared to 0.92 percent; and 0.18 percent to 0.24 percent in Arua-Yumbe, compared with 0.21 percent. The true ratio of stage 1 to stage 2 cases was around two-three times higher than that observed due to stage misclassification. The estimated detected fraction was between 42.2 percent and 84.0 percent in Kiri, 52.5 percent to 79.9 percent in Adjumani and 59.3 percent to 88.0 percent in Arua-Yumbe. In these well-resourced projects, a moderate to high fraction of cases appeared to be detected through mass screening. True prevalence differed little from observed prevalence for monitoring purposes. We discuss some limitations to our model that illustrate several difficulties of estimating the unseen burden of neglected tropical diseases.

16348. **Kambire, R., Lingue, K., Courtin, F., Sidibe, I., Kiendrebeogo, D., N'Gouan K, E., Ble, L., Kaba, D., Koffi, M., Solano, P., Bucheton, B. & Jamonneau, V., 2012.** Sleeping sickness surveillance in Côte d'Ivoire and Burkina Faso. *Parasite*, **19** (4): 389-396.

Programme National de Lutte contre la Trypanosomiase Humaine Africaine

(PNLTHA) au Burkina-Faso, 03 BP 7009 Ouagadougou, Burkina Faso.

The objective of this paper is to describe recent data from Burkina Faso and Cote d'Ivoire on human African trypanosomosis medical monitoring in order to (i) update the disease situation in these two countries that have been sharing important migratory, economic and epidemiological links for more than a century and (ii) to define the future strategic plans to achieve the goal of a sustainable control/elimination process. Results of active and passive surveillance indicate that all sleeping sickness patients diagnosed these last years in Burkina Faso were imported cases from Côte d'Ivoire. Nevertheless the re-introduction of the parasite is effective and the risk of a resumption of transmission exists. In Côte d'Ivoire, few cases are still diagnosed in several historical foci and the fear exists that the disease could re-emerge in these foci or spread to other areas. In order to achieve a sustainable elimination of sleeping sickness in these two countries, control entities have to adapt their strategy to the different epidemiological contexts. With the exception of specific cases, the current disease prevalence no longer justifies the use of expensive medical surveys by exhaustive screening of the population. New disease control strategies, based on the exchange of epidemiological information between the two countries and integrated with the regular national health systems are required to target priority intervention areas. Follow-up in time of both treated patients and serological suspects that are potential asymptomatic carriers of parasite is also important. In parallel, researchers need to better characterize the respective roles of the human and animal reservoir in the maintenance of transmission and evaluate the different control strategies taken by National Control Programmes in term of cost/effectiveness to help optimize them.

16349. **Mitashi, P., Hasker, E., Lejon, V., Kande, V., Muyembe, J. J., Lutumba, P. & Boelaert, M., 2012.** Human African trypanosomiasis diagnosis in first-line health services of endemic countries, a systematic review. *PLoS Neglected Tropical Diseases*, **6** (11): e1919.

Department of Tropical Medicine, Parasitology Service, Faculty of Medicine, Kinshasa University, Kinshasa, Democratic Republic of Congo; and Department of Public Health, Group Epidemiology and Disease Control, Institute of Tropical Medicine, Antwerp, Belgium. [mboelaert@itG.be].

While the incidence of human African trypanosomiasis (HAT) is decreasing, the control approach is shifting from active population screening by mobile teams to passive case detection in primary care centres. We conducted a systematic review of the literature between 1970 and 2011 to assess which diagnostic tools are most suitable for use in first-line health facilities in endemic countries. Our search retrieved 16 different screening and confirmation tests for HAT. The thermostable format of the card agglutination test for trypanosomiasis (CATT test) was the most appropriate screening test. Lateral flow antibody detection tests could become alternative screening tests in the near future. Confirmation of HAT diagnosis still depends on visualizing the parasite in direct microscopy. All other currently available confirmation tests are either technically too demanding and/or lack sensitivity and thus rather inappropriate for use at health centre level. Novel applications of molecular tests may have potential for use at district hospital level.

16350. **Namangala, B., Hachaambwa, L., Kajino, K., Mweene, A. S., Hayashida, K.,**

Simuunza, M., Simukoko, H., Choongo, K., Chansa, H., Lakhi, S., Moonga, L., Chota, A., Ndebe, J., Nsakashalo-Senkwe, M., Chizema, E., Kasonka, L. & Sugimoto, C., 2012. The use of loop-mediated isothermal amplification (LAMP) to detect the re-emerging human African trypanosomiasis (HAT) in the Luangwa and Zambezi valleys. *Parasites & Vectors*, **5** (1): 282.

Department of Paraclinical studies, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia; Department of Internal Medicine, University Teaching Hospital, PB RW 1X, Lusaka, Zambia; Research Centre for Zoonosis Control, Hokkaido University, Kita-ku, Sapporo, 001-0020, Japan; Department of Disease Control, School of Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia; Department of Biomedical Sciences, School of Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia; and Ministry of Health, Ndeke House, P.O. Box 30205, Lusaka, Zambia. [b.namangala@unza.zm].

Loop-mediated isothermal amplification (LAMP) is a novel strategy which amplifies DNA with high sensitivity and rapidity under isothermal conditions. In the present study, the performance of the repetitive insertion mobile element (RIME)-LAMP and human serum resistance-associated gene (SRA)-LAMP assays were evaluated using clinical specimens obtained from four male patients from Luangwa and Zambezi valleys in Zambia and Zimbabwe, respectively. The cases reported in this preliminary communication were all first diagnosed by microscopy, through passive surveillance, and confirmed by both RIME-LAMP and SRA-LAMP. A good correlation between microscopy and LAMP was observed and contributed to staging and successful treatment of patients. RIME-LAMP and SRA-LAMP complemented each other well in all the cases. It is concluded that both RIME-LAMP and SRA-LAMP were able to detect *Trypanosoma brucei rhodesiense* DNA in patient blood and CSF and hence confirmed HAT in the parasitaemic patients. Our study indicates that the LAMP technique is a potential tool for HAT diagnosis and staging and may be useful for making therapeutic decisions. However, no statistically significant conclusion may be drawn due to the limited sample size used in the present study. It is thus imperative to conduct a detailed study to further evaluate the potential of LAMP as a bedside diagnostic test for HAT.

16351. **Reid, H., Kibona, S., Rodney, A., McPherson, B., Sindato, C., Malele, I., Kinung'hi, S., Jennaway, M., Changalucha, J., Blake, B. & Vallely, A., 2012.** Assessment of the burden of human African trypanosomiasis by rapid participatory appraisal in three high-risk villages in Urambo District, Northwest Tanzania. *African Health Sciences*, **12** (2): 104-113.

Australian Centre for International and Tropical Health, School of Population Health, University of Queensland, Herston, Brisbane, Australia; National Institute for Medical Research, Tabora Centre, PO Box, Tabora, Tanzania; National Institute for Medical Research, Mwanza Centre, PO Box 1462, Mwanza, Tanzania; and Tsetse & Trypanosomiasis Research Institute (TTRI), PO Box 1026 Tanga, Tanzania. [avallely@kirby.unsw.edu.au].

The public health and socio-economic burden of human African trypanosomiasis (HAT) in East Africa is not well documented. Understanding the epidemiology and impact of HAT in such settings is difficult due to a lack of robust surveillance and reporting systems, restricting

evidence-based policy development and contributing to the continued neglect of this disease. To investigate the burden of HAT in Urambo District, Tanzania in order to inform future public health policy, a rapid participatory appraisal (RPA) using a combination of qualitative and quantitative methods was conducted, that included key informant interviews, hospital record analysis, and tools adapted from participatory learning and action. Three villages adjacent to Ugala Game Reserve appeared to be the most affected. High levels of under-reporting were noted due to a lack of diagnostic tools at peripheral health care facilities and limited access to specialist services. Community stakeholders perceived the health and socio-economic burden of HAT to be similar to that of malaria. In conclusion, the burden of HAT in remote rural communities is difficult to capture through routine surveillance systems alone. The RPA represents an efficient mechanism for engaging communities in public health action for trypanosomiasis control in northwest Tanzania.

16352. **Simarro, P. P., Cecchi, G., Franco, J. R., Paone, M., Diarra, A., Ruiz-Postigo, J. A., Fevre, E. M., Mattioli, R. C. & Jannin, J. G., 2012.** Estimating and mapping the population at risk of sleeping sickness. *PLoS Neglected Tropical Diseases*, **6** (10): e1859.

Control of Neglected Tropical Diseases, Innovative and Intensified Disease Management, World Health Organization, Geneva, Switzerland; Animal Production and Health Division, Food and Agriculture Organization of the United Nations, Rome, Italy; Regional Office for Africa, World Health Organization, Brazzaville, Congo; Regional Office for the Eastern Mediterranean, World Health Organization, Cairo, Egypt; Centre for Immunity, Infection and Evolution, Institute of Immunology and Infection Research, School of Biological Sciences, Ashworth Laboratories, University of Edinburgh, Edinburgh, UK and Foundation for Innovative New Diagnostics (FIND), Switzerland. [simarrop@who.int].

Human African trypanosomiasis (HAT), also known as sleeping sickness, persists as a public health problem in several sub-Saharan countries. Evidence-based, spatially explicit estimates of population at risk are needed to inform planning and implementation of field interventions, monitor disease trends, raise awareness and support advocacy. Comprehensive, geo-referenced epidemiological records from HAT-affected countries were combined with human population layers to map five categories of risk, ranging from "very high" to "very low," and to estimate the corresponding at-risk population. Approximately 70 million people distributed over a surface of 1.55 million km^2 are estimated to be at different levels of risk of contracting HAT. *Trypanosoma brucei gambiense* accounts for 82.2 percent of the population at risk, the remaining 17.8 percent being at risk of infection from *T. b. rhodesiense*. Twenty-one million people live in areas classified as moderate to very high risk, where more than 1 HAT case per 10 000 inhabitants per annum is reported. Updated estimates of the population at risk of sleeping sickness were made, based on quantitative information on the reported cases and the geographic distribution of human population. Due to substantial methodological differences, it is not possible to make direct comparisons with previous figures for at-risk population. By contrast, it will be possible to explore trends in the future. The presented maps of different HAT risk levels will help to develop site-specific strategies for control and surveillance, and to monitor progress achieved by on-going efforts aimed at the elimination of sleeping sickness.

16353. **Tiberti, N., Hainard, A., Lejon, V., Courtioux, B., Matovu, E., Enyaru, J. C., Robin, X., Turck, N., Kristensson, K., Ngoyi, D. M., Vatunga, G. M., Krishna, S., Buscher, P., Bisser, S., Ndung'u, J. M. & Sanchez, J. C., 2012.** Cerebrospinal fluid neopterin as marker of the meningo-encephalitic stage of *Trypanosoma brucei gambiense* sleeping sickness. *PLoS One*, **7** (7): e40909.

Biomedical Proteomics Research Group, Department of Human Protein Sciences, University of Geneva, Geneva, Switzerland. [Jean-Charles.Sanchez@unige.ch].

Sleeping sickness, or human African trypanosomiasis (HAT), is a protozoan disease that affects rural communities in sub-Saharan Africa. Determination of the disease stage, essential for correct treatment, represents a key issue in the management of patients. In the present study we evaluated the potential of CXCL10, CXCL13, ICAM-1, VCAM-1, MMP-9, B2MG, neopterin and IgM to complement current methods for staging *Trypanosoma brucei gambiense* patients. Five hundred and twelve *T. b. gambiense* HAT patients originated from Angola, Chad and the Democratic Republic of the Congo (DRC.). Their classification as stage 2 (S2) was based on the number of white blood cells (WBC) (>5/µL) or presence of parasites in the cerebrospinal fluid (CSF). The CSF concentration of the eight markers was first measured on a training cohort encompassing 100 patients (44 S1 and 56 S2). IgM and neopterin were the best in discriminating between the two stages of disease with 86.4 percent and 84.1 percent specificity respectively, at 100 percent sensitivity. When a validation cohort (412 patients) was tested, neopterin (14.3 nmol/L) correctly classified 88 percent of S1 and S2 patients, confirming its high staging power. On this second cohort, neopterin also predicted both the presence of parasites, and of neurological signs, with the same ability as IgM and WBC, the current reference for staging. This study has demonstrated that neopterin is an excellent biomarker for staging *T. b. gambiense* HAT patients. A rapid diagnostic test for detecting this metabolite in CSF could help in more accurate stage determination.

16354. **Wise, E., Easom, N., Watson, J., Bailey, R. & Brown, M., 2012.** Lesson of the month: a psychiatric diagnosis overturned by a blood film. *Clinical Medicine*, **12** (3): 295-296.

Hospital for Tropical Diseases, University College London Hospitals NHS Trust, London, UK. [emilywise@doctors.org.uk].

In September 2010, a 55-year-old lady presented to her GP following a fortnight's holiday in Mana Pools National Park, Zimbabwe. She had taken antimalarial prophylaxis. On returning to the UK she developed fevers, malaise and was non-specifically “unwell”. According to her flat-mate, “she could feed the cat and make cheese-on-toast, but was good for little else”. As the patient had previously experienced a depressive psychosis, her GP suspected that her symptoms were psychiatric and commenced treatment with antipsychotic and antidepressant medications. The patient continued to deteriorate and two weeks after her initial presentation a blood film was performed to exclude malaria. It revealed trypanosomes. The patient was diagnosed with human African trypanosomiasis (HAT), also known as sleeping sickness, and was transferred to the Hospital for Tropical Diseases. On arrival, the patient was able to converse but was uninhibited, had a fluctuating orientation and had difficulty with tasks such as operating her mobile phone. She recalled receiving multiple tsetse fly bites while kayaking in Zimbabwe. She had a grape-sized submandibular lymph node, but no chancre. Daytime somnolence and urinary incontinence were observed. Initial

investigations revealed haemoglobin 7.5 g/L, white cell count 2.75×10^9/L, C-reactive protein 122 mg/L, albumin 19 g/L and normal renal and liver function. Viable motile protozoa were seen on a wet preparation. Thick and thin films with modified rapid Field's staining revealed numerous trypanosomes. The patient was presumed, on the basis of the country of acquisition and her acute presentation, to have been infected by *Trypanosoma brucei rhodesiense*. Her *T. b. rhodesiense* immunofluorescent antibody (IFAT) was positive at one in 200. Suramin was commenced to treat stage I haemolymphatic *T. b. rhodesiense*. Cerebrospinal fluid (CSF) examination on the 13th day of treatment (when the peripheral parasitaemia had resolved) demonstrated 14 lymphocytes/μL, with no trypanosomes seen. World Health Organisation guidelines advocate treatment for stage II encephalopathic *T. b. rhodesiense* if the CSF white cell count is greater than 5μL, and therefore melarsoprol with adjunctive prednisolone was commenced (suramin cannot penetrate the blood brain barrier). Subsequent IFAT CSF analysis did not detect trypanosome antibodies and it is questionable whether there was central nervous system (CNS) involvement. Following full resolution of symptoms, the patient was discharged from hospital in November 2010. Unfortunately, she developed a bilateral pedal paraesthesia, which was most likely suramin-induced, and an axonal peripheral neuropathy was confirmed by nerve conduction studies.

HAT has two disease stages: an initial haemolymphatic stage and a subsequent meningoencephalitic stage, during which the parasites invade the CNS. In cases of *T. b. rhodesiense* infection, CNS involvement occurs acutely, after weeks or months. *T. b. gambiense* causes a more insidious course, with CNS involvement occurring months to years after the initial inoculation. CNS infection is characterised by deregulation of the circadian sleep–wake cycle, movement disorders, seizures and, without treatment, the invariable progression to coma and death. Psychiatric and behavioural disorders, including apathy and inactivity, aggression and psychosis, can also be observed in people with meningoencephalitic HAT. In rural Africa, such presentations are frequently interpreted by the local community as the afflicted being possessed by evil spirits, whereas in the west, people who have this disease have been mistakenly admitted to psychiatric institutions. Both strains of HAT are difficult to diagnose and currently available treatments are less than ideal. In the case described, the patient was suspected of having stage II encephalopathic *T. b. rhodesiense*, for which the recommended treatment is intravenous melarsoprol. Melarsoprol is an arsenic-containing drug that should not be commenced without confirmation of cerebral infection as it causes an irreversible encephalopathy in 10 percent of those who receive it. This encephalopathy is fatal in 50 percent of those who incur it. Administration of prednisolone can reduce the risk of encephalopathy, but the development of less toxic drugs for this neglected tropical disease is clearly long overdue. Standard practice is to administer suramin until the peripheral parasitaemia has cleared, before considering a lumbar puncture. This prevents both the mechanical dissemination of parasites from blood to CSF and the false diagnosis of stage II disease if the CSF is inadvertently contaminated with blood. In our patient, using this precaution resulted in a 13-day delay before the CSF was examined. In the field in Africa, a lumbar puncture is usually performed after the first dose of suramin. The investigation and management of HAT remains controversial, largely because of the toxicity of melarsoprol. In conclusion, “psychiatric” symptoms in a patient returning from sub-Saharan Africa indicate a protozoan infection of the CNS until proven otherwise. A competent examination of a blood film is required in the first instance. The causative organism in this case was the trypanosome, which only rarely infects travellers, rather than the more commonly encountered malarial parasite.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **35**: 16353, 16354]

16355. **Bucheton, B., MacLeod, A. & Jamonneau, V., 2011.** Human host determinants influencing the outcome of *Trypanosoma brucei gambiense* infections. *Parasite Immunology*, **33** (8): 438-447.

Institut de Recherche pour le Développement (IRD), Unité Mixte de Recherche IRD-CIRAD 177, Campus International de Baillarguet, Montpellier, France. [Bruno.bucheton@ird.fr].

Since first identified, human African trypanosomiasis (HAT) or sleeping sickness has been described as invariably fatal. Increasing data however argue that infection by *Trypanosoma brucei gambiense*, the causative agent of HAT, results in a wide range of outcomes in its human host and importantly that a number of subjects in endemic areas are apparently able to control infection to low levels, undetectable by the classical parasitological tests used in the field. Thus, trypanotolerance seems to occur in humans as has already been described in cattle or in the rodent experimental models of infection. This review focuses on the description of the diversity of outcomes resulting from *T. b. gambiense* in humans and on the host factors involved. The consequences/impacts on HAT epidemiology resulting from this diversity are also discussed with regard to implementing sustainable HAT control strategies.

16356. **Burchmore, R., 2012.** Parasites in the brain? The search for sleeping sickness biomarkers. *Expert Review of Anti Infective Therapy*, **10** (11): 1283-1286.

Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK. [richard.burchmore@glasgow.ac.uk].

Human African trypanosomiasis (HAT) is a fatal parasitic disease that progresses from an early stage (stage 1), where parasites multiply in the haemolymphatic system, to a late stage (stage 2) disease, where parasites have become manifest within the CNS. Patients with stage 1 disease are treated with relatively safe drugs, but stage 2 disease requires treatment with drugs that are very toxic or difficult to administer. Thus, it is important to determine the stage of HAT infection before treating. HAT staging currently involves microscopic examination of cerebrospinal fluid (CSF), looking for the presence of parasites, or for more than five white blood cells per μL CSF. This article tested the specificity and selectivity of eight potential CSF markers for stage 2 HAT, by analysis of levels in 400 CSF samples from patients diagnosed with stage 1 or stage 2 HAT by WHO protocols. Two of these markers gave results that were comparable with those obtained by conventional diagnosis. The potential for and challenges of developing improved staging for HAT are discussed.

16357. **Maclean, L., Reiber, H., Kennedy, P. G. & Sternberg, J. M., 2012.** Stage progression and neurological symptoms in *Trypanosoma brucei rhodesiense* sleeping sickness: role of the CNS inflammatory response. *PLoS Neglected Tropical Diseases*, **6** (10): e1857.

Centre for Immunology and Infection, Department of Biology, Hull York Medical School, University of York, York, UK. [j.sternberg@abdn.ac.uk].

Human African trypanosomiasis progresses from an early (haemolymphatic) stage, through CNS invasion to the late (meningoencephalitic) stage. In experimental infections disease progression is associated with neuroinflammatory responses and neurological symptoms, but this concept requires evaluation in African trypanosomiasis patients, where correct diagnosis of the disease stage is of critical therapeutic importance. This was a retrospective study on a cohort of 115 *T. b. rhodesiense* HAT patients recruited in eastern Uganda. Paired plasma and CSF samples allowed the measurement of peripheral and CNS immunoglobulin and of CSF cytokine synthesis. Cytokine and immunoglobulin expression were evaluated in relation to disease duration, stage progression and neurological symptoms. Neurological symptoms were not related to stage progression (with the exception of moderate coma). Increases in CNS immunoglobulin, IL-10 and TNF-alpha synthesis were associated with stage progression and were mirrored by a reduction in TGF-beta levels in the CSF. There were no significant associations between CNS immunoglobulin and cytokine production and neurological signs of disease with the exception of moderate coma cases. Within the study group we identified diagnostically early stage cases with no CSF pleocytosis but intrathecal immunoglobulin synthesis, and diagnostically late stage cases with marginal CSF pleocytosis and no detectable trypanosomes in the CSF. Our results demonstrate that there is not a direct linkage between stage progression, neurological signs of infection and neuroinflammatory responses in *rhodesiense* HAT. Neurological signs are observed in both early and late stages, and while intrathecal immunoglobulin synthesis is associated with neurological signs, these are also observed in cases lacking a CNS inflammatory response. While there is an increase in inflammatory cytokine production with stage progression, this is paralleled by increases in CSF IL-10. As stage diagnostics, the CSF immunoglobulins and cytokines studied do not have sufficient sensitivity to be of clinical value.

16358. **Meltzer, E., Leshem, E., Steinlauf, S., Michaeli, S., Sidi, Y. & Schwartz, E., 2012.** Human African trypanosomiasis in a traveller: diagnostic pitfalls. *American Journal of Tropical Medicine & Hygiene*, **87** (2): 264-266.

Department of Medicine C, Sheba Medical Center, Tel Hashomer, Israel. [leshem@gmail.com].

An Israeli traveller returning from Tanzania presented with a relapsing febrile illness. A diagnosis of *Trypanosoma brucei rhodesiense* infection was established by blood smear after nearly a month. Blood polymerase chain reaction failed to provide an early diagnosis of human African trypanosomiasis. Recognition of suggestive signs should prompt physicians to perform repeated tests before ruling out human African trypanosomiasis.

16359. **Stephens, N. A., Kieft, R., Macleod, A. & Hajduk, S. L., 2012.** Trypanosome resistance to human innate immunity: targeting Achilles' heel. *Trends in Parasitology*, **28** (12): 539-545.

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

Trypanosome lytic factors (TLFs) are powerful, naturally occurring toxins in humans that provide sterile protection against infection by several African trypanosomes. These trypanocidal complexes predominantly enter the parasite by binding to the trypanosome haptoglobin/haemoglobin receptor (HpHbR), trafficking to the lysosome, causing membrane damage and, ultimately, cell lysis. Despite TLF-mediated immunity, the parasites that cause human African Trypanosomiasis (HAT), *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, have developed independent mechanisms of resistance to TLF killing. In this review we describe the parasite defences that allow trypanosome infections of humans and discuss how targeting these apparent strengths of the parasite may reveal their Achilles' heel, leading to new approaches in the treatment of HAT.

(c) TREATMENT

[See also **35**: 16294, 16305, 16308]

16360. **Alirol, E., Schrumpf, D., Amici Heradi, J., Riedel, A., de Patoul, C., Quere, M. & Chappuis, F., 2013.** Nifurtimox-eflornithine combination therapy for second-stage *gambiense* human African trypanosomiasis: Médecins Sans Frontières experience in the Democratic Republic of the Congo. *Clinical Infectious Diseases*, **56** (2): 195-203.

Division of International and Humanitarian Medicine, University Hospitals of Geneva. [info.medint@hcuge.ch].

Existing diagnostic and treatment tools for human African trypanosomiasis (HAT) are limited. The recent development of nifurtimox-eflornithine combination therapy (NECT) has brought new hopes for patients in the second stage. While NECT has been rolled out in most endemic countries, safety data are scarce and derive only from clinical trials. The World Health Organization (WHO) coordinates a pharmaco- vigilance programme to collect additional data on NECT safety and efficacy. We report here the results of 18 months of experience of NECT use in treatment centres run by Médecins Sans Frontières in the Democratic Republic of the Congo (DRC). This cohort study included 684 second-stage HAT patients (including 120 children) treated with NECT in Doruma and Dingila hospitals, north-eastern DRC, between January 2010 and June 2011. All treatment-emergent adverse events (AEs) were recorded and graded according to the Common Terminology Criteria for Adverse Events version 3.0. Safety and efficacy data were retrieved from the WHO pharmaco-vigilance forms and from Epitryps, a program monitoring database. Eighty-six percent of the patients experienced at least 1 AE during treatment. On average, children experienced fewer AEs than adults. Most AEs were mild (37.9 percent) or moderate (54.7 percent). Severe AEs included vomiting (n = 32), dizziness (n = 16), headache (n = 11), and convulsions (n = 11). The in-hospital case fatality rate was low (0.15 percent) and relapses were rare (n = 14). In comparison with previous treatments, NECT was effective, safe, and well tolerated in non-trial settings in DRC, further supporting the roll-out of NECT as first-line treatment in second-stage *Trypanosoma brucei gambiense* HAT. Tolerance was particularly good in children.

16361. **Hasker, E., Mpanya, A., Makabuza, J., Mbo, F., Lumbala, C., Kumpel, J., Claeys, Y., Kande, V., Ravinetto, R., Menten, J., Lutumba, P. & Boelaert, M., 2012.** Treatment outcomes for human African trypanosomiasis in the Democratic

Republic of the Congo: analysis of routine program data from the world's largest sleeping sickness control programme. *Tropical Medicine & International Health*, **17** (9): 1127-1132.

Epidemiology and Disease Control Unit, Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium. [ehasker@itgbe].

To enable the human African trypanosomiasis (HAT) control programme of the Democratic Republic of the Congo to generate data on treatment outcomes, an electronic database was developed. The database was piloted in two provinces, Bandundu and Kasai Oriental. In this study, we analysed routine data from the two provinces for the period 2006-2008. Data were extracted from case declaration cards and monthly reports available at national and provincial HAT coordination units and entered into the database. Data were retrieved for 15 086 of 15 741 cases reported in the two provinces for the period (96 percent). Compliance with post-treatment follow-up was very poor in both provinces; only 25 percent had undergone at least one post-treatment follow-up examination, <1 percent had undergone the required four follow-up examinations. Relapse rates among those presenting for follow-up were high in Kasai (18 percent) but low in Bandundu (0.3 percent). It is concluded that high relapse rates in Kasai and poor compliance with post-treatment follow-up in both provinces are important problems that the HAT control programme urgently needs to address. Moreover, by analogy with tuberculosis control programmes, HAT control programmes need to adopt a recording and reporting routine that includes reporting on treatment outcomes.

16362. **Kennedy, P. G., 2012.** An alternative form of melarsoprol in sleeping sickness. *Trends in Parasitology*, **28** (8): 307-310.

Department of Neurology, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Southern General Hospital, Glasgow G51 4TF, Scotland, UK. [Peter.Kennedy@glasgow.ac.uk].

Human African trypanosomiasis (HAT), or sleeping sickness, is a major threat to human health throughout sub-Saharan Africa. Almost always fatal if untreated or inadequately treated, a commonly used drug for treating late-stage HAT, and the only drug for late-stage *Trypanosoma brucei rhodesiense*, is intravenous melarsoprol, which kills 5 percent of patients receiving it. Melarsoprol cyclodextrin inclusion complexes have been tested in a highly reliable mouse model of HAT. These complexes increase the oral bioavailability of melarsoprol making them effective orally and both curative and nontoxic in doses that are equivalent to those of intravenous melarsoprol. It is argued that a small clinical trial of this drug in HAT is justified to potentially improve the outcome of patients with late-stage *rhodesiense* disease.

16363. **Kuepfer, I., Schmid, C., Allan, M., Edielu, A., Haary, E. P., Kakembo, A., Kibona, S., Blum, J. & Burri, C., 2012.** Safety and efficacy of the 10-day melarsoprol schedule for the treatment of second stage *rhodesiense* sleeping sickness. *PLoS Neglected Tropical Diseases*, **6** (8): e1695.

Swiss Tropical and Public Health Institute, Basel, Switzerland.

[irene.kuepfer@unibas.ch].

This study set out to assess the safety and efficacy of a 10-day melarsoprol schedule in second stage *T. b. rhodesiense* patients and the effect of suramin pre-treatment on the incidence of encephalopathic syndrome (ES) during melarsoprol therapy. It involved sequential conduct of a proof-of-concept trial (n = 60) and a utilization study (n = 78) using historic controls as comparator. Two trial centres in the *T. b. rhodesiense* endemic regions of Tanzania and Uganda were used, the participants being consenting patients with confirmed second stage disease and a minimum age of 6 years. Unconscious and pregnant patients were excluded. The primary outcome measures were safety and efficacy at end of treatment. The secondary outcome measure was efficacy during follow-up after 3, 6 and 12 months. The incidence of ES in the trial population was 11.2 percent (CI 5-17 percent) and 13 percent (CI 9-17 percent) in the historic data. The respective case fatality rates were 8.4 percent (CI 3-13.8 percent) and 9.3 percent (CI 6-12.6 percent). All patients discharged alive were free of parasites at end of treatment. Twelve months after discharge, 96 percent of patients were clinically cured. The mean hospitalization time was reduced from 29 to 13 days ($p<0.0001$) per patient. It is concluded that the 10-day melarsoprol schedule does not expose patients to a higher risk of ES or death than does treatment according to national schedules in current use. The efficacy of the 10-day melarsoprol schedule was highly satisfactory. No benefit could be attributed to the suramin pre-treatment.

16364. **Porcheddu, A., Giacomelli, G. & De Luca, L., 2012.** New pentamidine analogues in medicinal chemistry. *Current Medicinal Chemistry,* **19**(34): 5819-5836.

Dipartimento di Chimica e Farmacia, Universita degli Studi di Sassari Via Vienna 2, 07100 Sassari, Italy. [anpo@uniss.it].

Sixty years after its introduction, 1,5-bis(4-amidinophenoxy)pentane (pentamidine) is still one of the most used drugs for the treatment of the first stage of human African trypanosomiasis and other neglected diseases such as malaria and leishmaniasis. These protozoan infections are prevalent in the poorest world areas such as sub-Saharan Africa and other developing countries; however the increasing immigration from these countries to the richest parts of the world and the overlap of HIV with parasitic infections result in a growing number of cases in developed nations. A great effort has been made to develop new generations of diamidines for the treatment of these infections transmitted by insects. This review summarises the synthesis and evaluation of pentamidine analogues reported in the last years in the effort to find new drugs with better pharmaceutical activity, higher lipophilicity and lower cytotoxicity.

16365. **Schmid, C., Kuemmerle, A., Blum, J., Ghabri, S., Kande, V., Mutombo, W., Ilunga, M., Lumpungu, I., Mutanda, S., Nganzobo, P., Tete, D., Mubwa, N., Kisala, M., Blesson, S. & Mordt, O. V., 2012.** In-hospital safety in field conditions of nifurtimox eflornithine combination therapy (NECT) for *T. b. gambiense* sleeping sickness. *PLoS Neglected Tropical Diseases*, **6** (11): e1920.

Department of Medicines Research, Swiss Tropical and Public Health Institute, Basel, Switzerland; and University of Basel, Basel, Switzerland. [ovalverde@dndi.org].

Trypanosoma brucei (T. *b*.) *gambiense* human African trypanosomiasis (HAT; sleeping sickness) is a fatal disease. Until 2009, available treatments for 2nd stage HAT were complicated to use, expensive (eflornithine monotherapy), or toxic, and insufficiently effective in certain areas (melarsoprol). Recently, nifurtimox-eflornithine combination therapy (NECT) demonstrated good safety and efficacy in a randomised controlled trial (RCT) and was added to the World Health Organisation (WHO) essential medicines list (EML). Documentation of its safety profile in field conditions will support its wider use. In a multicentre, open label, single arm, phase IIIb study of the use of NECT for 2nd stage *T. b. gambiense* HAT, all patients admitted to the trial centres who fulfilled inclusion criteria were treated with NECT. The primary outcome was the proportion of patients discharged alive from hospital. Safety was further assessed based on treatment emergent adverse events (AEs) occurring during hospitalisation. Patients (n=629) were treated in six HAT treatment facilities in the Democratic Republic of the Congo (DRC), including 100 children under 12, 14 pregnant and 33 breast-feeding women. The proportion of patients discharged alive after treatment completion was 98.4 percent (619/629; 95 percentCI [97.1 percent; 99.1 percent]). Of the 10 patients who died during hospitalisation, 8 were already in a bad or very bad health condition; one death was assessed as unlikely related to treatment. No major or unexpected safety concerns arose in any patient group. Most common AEs were gastro-intestinal (61 percent), general (46 percent), nervous system (mostly central; 34 percent) and metabolic disorders (26 percent). The overall safety profile was similar to previously published findings. In field conditions and in a wider population, including children, NECT displayed a similar tolerability profile to that described in more stringent clinical trial conditions. The in-hospital safety was comparable to published results, and long-term efficacy will be confirmed after 24 months follow-up.

6. ANIMAL TRYPANOSOMOSIS

(a) SURVEY AND DISTRIBUTION

[See also **35**: 16342, 16343, 16390, 16437]

16366. **Berlin, D., Nasereddin, A., Asmi, K., Ereqat, S., Abdeen, Z., Eyal, O. & Baneth, G.**, 2012. Prevalence of *Trypanosoma evansi* in horses in Israel evaluated by serology and reverse dot blot. *Research in Veterinary Science*, **93**(3): 1226-1230.

Veterinary Teaching Hospital, Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel; Al-Quds Nutrition and Health Research Center, Faculty of Medicine, Al-Quds University, Abu-Deis, P.O. Box 20760, West Bank, Palestine; and Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel. [berlin@agri.huji.ac.il].

Trypanosoma evansi is the cause of surra in horses, camels and other domestic animals. Following the first outbreak of surra in horses and camels in Israel in 2006, a survey of the prevalence of the parasite in the Israeli horse population was conducted using serology, PCR followed by the reverse dot blot (RDB) technique and blood smear microscopy. In total, 614 horses from seven regions were sampled. The CATT/*T. evansi* kit was used for serology for

all the horses. Horses from the Arava and Dead Sea region where the first outbreak occurred, were sampled again one year later and both samples were subjected to serology and the RDB technique. The country-wide seroprevalence was 4.6 percent (28/614). The seroprevalence in the Arava and Dead Sea region was 6.5 percent (9/139) in the first sampling compared with 4.1 percent (5/122) in the second, whereas the prevalence of RDB-positivity was 18.7 percent (26/139) in the first sampling and only 0.8 percent (1/122) in the second. All horses were asymptomatic except for one horse from the Arava and Dead Sea region that demonstrated clinical signs of surra combined with positive serology and RDB. The results of this study indicated that surra is prevalent in most regions of the country and thus should be considered an important differential diagnosis in horses and other domestic animals in Israel with chronic weight loss, oedema or neurological signs.

16367. **Elshafie, E. I., Sani, R. A., Hassan, L., Sharma, R., Bashir, A. & Abubakar, I. A., 2012.** Seroprevalence and risk factors of *Trypanosoma evansi* infection in horses in Peninsular Malaysia. *Research in Veterinary Science*. **E Publication ahead of print, September 25.**

Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Selangor, Malaysia; Central Veterinary Research Laboratories, Al Amarat, P.O. Box 8067, Khartoum, Sudan. [rehana@ve*T*.upm.edu.my].

A cross-sectional study was designed to assess the seroprevalence and risk factors associated with *Trypanosoma evansi* infection among horses, using a total of 527 blood samples obtained from eight states in peninsular Malaysia. A structured questionnaire was used to collect data on risk factors associated with *T. evansi* seroprevalence. The overall seroprevalence detected by the card agglutination test for *T. evansi* (CATT/*T. evansi*) was 13.90 percent (73/527, CI: 11.2-17.1 percent). Female and exogenous horses showed a higher risk in association with the disease seroprevalence compared with other groups. The majority of the horse owners were not familiar with surra (85.30 percent). However, most of them were very cautious with the health of their animals. In conclusion, this study showed that *T. evansi* occurred at a low frequency among horses in peninsular Malaysia, and the good management system adopted by horse owners was probably responsible for the low *T. evansi* occurrence.

16368. **Fikru, R., Goddeeris, B. M., Delespaux, V., Moti, Y., Tadesse, A., Bekana, M., Claes, F., De Deken, R. & Buscher, P., 2012.** Widespread occurrence of *Trypanosoma vivax* in bovines of tsetse- as well as non-tsetse-infested regions of Ethiopia: a reason for concern? *Veterinary Parasitology*, **190** (3-4): 355-361.

College of Veterinary Medicine, Addis Ababa University, P.O. Box 34, Debre Zeit, Ethiopia. [pbuscher@itg.be].

A cross-sectional study was undertaken to assess the prevalence of bovine trypanosomosis in some tsetse-infested and tsetse-free areas of Ethiopia. From August 2010 until April 2011, a total of 1 524 animals were examined parasitologically and compared by the haematocrit centrifugation technique (Woo test) and polymerase chain reaction (ITS-1 PCR). The ITS-1 PCR was more sensitive and more accurate in species identification than the Woo test. In ITS-1 PCR, an overall trypanosome prevalence of 31.0 percent was observed that is significantly ($p<0.001$) higher than in the Woo test (5.3 percent). *Trypanosoma vivax* was the predominant

taxon (24.9 percent), followed by *T. theileri* (6.0 percent), *T. congolense* (2.9 percent) and Trypanozoon (1.6 percent). Mixed infections were quite common (14 percent of all infections). The overall prevalence of trypanosome infections in the tsetse area (32.4 percent) was not different from the non-tsetse area (30.5 percent); neither were the prevalences of *T. vivax* in both areas (respectively 22.6 percent and 25.7 percent). With these high prevalences, bovine trypanosomosis continues to hinder animal production and productivity in Ethiopia, both in tsetse-infested and non-infested parts of the country. Attempts to control African trypanosomosis should also pay attention to mechanically transmitted pathogenic trypanosomes and should adopt the most advanced molecular tests for species identification.

16369. **Takeet, M. I., Fagbemi, B. O., Donato, M. D., Yakubu, A., Rodulfo, H. E., Peters, S. O., Wheto, M. & Imumorin, I. G., 2012.** Molecular survey of pathogenic trypanosomes in naturally infected Nigerian cattle. *Research in Veterinary Science*. **E Publication ahead of print, December 11.**

Dept. of Animal Science, Cornell University, Ithaca, NY 14853, USA; Dept. of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria; and Dept. of Veterinary Microbiology and Parasitology, University of Agriculture, Abeokuta, Nigeria. [takeetm@yahoo.com].

Microscopy and polymerase chain reaction (PCR) were used to survey pathogenic trypanosome infection in naturally infected Nigerian cattle. In 411 animals sampled, microscopy detected 15.1 percent positive infection of at least one of *Trypanosoma brucei*, *Trypanosoma congolense* or *Trypanosoma vivax*, while PCR detected 63.7 percent positive infections of at least one of those species as well as *Trypanosoma evansi*. PCR detected 4.4 percent, 48.7 percent, 26.0 percent and 0.5 percent respectively of *T. brucei*, *T. congolense*, *T. vivax* and *T. evansi* infections. All of the *T. congolense* detected were savannah type, except for two forest type infections. Prevalence of mixed infections was 13.9 percent, being primarily co-infection by *T. congolense* and *T. vivax* while prevalence of mixed infections by *T. evansi*, *T. vivax* and *T. congolense* was 1.5 percent. Microscopy showed poor sensitivity but specificity greater than 94 percent. Infection rates were much higher in southern than in northern Nigeria. Infections were lowest in N'dama compared with Muturu, Sokoto Gudali and White Fulani breeds. Animals with *T. vivax* mono-infection and mixed infections showed significantly lower packed cell volume (PCV) values. Those infected with any *Trypanosoma* species with <200 parasites/µL showed higher PCV values than those infected with >200 parasites/µL. The new finding of savannah- and forest- type *T. congolense* in Nigeria and the relatively high abundance of mixed infections are of significant clinical relevance. This study also suggests that *T. congolense* is the most prevalent species in Nigeria.

(b) PATHOLOGY AND IMMUNOLOGY

16370. **Almeida Kde, S., Costa, A. F., Silva, P. C., Fagliari, J. J., Machado, R. Z. & Nascimento, A. A., 2012.** Acute phase proteins: a potential approach for diagnosing chronic infection by *Trypanosoma vivax*. *Revista Brasiliera de Parasitologia Veterinaria*, **21** (2): 97-100.

Laboratorio de Higiene e Saude Publica, Escola de Medicina Veterinaria e Zootecnia, Universidade Federal do Tocantins, Araguaina, TO, Brazil.

[katyanesalmeida@gmail.com].

The present study aimed to assess potential changes in acute phase proteins in sheep experimentally infected with *Trypanosoma vivax*. Eight male sheep were studied, four being used as controls and four infected with 10^5 *T. vivax* trypomastigotes. Blood samples were collected on two occasions before infection and then at 5, 7, 9, 11, 13, 15, 20, 30, 45, 60, 75, 90, 105 and 120 days post-infection (dpi). Blood samples were centrifuged and acute phase proteins were then separated by electrophoresis on acrylamide gel containing sodium dodecyl sulphate. Protein concentrations were determined by computer-assisted densitometry. Total protein was determined colorimetrically by the biuret method. Trypanosomes were counted daily using a 5 mL aliquot of blood smear on a glass slide under a 22 x 22 mm coverslip. Parasites were counted in 100 microscopic fields (40x magnification), and then multiplied by a correction factor. The results were expressed as parasites per mL of blood. For statistical analyses, we used the Wilcoxon test at 5 percent significance level. Reductions in several acute phase proteins and increases in antitrypsin and transferrin were found. This can be used for the diagnosis of *T. vivax* infection, especially chronic infections.

16371. **Alves, A. S., Mouta-Confort, E., Figueiredo, F. B., Oliveira, R. V., Schubach, A. O. & Madeira, M. F., 2012.** Evaluation of serological cross-reactivity between canine visceral leishmaniasis and natural infection by *Trypanosoma caninum*. *Research in Veterinary Science*, **93** (3): 1329-1333.

Programa de pos-graduacao em Pesquisa Clinica em Doencas Infecciosas, Instituto de Pesquisa Clinica Evandro Chagas, Fundacao Oswaldo Cruz, Av. Brasil 4365, 21040-900 Rio de Janeiro, RJ, Brazil. [fatima.madeira@ipec.fioCruz.br].

In order to evaluate if the presence of *Trypanosoma caninum* can confuse diagnosis of canine visceral leishmaniasis (CVL), we investigated the serological status of dogs infected by *T. caninum* and assessed the serological cross-reactivity with CVL. A set of 117 serum samples from dogs infected by *T. caninum, Leishmania chagasi* and uninfected dogs (n=39 in each group) was tested using commercial kits–indirect immunofluorescence (IFI-LVC), ELISA (EIE-LVC) and immunochromatographic test (DPP), and in-house tests with *T. caninum* (IIF-Tc and ELISA-Tc) and *L. chagasi* antigens (IIF-Lc and ELISA-Lc). IIF-Tc and ELISA-Tc had sensitivities of 64.1 percent and 94.9 percent and specificities of 23.1 percent and 35.9 percent, respectively. The sensitivity of the IFI-LVC, EIE-LVC and DPP tests was 100 percent and specificities were 70.5 percent, 68 percent and 97.5 percent respectively. The concordance between the tests was considered as satisfactory. The specificities of IFI-LVC, EIE-LVC and DPP were higher when the group Tc was excluded, with significant values for IFI-LVC (chi^2=4.36, p-value=0.036), thus suggesting that infection with *T. caninum* can confuse the diagnosis of CVL.

16372. **Cadioli, F. A., Barnabe Pde, A., Machado, R. Z., Teixeira, M. C., Andre, M. R., Sampaio, P. H., Fidelis Junior, O. L., Teixeira, M. M. & Marques, L. C., 2012.** First report of *Trypanosoma vivax* outbreak in dairy cattle in Sao Paulo State, Brazil. *Revista Brasiliera de Parasitologia Veterinaria*, **21** (2): 118-124.

Departamento Clinica, Cirurgia e Reproducao Animal, Faculdade de Odontologia Curso de Medicina Veterinaria, Universidade Estadual Paulista, Rua Clovis Pestana

793, Aracatuba, SP, Brazil. [fabianocadioli@fmva.unesP.br].

This is the first description of a *Trypanosoma vivax* outbreak in the State of Sao Paulo (municipality of Lins). Fever, jaundice, decreased milk production, weight loss, profuse diarrhoea, abortion, anaemia, leukocytosis and hyperfibrinogenemia were observed in the affected animals. Thirty-one cows and calves died out of a total of 1 080 in the herd. Three cows showed neurological symptoms like dysmetria, ataxia, muscle weakness, ptyalism, lymph node enlargement and submandibular oedema. Flagellated haemoparasites were observed in blood smears. The species was diagnosed as *T. vivax* by means of PCR. This *T. vivax* strain showed resistance to diaminazene aceturate and the infection spread quickly within the herd. From the ELISA test, 599 serum samples (98.36 percent) were positive for anti-*T. vivax* IgG antibodies. This outbreak occurred during a very dry period, which indicates that other factors were involved in the outbreak, such as large populations of *Haematobia irritans* and *Stomoxys calcitrans*. The increases in these populations may have been due to the use of biosolid waste from sugar and ethanol plants in the sugar cane plantations surrounding the dairy farm.

16373. **Defontis, M., Richartz, J., Engelmann, N., Bauer, C., Schwierk, V. M., Buscher, P. & Moritz, A., 2012.** Canine *Trypanosoma evansi* infection introduced into Germany. *Veterinary Clinical Pathology*, **41** (3): 369-374.

Department of Veterinary Clinical Sciences, Small Animal Clinic, Justus-Liebig University Giessen, Giessen, Germany. [m_defontis@hotmail.com].

A 9-year-old male Jack Russell terrier with a history of travel to Thailand was presented with chronic lethargy, weight loss, unilateral anterior uveitis, pancytopaenia, hyperglobulinaemia, and proteinuria. Numerous trypomastigotes were found on a blood smear, and using molecular methods the parasite was identified as *Trypanosoma evansi*. After initial response to treatment, the dog experienced a relapse with central neurologic signs 88 days after initial presentation and died. Antibodies to *T. evansi* were detected in both serum and cerebrospinal fluid (CSF) using a card agglutination test (CATT/*T. evansi*), and PCR analysis of CSF for *T. evansi* was positive. Findings at necropsy included marked non-purulent meningoencephalitis. Chronic infection with *T. evansi* in a dog that returned to Germany following international travel highlights the risk associated with introduction of foreign animal diseases to Europe and the possibility of these infections becoming endemic. Detection of chronic infection and curative therapy of trypanosomiasis are challenging, and infection is usually fatal in the dog.

16374. **Desquesnes, M., Ravel, S., Deschamps, J. Y., Polack, B. & Roux, F., 2012.** Atypical hyperpachymorph *Trypanosoma* (Nannomonas) *congolense* forest-type in a dog returning from Senegal. *Parasite*, **19** (3): 239-247.

Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UMR Intertryp, 34398 Montpellier, France. [marc.desquesnes@cirad.fr].

Trypanosoma congolense forest-type was identified by PCR in France, in a dog returning from Senegal. This paper describes the morphological features of the parasite on Giemsa-

stained smears. Slender forms and "latent bodies" represent 30.4 percent and 20.4 percent, respectively. Some rosettes have been observed (0.8 percent). The predominant form (48.4 percent) is stumpy, close to "*montgomeryi*-form", but it is unusually broad, with a width/length ratio (WLr) of 0.40-0.55, while that of "*montgomeryi*-forms" is close to 0.3. To the best of our knowledge, this is the first description of such a form of *T. congolense* (Nannomonas). Also unusual, the shape of the cytoplasm appears to be tightened by an "S-" or "C-" shaped flagellum. We propose naming this peculiar morphotype "hyperpachymorph", and adding its description to that of *T. congolense* forest-type. Thus *T.* (Nannomonas) forms would include: sphaeromorph or "latent body-form" (globular), hyperleptomorph (*rodhaini*-form, very long and slender, with a free flagellum); leptomorph (*simiae*-form, slender, with a free flagellum); isomorph (*congolense*-form, short, generally without a free flagellum); pachymorph (*montgomeryi*-form, short and stout; 0.25 < WLr < 0.34, without a free flagellum), and hyperpachymorph ("hyper *montgomeryi*-form", short and very stout; 0.35 < WLr < 0.7, without a free flagellum).

16375. **Omeje, J. N. & Anene, B. M., 2012.** Comparative serum biochemical changes induced by experimental infection of *T. brucei* and *T. congolense* in pigs. *Veterinary Parasitology*, **190** (3-4): 368-374.

Department of Veterinary Medicine, University of Abuja, Nigeria. [judexnd@yahoo.co.uk].

A comparative evaluation of the serum biochemical parameters was carried out in groups of young pigs aged 3-5 months experimentally infected with single infection of *Trypanosoma brucei*, *Trypanosoma congolense*, and a mixed infection of the two species. All the parameters studied (alanine amino transferase, aspartate amino transferase, albumin, globulin, cholesterol and creatinine) with the exception of total protein and urea differed significantly ($p<0.05$) between the infected groups and uninfected control group. Serum concentrations of alanine amino transferase, aspartate amino transferase, creatinine and globulin were increased whereas albumin and cholesterol decreased, except for the *T. congolense* group that had similar cholesterol levels as the control group. There was no significant variation ($p>0.05$) in the parameters within the infected groups except that creatinine was elevated in the *T. brucei* group. Administration of diminazene aceturate on day 42 post infection restored alanine amino transferase, aspartate amino transferase and albumin to normal values unlike the other parameters. It was thus concluded that trypanosome infection in pigs could lead to some significant alterations in serum biochemical values and that these were not influenced by individual parasite species or by mixed infection.

16376. **Ranjithkumar, M., Malik, T. A., Saxena, A., Dan, A., Sakthivel, P. C. & Dey, S., 2012.** Hyperlipidaemia in trypanosomiasis of naturally infected horses: possible cachexia-anorexia syndrome? *Tropical Animal Health & Production*. **E publication ahead of print, July 27.**

Division of Medicine, Indian Veterinary Research Institute, Izatnagar, 243122, India.

Trypanosomiasis caused by *Trypanosoma evansi* commonly produces a wasting disease with signs of emaciation and cachexia mainly at the end stage. The present study was conducted to explore the possible hyperlipaemia or hyperlipidaemia and its association with

cachexia-anorexia in equine trypanosomiasis. Out of the fifteen confirmed animals, none of the plasma samples was opaque. There were significant increases in plasma triglyceride, total cholesterol and blood urea nitrogen and a highly significant increase in low-density lipoprotein (LDL) levels. Mild increases in high-density lipoprotein (HDL) and very low-density lipoprotein levels were observed, while the relative percentage of HDL and LDL was altered with high significance. A moderate increase in triglyceride and highly significant increase in LDL might be the reasons for retention of appetite and lipolysis. Possible protein breakdown and presence of lipolysis might be the reasons for cachexia in equine trypanosomiasis.

16377. **Rodrigues, C. M., Olinda, R. G., Silva, T. M., Vale, R. G., da Silva, A. E., Lima, G. L., Garcia, H. A., Teixeira, M. M. & Batista, J. S., 2013.** Follicular degeneration in the ovaries of goats experimentally infected with *Trypanosoma vivax* from the Brazilian semi-arid region. *Veterinary Parasitology*, **191** (1-2): 146-153.

Department of Animal Sciences, Federal Rural University of the Semiarid (UFERSA), Av. Francisco Mota, Br 110, Km 47-59, 59625-900 Mossoro, RN, Brazil. [jaelsoares@hotmail.com].

Infection by *Trypanosoma vivax* and other African trypanosomes plays an important role in reproductive disorders in male and female livestock. Outbreaks of *T. vivax* in the semi-arid region of north-eastern Brazil are characterized by wasting disease in cattle, sheep and goats with haematological, cardiac and nervous compromises in addition to reproductive failures. Similar to reports from Africa, we previously observed a reduction in fertility rates and severe testicular degeneration and epididymitis in male sheep infected with *T. vivax* from this region. Although anoestrus is frequently reported in goats and sheep infected with *T. vivax*, the effects of this infection on the female reproductive organs need clarification. In this study, we addressed this issue through a histopathological evaluation of ovarian follicular morphology and classification in goats experimentally infected with a *T. vivax* isolate from the Brazilian semi-arid region. The infected animals presented typical clinical signs of trypanosomosis by *T. vivax*, including anaemia, hyperthermia, pallour of the mucous membranes, enlarged lymph nodes, and progressive loss of weight. All the infected goats remained anoestrus throughout the experimental period and exhibited important disturbances in the ovaries, evidenced by reduced size and a smooth surface without follicles or *corpora lutea*, and abnormal follicular development. In addition, we detected *T. vivax* DNA in the ovarian tissues of the infected goats using PCR,. Our findings contribute to understanding the female reproductive failure associated with trypanosomosis caused by *T. vivax*.

(c) TRYPANOTOLERANCE

[See also **35**: 16399]

16378. **Chiejina, S. N. & Behnke, J. M., 2011.** The unique resistance and resilience of the Nigerian West African Dwarf goat to gastrointestinal nematode infections. *Parasites & Vectors*, **4**: 12.

Faculty of Veterinary Medicine, University of Nigeria, Nsukka Nigeria. [jerzy.behnke@nottingham.ac.uk].

West African Dwarf (WAD) goats serve an important role in the rural village economy of West Africa, especially among smallholder livestock owners. They have been shown to be trypanotolerant and to resist infections with *Haemonchus contortus* more effectively than any other known breed of goat. In this paper we review what is known about the origins of this goat breed, explain its economic importance in rural West Africa and review the current status of our knowledge about its ability to resist parasitic infections. We suggest that its unique capacity to show both trypanotolerance and resistance to gastrointestinal (GI) nematode infections is immunologically based and genetically endowed, and that knowledge of the underlying genes could be exploited to improve the capacity of more productive wool and milk producing, but GI nematode susceptible, breeds of goats to resist infection, without recourse to anthelmintics. Either conventional breeding allowing introgression of resistance alleles into susceptible breeds, or transgenesis could be exploited for this purpose. Appropriate legal protection of the resistance alleles of WAD goats might provide a much needed source of revenue for the countries in West Africa where the WAD goats exist and where currently living standards among rural populations are among the lowest in the world.

16379. **Nganga, J. K., Soller, M. & Iraqi, F. A., 2010.** High resolution mapping of trypanosomosis resistance loci Tir2 and Tir3 using F12 advanced intercross lines with major locus Tir1 fixed for the susceptible allele. *BMC Genomics*, **11**: 394.

International Livestock Research Institute, PO Box 30709, Nairobi, Kenya. [fuadi@pos*T*.tau.ac.il].

Trypanosomosis is the most economically important disease constraint to livestock productivity in Africa. A number of trypanotolerant cattle breeds are found in West Africa, and identification of the genes conferring trypanotolerance could lead to effective means of genetic selection for trypanotolerance. In this context, high resolution mapping in mouse models is a promising approach to identifying the genes associated with trypanotolerance. In previous studies, using F2 C57BL/6J x A/J and C57BL/6J x BALB/cJ mouse resource populations, trypanotolerance QTL were mapped within a large genomic interval of 20-40 cM to chromosomes MMU17, 5 and 1, and denoted Tir1, Tir2 and Tir3 respectively. Subsequently, using F6 C57BL/6J x A/J and C57BL/6J x BALB/cJ F6 advanced intercross lines (AIL), Tir1 was fine mapped to a confidence interval (CI) of less than 1 cM, while Tir2 and Tir3, were mapped within 5-12 cM. Tir1 represents the major trypanotolerance QTL. In order to improve map resolutions of Tir2 and Tir3, an F12 C57BL/6J x A/J AIL population fixed for the susceptible alleles at Tir1 QTL was generated. An F12 C57BL/6J x A/J AIL population, fixed for the resistant alleles at Tir1 QTL was also generated to provide an additional estimate of the gene effect of Tir1. The AIL populations homozygous for the resistant and susceptible Tir1 alleles and the parental controls were challenged with *T. congolense* and followed for survival times over 180 days. Mice from the two survival extremes of the F12 AIL population fixed for the susceptible alleles at Tir1 were genotyped with a dense panel of microsatellite markers spanning the Tir2 and Tir3 genomic regions and QTL mapping was performed. Tir2 was fine mapped to less than 1 cM CI while Tir3 was mapped to three intervals named Tir3a, Tir3b and Tir3c with 95 percent confidence intervals (CI) of 6, 7.2 and 2.2 cM, respectively. The mapped QTL regions encompass genes that are vital to innate immune response and can be potential candidate genes for the underlying QTL.

16380. **Noyes, H., Brass, A., Obara, I., Anderson, S., Archibald, A. L., Bradley, D. G.,**

Fisher, P., Freeman, A., Gibson, J., Gicheru, M., Hall, L., Hanotte, O., Hulme, H., McKeever, D., Murray, C., Oh, S. J., Tate, C., Smith, K., Tapio, M., Wambugu, J., Williams, D. J., Agaba, M. & Kemp, S. J., 2011. Genetic and expression analysis of cattle identifies candidate genes in pathways responding to *Trypanosoma congolense* infection. *Proceedings of the National Academy of Sciences USA*, **108** (22): 9304-9309.

School of Biological Sciences, University of Liverpool, Liverpool L69 7ZB, UK. [kempsj@liv.ac.uk].

African bovine trypanosomiasis caused by *Trypanosoma* spp. is a major constraint on cattle productivity in sub-Saharan Africa. Some African *Bos taurus* breeds are highly tolerant of infection, but the potentially more productive *Bos indicus* Zebu breeds are much more susceptible. Zebu cattle are well adapted for plowing and haulage, and increasing their tolerance of trypanosomiasis could have a major impact on crop cultivation as well as dairy and beef production. We used three strategies to obtain short lists of candidate genes within QTL that were previously shown to regulate response to infection. We analysed the transcriptomes of trypanotolerant N'Dama and susceptible Boran cattle after infection with *Trypanosoma congolense*. We sequenced EST libraries from these two breeds to identify polymorphisms that might underlie previously identified quantitative trait loci (QTL), and we assessed QTL regions and candidate loci for evidence of selective sweeps. The scan of the EST sequences identified a previously undescribed polymorphism in ARHGAP15 in the Bta2 trypanotolerance QTL. The polymorphism affects gene function *in vitro* and could contribute to the observed differences in expression of the MAPK pathway *in vivo*. The expression data showed that TLR and MAPK pathways responded to infection, and the former contained TICAM1, which is within a QTL on Bta7. Genetic analyses showed that selective sweeps had occurred at TICAM1 and ARHGAP15 loci in African taurine cattle, making them strong candidates for the genes underlying the QTL. Candidate QTL genes were identified in other QTL by their expression profile and the pathways in which they participate.

16381. **Orenge, C. O., Munga, L., Kimwele, C. N., Kemp, S., Korol, A., Gibson, J. P., Hanotte, O. & Soller, M., 2012.** Trypanotolerance in N'Dama x Boran crosses under natural trypanosome challenge: effect of test-year environment, gender, and breed composition. *BMC Genetics*, **13**: 87.

South Eastern University College (A constituent college of the University of Nairobi), P.O. Box 170-90200, Kitui, Kenya. [orengeo@gmail.com].

Trypanosomosis, a protozoal disease affecting livestock, transmitted by *Glossina* (tsetse) flies is a major constraint to agricultural production in sub-Saharan Africa. It is accepted that utilization of the native trypanotolerance exhibited in some of the African cattle breeds to improve trypanotolerance of more productive but susceptible breeds will offer a cost-effective and sustainable solution to the problem. The success of this approach is based on the premise that quantitative trait loci previously identified under relatively controlled situations confer useful trypanotolerance under natural field situations. As part of a study to authenticate this hypothesis, a population of 192 cattle, consisting of six batches of N'Dama and Kenya-Boran backcross animals [(N'Dama x Kenya-Boran) x Kenya-Boran] born over the period 2002 to 2006 was constructed. Some of the batches also included pure Kenya-Boran cattle, or N'Dama

x Kenya- Boran F1 animals. Each batch was exposed as yearlings to natural field trypanosomosis challenge over a period of about one year; the entire challenge period extending from December 2003 to June 2007. Performance of the animals was evaluated by weekly or biweekly measurements of body weight, packed blood cell volume (PCV), parasitaemia score, and number of trypanocide treatments. From these basic data, 49 phenotypes were constructed reflecting the dynamics of body weight, packed cell volume (PCV) and parasitaemia under challenge. Females were distinctly more trypanotolerant than males. F1, backcross and pure Kenya- Boran animals ranked in that order with respect to trypanotolerance. Overall batch effects were highly significant ($p<0.001$) for most traits, and were generally more significant than the gender or genetic type effects. The superior trypanotolerance of the F1 animals was expressed in all three components of animal defence strategies against pathogens: Avoidance resistance, and tolerance. The results show that trypanotolerance derived from the N'Dama is expressed under field conditions; and that the trait is primarily additive in nature, being expressed in heterozygous condition and in a three-quarters Boran genetic background. The results further underscore the complexity of the trait in the field manifesting all three host disease-control strategies, and show the importance of gender and local environmental conditions in determining response to challenge.

16382. **Stein, J., Ayalew, W., Rege, E., Mulatu, W., Lemecha, H., Tadesse, Y., Tekle, T. & Philipsson, J., 2011.** Trypanosomosis and phenotypic features of four indigenous cattle breeds in an Ethiopian field study. *Veterinary Parasitology*, **178** (1-2): 40-47.

Dept of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, P.O. Box 7023, Uppsala, Sweden. [Jennie.Stein@hgen.slu.se].

We conducted a two-part study in the native home areas of four cattle breeds, Abigar, Gurage, Horro and Sheko, in south-western Ethiopia. The first part of the study investigated livestock keeper knowledge about trypanosomosis and trypanotolerance. For each breed 60 livestock keepers were interviewed, resulting in a total of 240 interviews. The second part of the study focused on biological evidence for trypanotolerance. Blood samples of about 100 head of cattle per breed were collected during peak trypanosomosis challenge period and analysed for packed cell volume (PCV) and parasitaemia. In addition individual body measurements of the sampled animals were taken and the keepers provided some information regarding their animals. Livestock keeper interviews revealed that trypanosomosis was considered a major problem in all areas (95-100 percent). Almost all Abigar livestock keepers knew how trypanosomosis is transmitted, whereas only 34-52 percent of the keepers of the other breeds had that knowledge. Most Sheko keepers (75 percent) knew of trypanotolerance and claimed to have trypanotolerant animals in their own herds. Among the other three breeds the knowledge of trypanotolerance was much less (8-18 percent). A majority of the keepers were interested in purchasing trypanotolerant animals. PCV was highest among Horro (26.2) and Sheko (25.1) cattle whereas Abigar had the lowest PCV (20.0). Sheko were least infected by trypanosomes (6 percent) and had the lowest number of trypanocidal treatments per year (one treatment/animal and year). Abigar cattle were most infected (23 percent) followed by Gurage (20 percent) and Horro (17 percent). Gurage had by far the highest number of treatments per animal and year (24). There were large differences between the number of cattle perceived by the keepers to be infected, and the number detected from blood sampled, among Abigar, Gurage and Horro. Sheko livestock keepers were better at correctly diagnosing trypanosomosis in their animals. It is concluded that Sheko cattle have higher

trypanotolerance attributes of the breeds investigated and a better use of this breed could improve cattle health and household welfare in tsetse-infested areas.

(d) TREATMENT

16383. **Harrill, A. H., Desmet, K. D., Wolf, K. K., Bridges, A. S., Eaddy, J. S., Kurtz, C. L., Hall, J. E., Paine, M. F., Tidwell, R. R. & Watkins, P. B., 2012.** A mouse diversity panel approach reveals the potential for clinical kidney injury due to DB289 not predicted by classical rodent models. *Toxicological Sciences*, **130** (2): 416-426.

Hamner-University of North Carolina Institute for Drug Safety Sciences, The Hamner Institutes for Health Sciences, Durham, North Carolina 27709, USA. [aharrill@thehamner.org].

DB289 is the first oral drug shown in clinical trials to have efficacy in treating African trypanosomiasis (African sleeping sickness). Mild liver toxicity was noted but was not treatment limiting. However, development of DB289 was terminated when several treated subjects developed severe kidney injury, a liability not predicted from preclinical testing. We tested the hypothesis that the kidney safety liability of DB289 would be detected in a mouse diversity panel (MDP) comprised of 34 genetically diverse inbred mouse strains. MDP mice received 10 days of oral treatment with DB289 or vehicle and classical renal biomarkers blood urea nitrogen (BUN) and serum creatinine (sCr), as well as urine biomarkers of kidney injury were measured. While BUN and sCr remained within reference ranges, marked elevations were observed for kidney injury molecule-1 (KIM-1) in the urine of sensitive mouse strains. KIM-1 elevations were not always coincident with elevations in alanine aminotransferase (ALT), suggesting that renal injury was not linked to hepatic injury. Genome-wide association analyses of KIM-1 elevations indicated that genes participating in cholesterol and lipid biosynthesis and transport, oxidative stress, and cytokine release may play a role in DB289 renal injury. Taken together, the data resulting from this study highlight the utility of using an MDP to predict clinically relevant toxicities, to identify relevant toxicity biomarkers that may translate into the clinic, and to identify potential mechanisms underlying toxicities. In addition, the sensitive mouse strains identified in this study may be useful in screening next-in-class compounds for renal injury.

16384. **Mungube, E. O., Diall, O., Baumann, M. P., Hoppenheit, A., Hinney, B., Bauer, B., Sanogo, Y., Maiga, B., Zessin, K. H., Randolph, T. F. & Clausen, P. H., 2012.** Best-bet integrated strategies for containing drug-resistant trypanosomes in cattle. *Parasites & Vectors*, **5**: 164.

Institute for Parasitology and Tropical Veterinary Medicine, Freie Universitat Berlin, Konigsweg 67, D-14163, Berlin, Germany. [clausen.ph@vetmed.fu-berlin.de].

African animal trypanosomosis is a major constraint to the rearing of productive livestock in the sub-humid Sudan-Sahel zone of West Africa where cotton is grown. Trypanosomosis is mainly controlled using trypanocidal drugs, but the effective use of drugs is threatened by the development of widespread resistance. This study tested integrated best-bet strategies for containment and/or reversal of trypanocide resistance in villages in south-east Mali where resistance has been reported. Four sentinel villages each from an intervention area (along the

road from Mali to Burkina Faso) and a control area (along the road from Mali to Côte d'Ivoire) were selected for the study. Tsetse control was based on deltamethrin-treated stationary attractive devices and targeted cattle spraying between March 2008 and November 2009. Trypanosome-positive cattle were selectively treated with 3.5 mg/kg diminazene aceturate. Strategic helminth control using 10 mg/kg albendazole was also undertaken. During the intervention, tsetse densities along drainage lines, trypanosome infections and faecal egg counts in risk cattle (3 to 12 months of age) were monitored. Catch reductions of 66.5 percent in *Glossina palpalis gambiensis* and 90 percent in *G. tachinoides* were observed in the intervention area. Trypanosome prevalence was significantly ($p < 0.05$) lower in the intervention area (2.3 percent; 1.3-3.6 percent) compared with the control area (17.3 percent; 14.8-20.1 percent). Albendazole treatment resulted in a faecal egg count reduction of 55.6 percent and reduced trypanosome infection risk (2.9 times lower than in the placebo group) although not significantly ($p > 0.05$). Further studies are required before confirming the existence of albendazole resistant strongyles in the study area. It is concluded that integration of best-bet strategies in areas of multiple drug resistance is expected to reduce trypanosome infection risk thus contributing to containment of trypanocidal drug resistance. Integrated best-bet strategies could therefore be considered a viable trypanosomosis control option especially in areas where multiple drug resistance has been reported.

16385. **Mungube, E. O., Vitouley, H. S., Allegye-Cudjoe, E., Diall, O., Boucoum, Z., Diarra, B., Sanogo, Y., Randolph, T., Bauer, B., Zessin, K. H. & Clausen, P. H., 2012.** Detection of multiple drug resistant *Trypanosoma congolense* populations in village cattle of south-east Mali. *Parasites & Vectors*, **5**: 155.

Institute for Parasitology and Tropical Veterinary Medicine, Freie Universitat Berlin, Konigsweg 67, D-14163 Berlin, Germany. [clausen.ph@vetmed.fu-berlin.de].

Tsetse fly-transmitted African animal trypanosomosis causes annual losses that run into billions of dollars. The disease is assumed to cause hunger and poverty in most sub-Saharan countries since it represents a serious impediment to sustainable livestock production. Both a cross-sectional and a longitudinal study were carried out from November to December 2007 to evaluate trypanosomosis risk and susceptibility of trypanosomes to trypanocidal drug treatment in village cattle populations in south-east Mali. Eight purposively selected villages participated in the study. In each village, eight traps deployed along drainage lines over 24 hour duration were used to catch tsetse. One hundred systematically selected cattle in the study villages were examined for trypanosomes. All trypanosome-positive cattle were randomly allocated into two treatment groups: a group treated with 0.5 mg/kg bw. isometamidium chloride (ISMM) and a group treated with 3.5 mg/kg bw. diminazene aceturate (DIM). The cattle were monitored for trypanosomes at day 14 and 28 post-treatment. Of the 796 cattle examined, 125 (15.7 percent) were trypanosome positive. Village trypanosome prevalences ranged between 11 percent and 19 percent. There were no significant ($p > 0.05$) differences in the village trypanosome prevalences. *Trypanosoma congolense* was the dominant trypanosome species accounting for 73 percent (91/125) of the infections and *T. vivax* the remainder. Twenty (31.7 percent) of the 63 cattle on 0.5 mg/kg bw. ISMM treatment were still positive 14 days post-treatment. Of the 43 aparasitaemic cattle monitored to day 28, 25.6 percent (11) became parasitaemic, resulting in a cumulative failure rate of 49.2 percent (31/63). *Trypanosoma congolense* accounted for 77.4 percent (24/31) of failed ISMM treatments. The 62 cattle treated with 3.5 mg/kg bw. DIM resulted in 30.6

percent (19/62) failed treatments. Although 42.2 percent (19/45) of *T. congolense* positive cattle did not respond to DIM treatment, all *T. vivax* positive cattle responded positively to DIM treatment. The overreliance on trypanocides in the control of trypanosomosis will ultimately lead to multiple drug resistant trypanosome populations as detected in villages in south-east Mali rendering the use of drugs doubtful. Effective alternative methods for trypanosomosis control ought to substitute chemotherapy to ensure sustainable cattle production in these villages. Since there is no single strategy for containing trypanocidal drug resistance, promotion of an integrated approach combining proven trypanosomosis control approaches in high trypanosomosis risk areas is most desirable. The best-bet strategy this study recommended for areas with multiple drug resistance included area-wide community tsetse control, control of co-infections to exploit self-cure against resistant trypanosome populations and the rational use of trypanocidal drugs which should be urgently promoted at all levels as a way of containing or reversing resistance.

16386. **Vitouley, H. S., Sidibe, I., Bengaly, Z., Marcotty, T., Van Den Abbeele, J. & Delespaux, V., 2012.** Is trypanocidal drug resistance a threat for livestock health and production in endemic areas? Food for thoughts from Sahelian goats infected by *Trypanosoma vivax* in Bobo Dioulasso (Burkina Faso). *Veterinary Parasitology*, **190** (3-4): 349-354.

Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), 01BP454 Bobo Dioulasso 01, Burkina Faso. [vdelespaux@itG.be]

Trypanocidal drug resistance is unanimously recognized as a threat for livestock production in regions where the prevalence of trypanosomosis is high. To assess the impact of the disease and the effect of drug resistance on the health of small ruminants, twelve *Trypanosoma vivax* isolates collected in six villages in the vicinity of Bobo Dioulasso (Burkina Faso) were injected into 12 groups of five Sahelian goats, two being treated with 3.5 mg/kg bw. diminazene aceturate (DA), two with 0.5mg/kg bw. isometamidium chloride (ISM) and one left untreated as control. Monitoring was performed every five days for 100 days to evaluate the parasitaemia by buffy coat examination, the haematocrit and the bodyweight. Among the 12 groups, six were additionally monitored using a trypanosome specific 18S-PCR-RFLP every five days from day 30 to day 100 to verify the complete clearance of the parasites from the blood of the hosts. In six groups of goats, trypanosomes disappeared completely after treatment, five groups showed relapses in at least one goat treated with ISM and one group showed relapses in one goat treated with DA and one with ISM. For the six groups that were screened using both microscopic examination and trypanosome specific 18S-PCR-RFLP, the following results were obtained: for the groups treated with DA, no relapses by microscopic examination and 83.3 percent (10/12) using the 18S-PCR-RFLP. For the groups treated with ISM, 25 percent (3/12) relapses by microscopic examination and 83.3 percent with the 18S-PCR-RFLP (10/12). The evolution of the PCV and bodyweight during the observation period from relapsing (either by microscopical examination or by 18S-PCR-RFLP diagnosis) and non-relapsing animals were compared. The relative average PCV in goats that relapsed microscopically decreased significantly more than in non-relapsing goats. This difference was not significant when relapses were detected using the trypanosome specific 18S-PCR-RFLP. This indicates that only the animals with the highest parasitaemia suffered from the infection. Relapses after treatment where the host controls the parasitaemia to a level below the sensitivity of the microscopical examination do

not affect bodyweight or PCV.

7. EXPERIMENTAL TRYPANOSOMOSIS

(a) DIAGNOSTICS

16387**. Luciani, M., Di Pancrazio, C., Di Febo, T., Tittarelli, M., Podaliri Vulpiani, M., Puglielli, M. O., Naessens, J. & Sacchini, F., 2012.** IgG antibodies from dourine infected horses identify a distinctive *Trypanosoma equiperdum* antigenic pattern of low molecular weight molecules. *Veterinary Immunology & Immunopathology,* **151(**1-2): 140-146.

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Via Campo Boario, 64100 Teramo, Italy. [f.sacchini@izs.it].

Diagnosis and control of dourine are strongly based on serological evidence, but knowledge of the humoral response of horses during infection is limited. In this study we developed a chemiluminescent immunoblotting (cIB) assay to characterise the *Trypanosoma equiperdum* antigen pattern recognised by IgGs from naturally or experimentally dourine-infected horses and analyse the kinetics of the IgG humoral response following infection. One compounding factor is that sera from uninfected animals often cross-react with *T. equiperdum* antigens. Development of the cIB assay was based on the hypothesis that serum IgGs from healthy and infected animals recognise different *T. equiperdum* antigen patterns. We used sera from eight naturally infected horses which had recovered from Italian outbreaks and two experimentally infected mares. In addition, sera from 10 healthy control animals, eight of which were CFT positive but IFA negative for dourine, were collected from disease-free regions. Sera were compared by the complement fixation test (CFT), indirect immune fluorescence (IFA) and the cIB assay. cIB analysis revealed that IgGs from infected horses, in contrast to IgGs from healthy horses, specifically recognise a *T. equiperdum* antigenic profile with low molecular weight bands ranging between 16 and 35kDa. A time course experiment indicated that IgGs specific for the 16-35kDa parasite protein fraction appear 17 days post-infection. The cIB assay confirmed all ten infected animals as positive and all controls as negative. This study demonstrated that analysis of IgGs by cIB can provide clear confirmation of trypanosome infection in horses, suggesting that this technique can be applied as a confirmatory serological test for dourine infection.

16388**. Menachery, A., Kremer, C., Wong, P. E., Carlsson, A., Neale, S. L., Barrett, M. P. & Cooper, J. M., 2012.** Counterflow dielectrophoresis for trypanosome enrichment and detection in blood. *Science Reports*, **2**: 775.

Department of Electronics and Electrical Engineering, The Bioelectronics Research Centre, College of Science and Engineering, Rankine Building, University of Glasgow, Glasgow G12 8LT, Scotland, UK. [Barrett@glasgow.ac].

Human African trypanosomiasis or sleeping sickness is a deadly disease endemic in sub-Saharan Africa, caused by single-celled protozoan parasites. Although it has been targeted for elimination by 2020, this will only be realized if diagnosis can be improved to enable

identification and treatment of afflicted patients. Existing techniques of detection are restricted by their limited field applicability, sensitivity and capacity for automation. Microfluidic-based technologies offer the potential for highly sensitive automated devices that could achieve detection at the lowest levels of parasitaemia and consequently help in the elimination programme. In this work we implement an electrokinetic technique for the separation of trypanosomes from both mouse and human blood. This technique utilises differences in polarisability between the blood cells and trypanosomes to achieve separation through opposed bi-directional movement (cell counterflow). We combine this enrichment technique with an automated image analysis detection algorithm, negating the need for a human operator.

16389**. Pruvot, M., Kamyingkird, K., Desquesnes, M., Sarataphan, N. & Jittapalapong, S., 2013.** The effect of the DNA preparation method on the sensitivity of PCR for the detection of *Trypanosoma evansi* in rodents and implications for epidemiological surveillance efforts. *Veterinary Parasitology*, **191** (3-4): 203-208.

Centre de Cooperation Internationale en Recherche Agronomique pour le Développement (CIRAD), UMR Intertryp, F-34398 Montpellier, France. [fvetspj@ku.ac.th].

Trypanosoma evansi is responsible for the most largely distributed animal trypanosomosis, affecting a wide range of wild and domestic animals. Its surveillance requires the implementation of standardized and reliable diagnostic tools. Although the development of polymerase chain reaction (PCR) tools has greatly improved our diagnostic capacity, factors affecting their sensitivity need to be acknowledged and accounted for in the interpretation of results. The targeted gene and the primer design have already been shown to greatly affect the sensitivity of a PCR, and the best performing sets of primers have been previously identified. However, the sensitivity of the PCR is also largely influenced by the DNA extraction or sample preparation method. In this paper, we selected six DNA extraction or blood sample preparation methods representative of what would be used in a budget-constrained setting: phenol-chloroform, Chelex®, Flexigen (Qiagen® kit), Genekam® kit and two original protocols using sodium hydroxide. We studied the effects of the preparation method on the detection limit of the subsequent PCR. Our results show that the extraction method strongly affects the PCR sensitivity. The classical phenol-chloroform extraction method allowed for the PCR with the lowest detection limit. Some combinations of extraction method and primer set had detection limits that were not compatible with their use as a reliable diagnostic test, and would severely reduce the performance of a surveillance programme. Therefore, we encourage laboratories to carefully select their sample preparation and PCR protocols, depending on the aimed sensitivity, cost, safety, time requirement and objectives.

16390**. Sharma, P., Juyal, P. D., Singla, L. D., Chachra, D. & Pawar, H., 2012.** Comparative evaluation of real time PCR assay with conventional parasitological techniques for diagnosis of *Trypanosoma evansi* in cattle and buffaloes. *Veterinary Parasitology*, **190** (3-4): 375-382.

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

For comparative evaluation, a real time PCR assay was standardized by using TaqMan primer and probe targeting the internal transcribed spacer 1 (ITS-1) region of rRNA for *Trypanosoma evansi* and sensitivity was evaluated by using DNA extracted from diethyleamino ethane cellulose purified trypanosomes and whole blood of trypanosome-infected mice. The minimum detection limit for purified l DNA was 0.01 ng (approximately 0.33 genomic DNA of *T. evansi*) whereas for whole blood the minimum detection limit was 0.1 ng (approximately 6.12 genomic DNA). *T. evansi*-infected mice blood samples were collected at different intervals post-infection and were analysed by conventional parasitological methods (CPT) viz. wet blood smear, thin blood smear, thick blood smear, quantitative buffy coat and real time PCR. It was found that TaqMan assay was twice as sensitive as CPT in case of *in vivo* infectivity in mice and gave a positive signal at 36 h post infection whereas QBC and blood smear examination were able to detect at 60 h and 72 h post-infection respectively. A total 109 (80 cattle and 29 buffaloes) blood samples was collected from in and around Ludhiana district and analysed by CPT and real time PCR. The overall prevalence of *T. evansi* by CPT in cattle and buffaloes was 2.75 per cent. The prevalence rate was 2.5 per cent in cattle and 3.45 per cent in buffaloes. By real time PCR overall prevalence was 12.84 per cent in cattle and buffaloes, with a prevalence rate of 12.50 per cent in cattle and 13.79 per cent in buffaloes.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **35**: 16436, 16443, 16449, 16471]

16391**. Alsford, S. & Horn, D., 2012.** Cell-cycle-regulated control of VSG expression site silencing by histones and histone chaperones ASF1A and CAF-1b in *Trypanosoma brucei*. *Nucleic Acids Research*, **40** (20): 10150-10160.

London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK. [david.horn@lshtm.ac.uk].

Antigenic variation in African trypanosomes involves monoallelic expression and reversible silencing of variant surface glycoprotein (VSG) genes found adjacent to telomeres in polycistronic expression sites (ESs). We assessed the impact on ES silencing of five candidate essential chromatin-associated factors that emerged from a genome-wide RNA interference viability screen. Using this approach, we demonstrate roles in VSG ES silencing for two histone chaperones. Defects in S-phase progression in cells depleted for histone H3, or either chaperone, highlight in particular the link between chromatin assembly and DNA replication control. S-phase checkpoint arrest was incomplete, however, allowing G(2)/M-specific VSG ES derepression following knockdown of histone H3. In striking contrast, knockdown of anti-silencing factor 1A (ASF1A) allowed for de-repression at all cell cycle stages, whereas knockdown of chromatin assembly factor 1b (CAF-1b) revealed de-repression predominantly in S-phase and G(2)/M. Our results support a central role for chromatin in maintaining VSG ES silencing. ASF1A and CAF-1b appear to play constitutive and DNA replication-dependent roles, respectively, in the recycling and assembly of chromatin. Defects in these functions typically lead to arrest in S-phase but defective cells can also progress through the cell cycle leading to nucleosome depletion and de-repression of telomeric VSG ESs.

16392**. Barry, J. D., Hall, J. P. & Plenderleith, L., 2012.** Genome hyperevolution and the success of a parasite. *Annals of the New York Academy of Sciences*, **1267**: 11-17.

Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary, & Life Sciences, University of Glasgow, Glasgow, UK. [dave.barry@glasgow.ac.uk].

The strategy of antigenic variation is to present a constantly changing population phenotype that enhances parasite transmission, through evasion of immunity arising within, or existing between, host animals. Trypanosome antigenic variation occurs through spontaneous switching among members of a silent archive of many hundreds of variant surface glycoprotein (VSG) antigen genes. As with such contingency systems in other pathogens, switching appears to be triggered through inherently unstable DNA sequences. The archive occupies subtelomeres, a genome partition that promotes hypermutagenesis and, through telomere position effects, singular expression of VSG. Trypanosome antigenic variation is augmented greatly by the formation of mosaic genes from segments of pseudo-VSG, an example of implicit genetic information. Hypermutation occurs apparently evenly across the whole archive, without direct selection on individual VSG, demonstrating second-order selection of the underlying mechanisms. Coordination of antigenic variation, and thereby transmission, occurs through networking of trypanosome traits expressed at different scales from molecules to host populations.

16393**. Caljon, G., Stijlemans, B., Saerens, D., Van Den Abbeele, J., Muyldermans, S., Magez, S. & De Baetselier, P., 2012.** Affinity is an important determinant of the anti-trypanosome activity of nanobodies. *PLoS Neglected Tropical Diseases*, **6** (11): e1902.

Department of Biomedical Sciences, Unit of Veterinary Protozoology, Institute of Tropical Medicine Antwerp, Antwerp, Belgium; Research Unit of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium; and Laboratory of Myeloid Cell Immunology, VIB, Brussels, Belgium. [gcaljon@itg.be].

The discovery of nanobodies (Nbs) with a direct toxic activity against African trypanosomes is a recent advancement towards a new strategy against these extracellular parasites. The anti-trypanosomal activity relies on perturbing the highly active recycling of the variant-specific surface glycoprotein (VSG) that occurs in the parasite's flagellar pocket. Here we expand the existing panel of Nbs with anti-*Trypanosoma brucei* potential and identify four categories based on their epitope specificity. We modified the binding properties of previously identified nanobodies Nb_An05 and Nb_An33 by site-directed mutagenesis in the paratope and found this to strongly affect trypanotoxicity despite retention of antigen-targeting properties. Affinity measurements for all identified anti-trypanosomal Nbs reveal a strong correlation between trypanotoxicity and affinity (K_D), suggesting that it is a crucial determinant for this activity. Half maximal effective (50 percent) affinity of 57 nM was calculated from the non-linear dose-response curves. In line with these observations, Nb humanizing mutations only preserved the trypanotoxic activity if the K_D remained unaffected. This study reveals that the binding properties of nanobodies need to be compatible with achieving an occupancy of >95 percent saturation of the parasite surface VSG in order to

exert an anti-trypanosomal activity. As such, Nb-based approaches directed against the VSG target would require binding to an accessible, conserved epitope with high affinity.

16394**. Costa, M. M., Silva, A. S., Paim, F. C., Franca, R., Dornelles, G. L., Thome, G. R., Serres, J. D., Schmatz, R., Spanevello, R. M., Goncalves, J. F., Schetinger, M. R., Mazzanti, C. M., Lopes, S. T. & Monteiro, S. G., 2012.** Cholinesterase as inflammatory markers in an experimental infection by *Trypanosoma evansi* in rabbits. *Anais da Academia Brasiliera Ciencias*, **84** (4): 1105-1113.

Laboratorio de Analises Clinicas Veterinaria (LACVet), Universidade Federal de Santa Maria, Santa Maria, RS, Brazil, 97105-900.

The aim of this study was to evaluate the role of cholinesterases as an inflammatory marker in acute and chronic infection by *Trypanosoma evansi* in experimentally infected rabbits. Twelve adult female New Zealand rabbits were used and divided into two groups with six animals each: control group (rabbits 1-6) and infected group (rabbits 7-12). The infected group received intraperitoneally 0.5 mL of blood from a rat containing 108 parasites per animal. Blood samples used for cholinesterase evaluation were collected on days 0, 2, 7, 12, 27, 42, 57, 87, 102 and 118 post-inoculation (PI). Increased activity ($p<0.05$) of butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) was observed in the blood on days 7 and 27, respectively and no differences were observed in cholinesterase activity in other periods. No significant difference in AChE activity ($p>0.05$) was observed in the encephalic structures. The increased activities of AChE and BChE probably have a pro-inflammatory purpose, attempting to reduce the concentration of acetylcholine, a neurotransmitter which has an anti-inflammatory property. Therefore, cholinesterases may be inflammatory markers in infection with *T. evansi* in rabbits.

16395**. Da Silva, A. S., Paim, F. C., Santos, R. C., Sangoi, M. B., Moresco, R. N., Lopes, S. T., Jaques, J. A., Baldissarelli, J., Morsch, V. M. & Monteiro, S. G., 2012.** Nitric oxide level, protein oxidation and antioxidant enzymes in rats infected by *Trypanosoma evansi*. *Experimental Parasitology*, **132** (2): 166-170.

Department of Animal Science, Universidade do Estado de Santa Catarina, Brazil. [aleksandro_ss@yahoo.com.br].

The aim of this study was to evaluate the nitric oxide (NO) level, protein oxidation and antioxidant enzymes in rats infected with *Trypanosoma evansi* and establish the association of NO levels with the degree of parasitaemia. Thirty-six male rats (Wistar) were divided into two groups with 18 animals each. Group A was not infected while Group B was intraperitoneally infected, receiving $7.5x10^6$ trypomastigotes per animal. Each group was divided into three subgroups with six rats each and blood was collected during different periods post-infection (PI), as follows: day 5 (A(5) and B(5)), day 15 (A(15) and B(15)) and day 30 PI (A(30) and B(30)). Blood samples were collected by cardiac puncture to estimate the levels of nitrites/nitrates (NO_x) and advanced oxidation protein products (AOPP) in serum, and superoxide dismutase (SOD) and catalase (CAT) activities in blood. On days 15 and 30 PI NO_x and AOPP levels were increased in serum of rats infected. Rodents infected with *T. evansi* showed a significant increase in SOD (days 5 and 15 PI) and CAT (day 30 PI) activities. Based on the physiological role of NO, we can conclude that its increased

concentration is related to an inflammatory response against the parasite, once a redox imbalance was observed during infection.

16396**. Eze, J. I., Orajaka, L. J., Okonkwo, N. C., Ezeh, I. O., Ezema, C. & Anosa, G. N., 2012.** Effect of probiotic (*Saccharomyces cerevisiae*) supplementation on immune response in *Trypanosoma brucei brucei* infected rats. *Experimental Parasitology*, **132** (4): 434-439.

Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. [jamesifeeze@yahoo.com].

The immunomodulatory effect of the probiotic (*Saccharomyces cerevisiae*) on *Trypanosoma brucei brucei* infected rats was studied. Thirty (30) rats divided into five groups (A-E) of six rats each were used for the study. Groups A, B and C rats received feed supplemented with *S. cerevisiae* (at 0.08, 0.12 and 0.16 percent /kg of feed, respectively) for the duration of the study. Groups D and E diets were not supplemented. All the rats in the five groups were immunized with 0.3ml of 10 percent sheep red blood cells (SRBC) at day 7 pre-supplementation, and booster doses given every 14 days thereafter. On day 28 post supplementation (PS), rats of groups A-D were infected with 1 x 10^6 of *T. brucei brucei* intraperitoneally. Supplementation resulted in increases in antibody titres to SRBC which later declined following *T. brucei brucei* infection, but remained higher than the pre-supplementation titres. At termination of the study (i.e. day 49 PS) supplemented groups had significantly ($p<0.05$) higher antibody titres than either the infected or the non-infected controls. The total and differential leucocyte counts followed a similar pattern with initial increases in counts following supplementation followed by reductions after *T. brucei brucei* infection. Supplementation also resulted in declines in parasitaemia with significant difference between the supplemented groups and the un-supplemented controls on day 42 post-infection. The results are an indication that probiotics can be used to ameliorate the immunosuppressive effect of *T. brucei brucei* infections.

16397**. Frevert, U., Movila, A., Nikolskaia, O. V., Raper, J., Mackey, Z. B., Abdulla, M., McKerrow, J. & Grab, D. J., 2012.** Early invasion of brain parenchyma by African trypanosomes. *PLoS One*, **7** (8): e43913.

Division of Medical Parasitology, Department of Microbiology, New York University School of Medicine, New York, New York, USA. [dgrab1@jhmi.edu].

Human African trypanosomiasis or sleeping sickness is a vector-borne parasitic disease that has a major impact on human health and welfare in sub-Saharan countries. Based mostly on data from animal models, it is currently thought that trypanosome entry into the brain occurs by initial infection of the choroid plexus and the circumventricular organs followed days to weeks later by entry into the brain parenchyma. However, *Trypanosoma brucei* bloodstream forms rapidly cross human brain microvascular endothelial cells *in vitro* and appear to be able to enter the murine brain without inflicting cerebral injury. Using a murine model and intravital brain imaging, we show that bloodstream forms of *T. b. brucei* and *T. b. rhodesiense* enter the brain parenchyma within hours, before a significant level of microvascular inflammation is detectable. Extravascular bloodstream forms were viable as

indicated by motility and cell division, and remained detectable for at least 3 days post infection suggesting the potential for parasite survival in the brain parenchyma. Vascular inflammation, as reflected by leukocyte recruitment and emigration from cortical microvessels, became apparent only with increasing parasitaemia at later stages of the infection, but was not associated with neurological signs. Extravascular trypanosomes were predominantly associated with post capillary venules suggesting that early brain infection occurs by parasite passage across the neuroimmunological blood brain barrier. Thus, trypanosomes can invade the murine brain parenchyma during the early stages of the disease before meningoencephalitis is fully established. Whether individual trypanosomes can act alone or require the interaction from a quorum of parasites remains to be shown. The significance of these findings for disease development is now testable.

16398**. Kateete, D., Alezuyo, C., Nanteza, A., Asiimwe, C. & Lubega, G., 2012.** *In vitro* trypanocidal activity of antibodies to bacterially expressed *Trypanosoma brucei* tubulin. *Iranian Journal of Parasitology*, **7** (3): 54-63.

School of Biomedical Sciences, Makerere University College of Health Sciences, Kampala, Uganda; and College of Veterinary Medicine, Animal Resources & Biosecurity, Makerere University, Kampala, Uganda.

There are only four drugs for treating African trypanosomiasis, a devastating disease in sub-Saharan Africa. With slow discovery of better drugs, vaccination is viewed as the best method of control. We previously showed that antibodies to native *Trypanosoma brucei brucei* tubulin inhibit the growth of trypanosomes in culture. Here, we aimed to determine the effect of antibodies to bacterially expressed trypanosome tubulin on *T. brucei brucei* growth. *T. brucei brucei* alpha and beta tubulin genes were individually expressed in *Escherichia coli* under the tryptophan promoter. Monoclonal tubulin antibodies reacted specifically with the expressed tubulins with no cross-reaction with the opposite tubulin. Rabbits were immunized with 450µg each of the concentrated recombinant tubulin, and production of antibodies assessed by ELISA and Western blotting. The effect of polyclonal antibodies on trypanosome growth was determined by culturing bloodstream *T. brucei brucei* in up to 25 percent of antisera. Low antisera dilutions (25 percent) from the immunized rabbits inhibited trypanosome growth. The most cytotoxic antisera were from one rabbit immunized with a mixture of both alpha and beta tubulins. However, the result was not reproduced in other rabbits and there was no apparent effect on growth at higher antisera dilutions. It is concluded that antibodies to bacterially expressed trypanosome tubulin are not effective at killing cultured bloodstream trypanosomes.

16399**. Okwor, I., Onyilagha, C., Kuriakose, S., Mou, Z., Jia, P. & Uzonna, J. E., 2012.** Regulatory T cells enhance susceptibility to experimental *Trypanosoma congolense* infection independent of mouse genetic background. *PLoS Neglected Tropical Diseases*, **6** (7): e1761.

Department of Medical Microbiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada. [uzonna@cc.umanitoba.ca].

BALB/c mice are highly susceptible while C57BL/6 are relatively resistant to experimental *Trypanosoma congolense* infection. Although regulatory T cells (Tregs) have been shown to

regulate the pathogenesis of experimental *T. congolense* infection, their exact role remains controversial. We wished to determine whether Tregs contribute to distinct phenotypic outcomes in BALB/c and C57BL/6 mice and if so how they operate with respect to control of parasitaemia and production of disease-exacerbating proinflammatory cytokines. BALB/c and C57BL/6 mice were infected intraperitoneally (i.p) with 10^3*T. congolense* clone TC13 and both the kinetics of Tregs expansion and intracellular cytokine profiles in the spleens and livers were monitored directly *ex vivo* by flow cytometry. In some experiments, mice were injected with anti-CD_{25} mAb prior or post *T. congolense* infection or adoptively (by intravenous route) given highly enriched naive CD_{25}+ T lymphocytes prior to *T. congolense* infection and the inflammatory cytokine/chemokine levels and survival were monitored. In contrast to a transient and non-significant increase in the percentages and absolute numbers of CD_4+CD_{25}+$Foxp_3$+ T cells (Tregs) in C57BL/6 mouse spleens and livers, a significant increase in the percentage and absolute numbers of Tregs was observed in spleens of infected BALB/c mice. Ablation or increasing the number of CD_{25}+ cells in the relatively resistant C57BL/6 mice by anti-CD_{25} mAb treatment or by adoptive transfer of CD_{25}+ T cells, respectively, ameliorates or exacerbates parasitaemia and production of proinflammatory cytokines. Collectively, our results show that regulatory T cells contribute to susceptibility in experimental murine trypanosomiasis in both the highly susceptible BALB/c and relatively resistant C57BL/6 mice.

16400**. Povelones, M. L., Gluenz, E., Dembek, M., Gull, K. & Rudenko, G., 2012.** Histone H1 plays a role in heterochromatin formation and VSG expression site silencing in *Trypanosoma brucei*. *PLoS Pathogens*, **8** (11): e1003010.

Division of Cell and Molecular Biology, Imperial College London, South Kensington, London, UK. [gloria.rudenko@imperial.ac.uk].

The African sleeping sickness parasite *Trypanosoma brucei* evades the host immune system through antigenic variation of its variant surface glycoprotein (VSG) coat. Although the *T. brucei* genome contains approximately 1 500 VSGs, only one VSG is expressed at a time from one of about 15 subtelomeric VSG expression sites (ESs). For antigenic variation to work, not only must the vast VSG repertoire be kept silent in a genome that is mainly constitutively transcribed, but the frequency of VSG switching must be strictly controlled. Recently it has become clear that chromatin plays a key role in silencing inactive ESs, thereby ensuring monoallelic expression of VSG. We investigated the role of the linker histone H1 in chromatin organization and ES regulation in *T. brucei*. *T. brucei* histone H1 proteins have a different domain structure to H1 proteins in higher eukaryotes. However, we show that they play a key role in the maintenance of higher order chromatin structure in bloodstream form *T. brucei* as visualised by electron microscopy. In addition, depletion of histone H1 results in chromatin becoming generally more accessible to endonucleases in bloodstream but not in insect form *T. brucei*. The effect on chromatin following H1 knock-down in bloodstream form *T. brucei* is particularly evident at transcriptionally silent ES promoters, leading to 6-8 fold de-repression of these promoters. *T. brucei* histone H1 therefore appears to be important for the maintenance of repressed chromatin in bloodstream form *T. brucei*. In particular H1 plays a role in downregulating silent ESs, arguing that H1-mediated chromatin functions in antigenic variation in *T. brucei*.

16401**. Stijlemans, B., Vankrunkelsven, A., Brys, L., Raes, G., Magez, S. & De**

Baetselier, P., 2010. Scrutinizing the mechanisms underlying the induction of anaemia of inflammation through GPI-mediated modulation of macrophage activation in a model of African trypanosomiasis. *Microbes & Infection*, **12** (5): 389-399.

Department of Molecular and Cellular Interactions, VIB, 1050 Brussel, Belgium. [bstijlem@vub.ac.be].

In animal trypanosomiasis the severity of infection is reflected by the degree of anaemia which resembles anaemia of inflammation, involving a skewed iron homeostasis leading to iron accumulation within the reticuloendothelial system. Myeloid cells (M cells) have been implicated in the induction and maintenance of this type of anaemia and modulation of M cells through the main trypanosome-derived glycosylphosphatidylinositol (GPI)-anchor could attenuate both anaemia and trypano-susceptibility in *Trypanosoma brucei*-infected mice. Herein the GPI-based treatment, allowing a straightforward comparison between trypanotolerance and susceptibility in *T. brucei*-infected C57Bl/6 mice, was further adopted to scrutinize mechanisms/pathways underlying trypanosome-elicited anaemia. Hereby, the following interlinkable observations were made in GPI-based treated (GBT) *T. brucei*-infected mice: (i) a reduced inflammatory cytokine production and increased IL-10 production associated with alleviation of anaemia and restoration of serum iron levels; (ii) a shift in increased liver expression of iron storage towards iron export genes; and (iii) increased erythropoiesis in the bone marrow and extramedullar sites (spleen) probably reflecting a normalized iron homeostasis and availability. Collectively, our results demonstrate that reprogramming macrophages towards an anti-inflammatory state alleviates anaemia of inflammation by normalizing iron homeostasis and restoring erythropoiesis.

16402**. Wolburg, H., Mogk, S., Acker, S., Frey, C., Meinert, M., Schonfeld, C., Lazarus, M., Urade, Y., Kubata, B. K. & Duszenko, M., 2012.** Late stage infection in sleeping sickness. *PLoS One*, **7** (3): e34304.

Institute of Pathology and Neuropathology, University of Tubingen, Tubingen, Germany. [michael.duszenko@uni-tuebingen.de].

At the turn of the 19th century, trypanosomes were identified as the causative agent of sleeping sickness and their presence within the cerebrospinal fluid of late stage sleeping sickness patients was described. However, no definitive proof of how the parasites reach the brain has been presented so far. Analysing electron micrographs prepared from rodent brains more than 20 days after infection, we present here conclusive evidence that the parasites first enter the brain via the choroid plexus from where they penetrate the epithelial cell layer to reach the ventricular system. Conversely, no trypanosomes were observed within the parenchyma outside blood vessels. We also show that brain infection depends on the formation of long slender trypanosomes and that the cerebrospinal fluid as well as the stroma of the choroid plexus is a hostile environment for the survival of trypanosomes which enter the pial space including the Virchow-Robin space via the subarachnoid space to escape degradation. Our data suggest that trypanosomes do not intend to colonize the brain but reside near or within the glia limitans, from where they can re-populate blood vessels and disrupt the sleep wake cycles.

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[See also **35**: 16439, 16472, 16483, 16486, 16493]

16403. **Abdelmohsen, U. R., Szesny, M., Othman, E. M., Schirmeister, T., Grond, S., Stopper, H. & Hentschel, U., 2012.** Antioxidant and anti-protease activities of diazepinomicin from the sponge-associated *Micromonospora* strain RV115. *Marine Drugs*, **10** (10): 2208-2221.

Julius-von-Sachs-Institute for Biological Sciences, University of Wurzburg, Julius-von-Sachs-Platz 3, Wurzburg 97082, Germany. [ute.hentschel@uni-wuerzburg.de].

Diazepinomicin is a dibenzodiazepine alkaloid with an unusual structure among the known microbial metabolites discovered so far. Diazepinomicin was isolated from the marine sponge-associated strain *Micromonospora* sp. RV115 and was identified by spectroscopic analysis and by comparison with literature data. In addition to its interesting preclinical broad-spectrum antitumour potential, we report here new antioxidant and anti-protease activities for this compound. Using the ferric reducing antioxidant power (FRAP) assay, a strong antioxidant potential of diazepinomicin was demonstrated. Moreover, diazepinomicin showed a significant antioxidant and protective capacity from genomic damage induced by the reactive oxygen species hydrogen peroxide in human kidney (HK-2) and human promyelocytic (HL-60) cell lines. Additionally, diazepinomicin inhibited the proteases rhodesain and cathepsin L at an IC_{50} of 70-90 µM. It also showed antiparasitic activity against trypomastigote forms of *Trypanosoma brucei* with an IC_{50} of 13.5 µM. These results showed unprecedented antioxidant and anti-protease activities of diazepinomicin, thus further highlighting its potential as a future drug candidate.

16404**. Abdel-Salam, O. M., Khadrawy, Y. A., Mohammed, N. A. & Youness, E. R., 2012.** The effect of gabapentin on oxidative stress in a model of toxic demyelination in rat brain. *Journal of Basic & Clinical Physiology & Pharmacology*, **23** (2): 61-68.

Department of Toxicology and Narcotics, National Research Centre, Dokki, Cairo, Egypt. [omasalam@hotmail.com].

16405. **Adeyemi, O. S. & Sulaiman, F. A., 2012.** Biochemical and morphological changes in *Trypanosoma brucei brucei*-infected rats treated with homidium chloride and diminazene aceturate. *Journal of Basic & Clinical Physiology & Pharmacology,* **23**(4): 179-183. **E Publication ahead of print, October 12.**

University of Ilorin, Department of Biochemistry, PMB 1515, Ilorin, Nigeria. [adeyemiy@run.edu.ng].

Chemotherapy which is one of the major methods for controlling trypanosomal infections is beset with several challenges including unwanted toxicity and limited efficacy. These factors and others underscore research efforts aimed at finding safer and more effective therapeutic agents for trypanosomiasis. Homidium chloride and diminazene aceturate are registered drugs for the treatment of animal trypanosomiasis. This study investigated and compared, in an experimental *Trypanosoma* infection, the effects of two trypanocides on the pathology of tissues and some biochemical indices in rats. Data revealed that the levels of

alkaline phosphatase, alanine transaminase and aspartate transaminase in infected positive animals were significantly ($p<0.05$) elevated relative to uninfected negative controls but showed no significant difference when compared with the trypanocide-treatment groups. The histopathological presentations in the infected and treatment groups are a demonstration of the inimical cellular alterations associated with *Trypanosoma brucei brucei* infection. The alterations to biochemical and morphological parameters observed in the infected as well as the treatment groups suggest shortcomings of the investigated trypanocides to alleviate pathology associated with *Trypanosoma brucei brucei* infection. We present evidence that further supports the urgent need for the development of safer and more effective trypanocides.

16406. **Al-Musayeib, N. M., Mothana, R. A., Al-Massarani, S., Matheeussen, A., Cos, P. & Maes, L., 2012.** Study of the *in vitro* antiplasmodial, antileishmanial and antitrypanosomal activities of medicinal plants from Saudi Arabia. *Molecules*, **17** (10): 11379-11390.

Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia. [nalmusayeib@ksu.edu.sa].

The present study investigated the *in vitro* antiprotozoal activity of sixteen selected medicinal plants. Plant materials were extracted with methanol and screened *in vitro* against erythrocytic schizonts of *Plasmodium falciparum*, intracellular amastigotes of *Leishmania infantum* and *Trypanosoma cruzi* and free trypomastigotes of *T. brucei*. Cytotoxic activity was determined against MRC-5 cells to assess selectivity. The criterion for activity was an IC_{50} < 10 µg/mL (<5 µg/mL for *T. brucei*) and a selectivity index of >/=4. Antiplasmodial activity was found in the extracts of *Prosopis juliflora* and *Punica granatum*. Antileishmanial activity against *L. infantum* was demonstrated in *Caralluma sinaica* and *Periploca aphylla*. Amastigotes of *T. cruzi* were affected by the methanol extract of *Albizia lebbeck pericarp, Caralluma sinaica, Periploca aphylla* and *Prosopius juliflora*. Activity against *T. brucei* was obtained in *Prosopis juliflora*. Cytotoxicity (MRC-5 IC_{50} < 10 µg/mL) and hence non-specific activities were observed for *Conocarpus lancifolius*.

16407. **Baker, N., Glover, L., Munday, J. C., Aguinaga Andres, D., Barrett, M. P., de Koning, H. P. & Horn, D., 2012.** Aquaglyceroporin 2 controls susceptibility to melarsoprol and pentamidine in African trypanosomes. *Proceedings of the National Academy of Sciences USA*, **109** (27): 10996-11001.

Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK. [david.horn@lshtm.ac.uk].

African trypanosomes cause sleeping sickness in humans, a disease that is typically fatal without chemotherapy. Unfortunately, drug resistance is common and melarsoprol-resistant trypanosomes often display cross-resistance to pentamidine. Although melarsoprol/pentamidine cross-resistance (MPXR) has been an area of intense interest for several decades, our understanding of the underlying mechanisms remains incomplete. Recently, a locus encoding two closely related aquaglyceroporins, AQP2 and AQP3, was linked to MPXR in a high-throughput loss-of-function screen. Here, we show that AQP2 has an unconventional "selectivity filter." AQP2-specific gene knockout generated MPXR trypanosomes but did not affect resistance to a lipophilic arsenical, whereas recombinant

AQP2 reversed MPXR in cells lacking native AQP2 and AQP3. AQP2 was also shown to be disrupted in a laboratory-selected MPXR strain. Both AQP2 and AQP3 gained access to the surface plasma membrane in insect life-cycle-stage trypanosomes but, remarkably, AQP2 was specifically restricted to the flagellar pocket in the bloodstream stage. We conclude that the unconventional aquaglyceroporin, AQP2, renders cells sensitive to both melarsoprol and pentamidine and that loss of AQP2 function could explain cases of innate and acquired MPXR.

16408. **Chianese, G., Fattorusso, E., Scala, F., Teta, R., Calcinai, B., Bavestrello, G., Dien, H. A., Kaiser, M., Tasdemir, D. & Taglialatela-Scafati, O., 2012.** Manadoperoxides, a new class of potent antitrypanosomal agents of marine origin. *Organic & Biomolecular Chemistry*, **10** (35): 7197-7207.

Dipartimento di Chimica delle Sostanze Naturali, Universita di Napoli Federico II, Napoli, Italy.

Chemical investigation of the marine sponge *Plakortis cfr. lita* afforded a library of endoperoxyketal polyketides, manadoperoxides B-K (3-5 and 7-13) and peroxyplakoric esters B_3 (6) and C (14). Eight of these metabolites are new compounds and some contain an unprecedented chlorine-bearing THF-type ring in the side chain. The library of endoperoxide derivatives was evaluated for *in vitro* activity against *Trypanosoma brucei rhodesiense* and *Leishmania donovani*. Some compounds, such as manadoperoxide B, exhibited ultrapotent trypanocidal activity (IC_{50} = 3 ng mL^{-1}) without cytotoxicity. Detailed examination of the antitrypanosomal activity data and comparison with those available in the literature for related dioxane derivatives enabled us to draw a series of structure-activity relationships. Interestingly, it appears that minor structural changes, such as a shift of the methyl group around the dioxane ring, can dramatically affect the antitrypanosomal activity. This information can be valuable to guide the design of optimized antitrypanosomal agents based on the dioxane scaffold.

16409. **Clark, R. L., Clements, C. J., Barrett, M. P., Mackay, S. P., Rathnam, R. P., Owusu-Dapaah, G., Spencer, J. & Huggan, J. K., 2012.** Identification and development of the 1,4-benzodiazepin-2-one and quinazoline-2,4-dione scaffolds as submicromolar inhibitors of HAT. *Bioorganic & Medicinal Chemistry*, **20** (20): 6019-6033.

Strathclyde Institute for Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, UK. [j.huggan@strath.ac.uk].

A library of 1,4-benzodiazepines has been synthesised and evaluated for activity against *Trypanosoma brucei*, a causative parasite of human African trypanosomiasis (HAT). The most potent of these derivatives has an MIC value of 0.97 µM. Herein we report the design, synthesis and biological evaluation of the above mentioned compounds.

16410. **De Rycker, M., O'Neill, S., Joshi, D., Campbell, L., Gray, D. W. & Fairlamb, A. H., 2012.** A static-cidal assay for *Trypanosoma brucei* to aid hit prioritisation for progression into drug discovery programmes. *PLoS Neglected Tropical Diseases*, **6** (11): e1932.

Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee, UK. [M.DeRycker@dundee.ac.uk].

Human African trypanosomiasis is a vector-borne disease of sub-Saharan Africa that causes significant morbidity and mortality. Current therapies have many drawbacks, and there is an urgent need for new, better medicines. Ideally such new treatments should be fast-acting cidal agents that cure the disease in as few doses as possible. Screening assays used for hit-discovery campaigns often do not distinguish cytocidal from cytostatic compounds and further detailed follow-up experiments are required. Such studies usually do not have the throughput required to test the large numbers of hits produced in a primary high-throughput screen. Here, we present a 384-well assay that is compatible with high-throughput screening and provides an initial indication of the cidal nature of a compound. The assay produces growth curves at ten compound concentrations by assessing trypanosome counts at 4, 24 and 48 hours after compound addition. A reduction in trypanosome counts over time is used as a marker for cidal activity. The lowest concentration at which cell killing is seen is a quantitative measure for the cidal activity of the compound. We show that the assay can identify compounds that have trypanostatic activity rather than cidal activity, and importantly, that results from primary high-throughput assays can overestimate the potency of compounds significantly. This is due to biphasic growth inhibition, which remains hidden at low starting cell densities and is revealed in our static-cidal assay. The assay presented here provides an important tool to follow-up hits from high-throughput screening campaigns and avoid progression of compounds that have poor prospects due to lack of cidal activity or overestimated potency.

16411. **Endeshaw, M., Zhu, X., He, S., Pandharkar, T., Cason, E., Mahasenan, K. V., Agarwal, H., Li, C., Munde, M., Wilson, W. D., Bahar, M., Doskotch, R. W., Kinghorn, A. D., Kaiser, M., Brun, R., Drew, M. E. & Werbovetz, K. A., 2012.** 8,8-dialkyldihydroberberines with potent antiprotozoal activity. *Journal of Natural Products*. **E Publication ahead of print, November 20.**

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

Semisynthetic 8,8-dialkyldihydroberberines (8,8-DDBs) were found to possess mid- to low-nanomolar potency against *Plasmodium falciparum* blood-stage parasites, *Leishmania donovani* intracellular amastigotes, and *Trypanosoma brucei brucei* bloodstream forms. For example, 8,8-diethyldihydroberberine chloride exhibited *in vitro* IC_{50} values of 77, 100, and 5.3 nM against these three parasites, respectively. In turn, two 8,8-dialkylcanadines, obtained by reduction of the corresponding 8,8-DDBs, were much less potent against these parasites *in vitro*. While the natural product berberine is a weak DNA binder, the 8,8-DDBs displayed no affinity for DNA, as assessed by changes in the melting temperature of poly(dA.dT) DNA. Selected 8,8-DDBs showed efficacy in mouse models of visceral leishmaniasis and African trypanosomiasis, with 8,8-dimethyldihydroberberine chloride reducing liver parasitaemia by 46 percent in *L. donovani*-infected BALB/c mice when given at an intraperitoneal dose of 10 mg/kg/day for five days. The 8,8-DDBs may thus serve as leads for discovering new antimalarial, antileishmanial and antitrypanosomal drug candidates.

16412. **Generaux, C. N., Ainslie, G. R., Bridges, A. S., Ismail, M. A., Boykin, D. W.,**

Tidwell, R. R., Thakker, D. R. & Paine, M. F., 2012. Compartmental and enzyme kinetic modelling to elucidate the biotransformation pathway of a centrally-acting antitrypanosomal prodrug. *Drug Metabolism & Disposition*. **E Publication ahead of print, December 6.**

The University of North Carolina at Chapel Hill, North Carolina, USA. [mpaine@unc.edu].

16413. **Hall, B. S., Meredith, E. L. & Wilkinson, S. R., 2012.** Targeting the substrate preference of a type I nitroreductase to develop antil quinone-based prodrugs. *Antimicrobial Agents & Chemotherapy*, **56** (11): 5821-5830.

Queen Mary Pre-Clinical Drug Discovery Group, School of Biological & Chemical Sciences, Queen Mary University of London, London, UK.

16414. **Harrington, J. M., Scelsi, C., Hartel, A., Jones, N. G., Engstler, M., Capewell, P., MacLeod, A. & Hajduk, S., 2012.** Novel African trypanocidal agents: membrane rigidifying peptides. *PLoS One*, 7 (9): e44384.

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

16415. **Kao, L. P., Ovchinnikov, D. & Wolvetang, E., 2012.** The effect of ethidium bromide and chloramphenicol on mitochondrial biogenesis in primary human fibroblasts. *Toxicology & Applied Pharmacology*, **261** (1): 42-49.

Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, Australia. [e.wolvetang@uq.edu.au].

16416. **Lama, R., Sandhu, R., Zhong, B., Li, B. & Su, B., 2012.** Identification of selective tubulin inhibitors as potential anti-trypanosomal agents. *Bioorganic & Medicinal Chemistry Letters*, **22** (17): 5508-5516.

Department of Chemistry, College of Sciences and Health Professions, Cleveland State University, 2121 Euclid Ave., Cleveland, OH 44115, USA. [b.su@csuohio.edu].

The potency of a series of sulphonamide tubulin inhibitors against the growth of *Trypanosoma brucei* (*T. brucei*), as well as human cancer and primary fibroblast cells were evaluated with the aim of determining whether compounds that selectively inhibit parasite proliferation could be identified. Several compounds showed excellent selectivity against *T. brucei* growth, and have the potential to be used for the treatment of human African trypanosomiasis. A *T. brucei* tubulin protein homology model was built based on the crystal structure of the bovine tubulin. The colchicine-binding domain, which is also the binding site of the tested sulfonamide tubulin inhibitors, showed clear differences between the tubulin structures and presumably explained the selectivity of the compounds.

16417**. Lavrado, J., Mackey, Z., Hansell, E., McKerrow, J. H., Paulo, A. & Moreira, R., 2012.** Antitrypanosomal and cysteine protease inhibitory activities of alkyldiamine cryptolepine derivatives. *Bioorganic & Medicinal Chemistry Letters*, **22** (19): 6256-6260.

iMed-UL, Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal. [rmoreira@ff.ul.pt].

Cryptolepine derivatives containing alkyldiamine side-chains, with potent inhibitory activity against *Trypanosoma brucei brucei* are reported. Compounds 2 showed improved activity and selectivity to *T. b. brucei* when compared with the lead compound. The most selective compound, 2k, presents a selectivity index value of 6 200 and an IC_{50} of 10nM against the parasite. These derivatives are also potent inhibitors of the trypanosome papain-like cysteine proteases cruzain, which could, at least in part, explain their anti-*Trypanosoma*l activity. Overall, these compounds with good activity and selectivity provide an encouraging starting point for the rational design of new and effective antitrypanosomal agents.

16418. **Lizzi, F., Veronesi, G., Belluti, F., Bergamini, C., Lopez-Sanchez, A., Kaiser, M., Brun, R., Krauth-Siegel, R. L., Hall, D. G., Rivas, L. & Bolognesi, M. L., 2012.** Conjugation of quinones with natural polyamines: toward an expanded antitrypanosomatid profile. *Journal of Medicinal Chemistry*, **55** (23): 10490-10500.

Department of Pharmacy and Biotechnologies, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy. [marialaura.bolognesi@unibo.it].

A combinatorial library of quinone-polyamine conjugates designed to optimize the antitrypanosomatid profile of hit compounds 1 and 2 has been prepared by a solid-phase approach. The conjugates were evaluated against the three most important human trypanosomatid pathogens (*Trypanosoma brucei rhodesiense, Trypanosoma cruzi*, and *Leishmania donovani*), and several showed promising activity. A subset also inhibited trypanothione reductase *in vitro* and induced oxidase activity of the enzyme. A highly potent analogue (7) was identified with activity against *T. brucei* at levels as low as 70 nM and a selectivity index of 72. Interestingly, the presence of a cadaverine tail confers to 7 the ability to target mitochondrial function in *Leishmania.* In fact, in *L. donovani* promastigotes, we verified for 7 a decrease of cytoplasmic ATP and mitochondrial potential. Therefore, the current results support the suitability of the conjugation approach for the development of novel polyamine conjugates with enhanced therapeutic potential.

16419**. Minagawa, N., 2012.** Mitochondria as targets of chemotherapy. *Yakugaku Zasshi*, **132** (10): 1093-1098.

Department of Health Chemistry, Niigata University of Pharmacy and Applied Life Sciences, 265-1 Higashijima, Akiha, Niigata, Japan. [minagawa@nupals.ac.jp].

Living organisms have developed a wide variety of energy metabolism to survive within their specialized environments. There is a remarkable diversity in mitochondrial electron transport systems, which might be potential targets for chemotherapy. Atovaquone, clinically used to treat malaria and pneumocystis pneumonia, is a specific inhibitor of the Qo site in the

cytochrome bc_1 complex of *Plasmodium falciparum* and *Pneumocystis jirovecii*. The phytopathogenic fungus *Ascochyta viciae* produces two antibiotics, ascochlorin and ascofuranone. Ascochlorin specifically binds to inhibit the electron transport of both Qi and Qo sites in cytochrome bc_1 complex. Besides the unique respiratory inhibition, further investigation is in progress to elucidate the effects on cancer cells. On the other hand, ascofuranone specifically inhibits cyanide-insensitive trypanosome alternative oxidase, which is a sole terminal oxidase in the mitochondrion of *Trypanosoma brucei*, the causative of African trypanosomiasis. *In vivo* studies suggest that ascofuranone is a promising candidate for chemotherapeutic agents to treat African trypanosomiasis.

16420. **Mothana, R. A., Al-Musayeib, N. M., Matheeussen, A., Cos, P. & Maes, L., 2012.** Assessment of the *in vitro* antiprotozoal and cytotoxic potential of 20 selected medicinal plants from the island of Soqotra. *Molecules*, **17** (12): 14349-14360.

Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia. [rmothana@ksu.edu.sa].

16421. **Navarro, G., Chokpaiboon, S., De Muylder, G., Bray, W. M., Nisam, S. C., McKerrow, J. H., Pudhom, K. & Linington, R. G., 2012.** Hit-to-lead development of the chamigrane endoperoxide merulin A for the treatment of African sleeping sickness. *PLoS One*, **7** (9): e46172.

Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, California, USA.

Human African trypanosomiasis (HAT) is an infectious disease with a large global health burden occurring primarily in Central and Eastern Africa. Most current treatments have poor blood brain barrier (BBB) penetration, which prevent them from targeting the most lethal stage of the infection. In addition, current therapeutics suffer from a variety of limitations ranging from serious side effects to difficulties with treatment administration. Therefore it is of crucial importance to find new treatments that are safe, affordable, and effective against both sub-species of *Trypanosoma brucei*. Semi-synthetic derivatization of the fungally-derived natural product merulin A_1 has led to the discovery of new development candidates for the protozoan parasite *T. brucei*, the causative agent of HAT. Creation of an initial SAR library based around the merulin scaffold revealed several key features required for activity, including the endoperoxide bridge, as well as one position suitable for further derivatization. Subsequent synthesis of a 20-membered analogue library, guided by the addition of acyl groups that improve the drug-like properties of the merulin A core, resulted in the development of compound 12 with an IC_{50} of 60 nM against *T. brucei*, and a selectivity index greater than 300-fold against HeLa and immortalized glial cells. We report the semi-synthetic optimization of the merulin class of endoperoxide natural products as development candidates against *T. brucei*. We have identified compounds with low nM antiparasitic activities and high selectivity indices against HeLa cells. These compounds can be produced economically in large quantities via a one-step derivatization from the microbial fermentation broth isolate, making them encouraging lead candidates for further development.

16422. **Nwodo, N. & Nwodo, O., 2012.** Antitrypanosomal potentials of the extract and fractions of *Abrus precatorius* seeds. *Asian Pacific Journal of Tropical Medicine*, **5**

(11): 857-861.

Department of Pharmaceutical Chemistry, University of Nigeria, Nsukka, Nigeria. [ngozi.nwodo@unn.edu.ng].

The objective of this study was to evaluate the *in vivo* trypanocidal activity of the methanol extract and fractions of *Abrus precatorius* seeds in mice. Parasitaemia was induced mice by intraperitoneal injection of $1.25x10^5$ *Trypanosoma* in normal saline. Five days later, when a high level of parasitaemia was established, treatment commenced for ten days. The mice were treated with 10, 20 and 40 mg/kg bodyweight of the extract and 5 and 10 mg/kg bodyweight of the fraction (F_2), respectively for five days. Diminazene aceturate at a dose of 3.5 mg/kg bodyweight for two days was used as the reference drug. The levels of parasitaemia and packed cell volume (PCV) of the animals were determined. At doses of 10, 20 and 40 mg/kg bodyweight the crude extract produced a sharp reduction in the level of parasitaemia in mice compared with the untreated group. The mice treated with F_2 at doses of 5 and 10 mg/kg bodyweight showed a sharp reduction in the level of parasitaemia which reached zero on day 9, and a gradual recovery from the 12^{th} day of treatment. This effect was comparable to that of the mice treated with 7 mg/kg of the standard drug diminazene aceturate. The PCV of the treated animals decreased gradually with time, but not as much as in the untreated group. Phytochemical screening revealed the presence of glycosides, alkaloids, carbohydrates, tannins and proteins in the *Abrus precatorius* powder while F_2 was rich in alkaloids. This study shows that both the extract and the fractions of *Abrus precatorius* seeds exhibited a promising trypanocidal property. Alkaloids may be responsible for the observed activity.

16423. **Nyunt, K. S., Elkhateeb, A., Tosa, Y., Nabata, K., Katakura, K. & Matsuura, H., 2012.** Isolation of antitrypanosomal compounds from *Vitis repens*, a medicinal plant of Myanmar. *Natural Product Communications*, **7** (5): 609-610.

Laboratory of Bioorganic Chemistry, Division of Applied Bioscience, Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan.

Bioactivity-guided fractionation of an ethanolic extract of *Vitis repens* led to the isolation of resveratrol (1), 11-O-acetyl bergenin (2), and stigmast-4-en-3-one (3). The compounds were examined for their *in vitro* activities against trypomastigotes of *Trypanosoma evansi*. Resveratrol showed antitrypanosomal activity with an IC_{50} value of 0.13 µM, whereas 11-O-acetyl bergenin and stigmast-4-en-3-one exhibited IC_{50} values of 0.17 and 0.15 µM, respectively.

16424. **Ochiana, S. O., Pandarinath, V., Wang, Z., Kapoor, R., Ondrechen, M. J., Ruben, L. & Pollastri, M. P., 2012.** The human Aurora kinase inhibitor danusertib is a lead compound for anti-trypanosomal drug discovery via target repurposing. *European Journal of Medicinal Chemistry*. **E publication ahead of print, July 31.**

Northeastern University, Department of Chemistry and Chemical Biology, Hurtig 102, 360 Huntington Avenue, Boston, MA 02115, USA.

New drugs for neglected tropical diseases such as human African trypanosomiasis (HAT) are needed, yet drug discovery efforts are not often focused on this area due to cost. Target

repurposing, achieved by the matching of essential parasite enzymes to those human enzymes that have been successfully inhibited by small molecule drugs, provides an attractive means by which new drug optimization programmes can be pragmatically initiated. In this report we describe our results in repurposing an established class of human Aurora kinase inhibitors, typified by danusertib 1, which we have observed to be an inhibitor of trypanosomal Aurora kinase 1 (TbAUK1) and effective in parasite killing *in vitro*. Informed by homology modelling and docking, a series of analogues of 1 were prepared that explored the scope of the chemotype and provided a nearly 25-fold improvement in cellular selectivity for parasite cells over human cells.

16425. **Phillips, E. A., Sexton, D. W. & Steverding, D., 2012.** Bitter melon extract inhibits proliferation of *Trypanosoma brucei* bloodstream forms *in vitro*. *Experimental Parasitology*. **Available online, 22 December.**

BioMedical Research Centre, Norwich Medical School, University of East Anglia, Norwich, UK. [dsteverding@hotmail.com].

Trypanosoma brucei is the causative agent of sleeping sickness, a fatal disease prevalent in sub-Saharan Africa. The few currently available drug treatments are dated and face problems with toxicity and resistance. For these reasons, there is an urgent need for the development of new chemotherapies for the treatment of sleeping sickness. In this study, we investigated the trypanocidal activity of bitter melon extract. Recently, it has been shown that bitter melon extracts display cytotoxic activity towards different cancer cell lines. However, agents exhibiting anti-tumour activity are usually also inhibiting the growth of *T. brucei*. Treatment of bloodstream forms of *T. brucei* with extracts prepared from Chinese and Indian bitter melon varieties resulted in a decrease in cell proliferation. In contrast, human myeloid leukaemia HL-60 cells were 3-6 times less sensitive to the extracts than trypanosomes. Initial fractionation of bitter melon extracts indicated that the trypanocidal activity of the extract is associated with at least two different classes of substances: one class of larger molecular weight compounds (> 3 kDa) causing rapid lysis of trypanosomes and one class of smaller molecular weight compounds (< 3 kDa) inducing accumulation of the parasites in the G_2-M phase of the cell cycle. Together, the results suggest that bitter melon is a promising source for trypanocidal agents which could be used as lead compounds for the development of novel anti-sleeping sickness drugs.

16426**. Pomel, S., Biot, C., Bories, C. & Loiseau, P. M., 2012.** Antiprotozoal activity of ferroquine. *Parasitological Research*. **E Publication ahead of print, November 15.**

Faculté de Pharmacie, UMR 8076 CNRS, Chimiothérapie Antiparasitaire, Univ. Paris-Sud, 92290, Chatenay-Malabry, France.

Ferroquine (FQ, SSR97193) is a synthetic compound currently in development for the treatment of malaria. The use of a single compound to treat several parasitoses would be very convenient for multi-infected patients and also for financial considerations. In this work, the activity of FQ was investigated on three other Protista parasites: Kinetoplastidae (*Leishmania* and *Trypanosoma*) and the cosmopolite parasite *Trichomonas vaginalis*. FQ exhibited a significant *in vitro* activity on *Trypanosoma brucei brucei* and *Trypanosoma brucei gambiense*, the agents of African trypanosomiasis in a range from 0.2 to 3.1 µM. *In vivo*, intraperitoneally administered FQ demonstrated a weak but significant trypanocidal activity at

100 µmol/kg, which is however higher than the maximum tolerated dose. The drop of the parasitaemia of the treated mice was significantly related to the amount of injected FQ. Furthermore, this organometallic compound was responsible for a delay in the appearance of bloodstream parasites at 50 µmol/kg. However, it was not able to cure infected mice. Although no synergy was identified *in vitro* between FQ and pentamidine, these results justify further investigations by evaluating analogues in this chemical series.

16427. **Redecke, L., Nass, K., Deponte, D. P., White, T. A., Rehders, D., Barty, A., Stellato, F., Liang, M., Barends, T. R., Boutet, S., Williams, G. J. and Messerschmidt, *et. al.,* 2012.** Natively inhibited *Trypanosoma brucei* cathepsin B structure determined by using an X-ray laser. *Science*. **E Publication ahead of print, November 29.**

Joint Laboratory for Structural Biology of Infection and Inflammation, Institute of Biochemistry and Molecular Biology, University of Hamburg, and Institute of Biochemistry, University of Lubeck, at Deutsches Elektronen-Synchrotron (DESY), Notkestrasse 85, 22607 Hamburg, Germany. [henry.chapman@desy.de].

16428**. Samant, B. S. & Nyangari, N., 2012.** A novel microwave synthesis of quinoline-3-carboxylic acid derivatives for treatment against human African trypanosomiasis. *Molecular Diversity*, **16** (4): 685-695.

Natural Product and Medicinal Chemistry (NPMC) Research Group, Division of Pharmaceutical chemistry, Faculty of Pharmacy, Rhodes University, Grahamstown, 6140, South Africa, [b.samant@ru.ac.za].

16429**. Sykes, M. L., Baell, J. B., Kaiser, M., Chatelain, E., Moawad, S. R., Ganame, D., Ioset, J. R. & Avery, V. M., 2012.** Identification of compounds with anti-proliferative activity against *Trypanosoma brucei brucei* strain 427 by a whole cell viability based HTS campaign. *PLoS Neglected Tropical Diseases*, **6** (11): e1896.

Discovery Biology, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Nathan, Queensland, Australia. [v.avery@griffith.edu.au].

Human African Trypanosomiasis (HAT) is caused by two trypanosome sub-species, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. Drugs available for the treatment of HAT have significant issues related to difficult administration regimes and limited efficacy across species and disease stages. Hence, there is considerable need to find new alternative and less toxic drugs. An approach to identify starting points for new drug candidates is high-throughput screening (HTS) of large compound library collections. We describe the application of an alamar blue based, 384-well HTS assay to screen a library of 87 296 compounds against the related trypanosome subspecies, *Trypanosoma brucei brucei* bloodstream form Lister 427. Primary hits identified against *T. b. brucei* were retested and the IC_{50} value compounds were estimated for *T. b. brucei* and a mammalian cell line HEK293, to determine a selectivity index for each compound. The screening campaign identified 205 compounds with greater than 10 times selectivity against *T. b. brucei*. Cluster analysis of these compounds, taking into account chemical and structural properties required for drug-like compounds, afforded a panel of eight compounds for further biological analysis. These

compounds had IC_{50} values ranging from 0.22 µM to 4 µM with associated selectivity indices ranging from 19 to greater than 345. Further testing against *T. b. rhodesiense* led to the selection of six compounds from five new chemical classes with activity against the causative species of HAT, which can be considered potential candidates for HAT early drug discovery. Structure-activity relationship (SAR) mining revealed components of those hit compound structures that may be important for biological activity. Four of these compounds have undergone further testing to (i) determine whether they are cidal or static *in vitro* at the minimum inhibitory concentration (MIC), and (ii) estimate the time to kill.

16430. **Tamborini, L., Pinto, A., Smith, T. K., Major, L. L., Iannuzzi, M. C., Cosconati, S., Marinelli, L., Novellino, E., Lo Presti, L., Wong, P. E., Barrett, M. P., De Micheli, C. & Conti, P., 2012.** Synthesis and biological evaluation of CTP synthetase inhibitors as potential agents for the treatment of African trypanosomiasis. *ChemMedChem*, **7** (9): 1623-1634.

Dipartimento di Scienze Farmaceutiche, Universita degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy. [paola.conti@unimi.it].

16431. **Thuita, J. K., Wang, M. Z., Kagira, J. M., Denton, C. L., Paine, M. F., Mdachi, R. E., Murilla, G. A., Ching, S., Boykin, D. W., Tidwell, R. R., Hall, J. E. & Brun, R., 2012.** Pharmacology of DB844, an orally active aza analogue of pafuramidine, in a monkey model of second stage human African trypanosomiasis. *PLoS Neglected Tropical Diseases*, **6** (7): e1734.

Trypanosomiasis Research Centre, Kenya Agricultural Research Institute (TRC-KARI), Kikuyu, Kenya. [thuitajk@yahoo.com].

Novel drugs to treat human African trypanosomiasis (HAT) are still urgently needed despite the recent addition of nifurtimox-eflornithine combination therapy (NECT) to WHO Model Lists of Essential Medicines against second stage HAT, where parasites have invaded the central nervous system (CNS). The pharmacology of a potential orally available lead compound, N-methoxy-6-{5-[4-(N-methoxyamidino) phenyl]-furan-2-yl}-nicotinamidine (DB844), was evaluated in a vervet monkey model of second stage HAT, following promising results in mice. DB844 was administered orally to vervet monkeys, beginning 28 days post-infection (DPI) with *Trypanosoma brucei rhodesiense* KETRI 2537. DB844 was absorbed and converted to the active metabolite 6-[5-(4-phenylamidinophenyl)-furanyl-2-yl]-nicotinamide (DB820), exhibiting plasma C_{max} values of 430 and 190 nM for DB844 and DB820, respectively, after the 14^{th} dose at 6 mg/kg bodyweight. A 100-fold reduction in blood trypanosome counts was observed within 24 h of the third dose and, at the end of treatment evaluation performed four days post the last drug dose, trypanosomes were not detected in the blood or cerebrospinal fluid of any monkey. However, some animals relapsed during the 300 days of post treatment monitoring, resulting in a cure rate of 3/8 (37.5 percent) and 3/7 (42.9 percent) for the 5 mg/kg x 10 days and the 6 mg/kg x 14 days dose regimens respectively. These DB844 efficacy data were an improvement compared with pentamidine and pafuramidine both of which were previously shown to be non-curative in this model of CNS stage HAT. These data show that synthesis of novel diamidines with improved activity against CNS-stage HAT was possible.

16432. **Udensi, U. K. & Fagbenro-Beyioku, A. F., 2012.** Effect of ivermectin on *Trypanosoma brucei brucei* in experimentally infected mice. *Journal of Vector Borne Diseases*, **49** (3): 143-150.

Centre for Bioinformatics and Computational Biology, Jackson State University, Jackson, USA. [udensik@yahoo.com].

Human African trypanosomiasis**,** otherwise known as sleeping sickness, is a neglected tropical disease of public health importance in West and Central Africa. In view of the adverse side effects of the antitrypanosomal drugs, the relatively few side effects observed in ivermectin use, and because both onchocerciasis and typanosomiasis occur in overlapping foci in Africa, it would be desirable if the ivermectin that has been used successfully on onchocerciasis management could also be used in the control and treatment of trypanosomiasis. In this study, prophylactic and therapeutic effects of ivermectin (Mectizan) were investigated in albino mice infected with a Nigerian strain of *Trypanosoma brucei brucei*. A 300 µg/ml/kg dose had the most effective impact because it showed the highest mean survival time of 12 days in both the treatment and prophylactic groups of mice. This dose also enhanced the defence capacity of the treated groups. It also had positive influence on the packed cell volume (PCV) and the state of anaemia in the trypanosome infected mice, hence, improving their survivability. Our report indicates that using the 300 µg/ml/kg dose of ivermectin increases the mean survival period from 5 to 12 days. This suggests that ivermectin could possibly be used in the treatment of trypanosomiasis. Further studies will be required to show whether proper treatment may entail a single dose, as used in this study; an increased number of doses, or combinations with other drugs.

16433**. Urbaniak, M. D., Mathieson, T., Bantscheff, M., Eberhard, D., Grimaldi, R., Miranda-Saavedra, D., Wyatt, P., Ferguson, M. A., Frearson, J. & Drewes, G., 2012.** Chemical proteomic analysis reveals the drugability of the kinome of *Trypanosoma brucei*. *ACS Chemical Biology*, **7** (11): 1858-1865.

Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, U.K. [m.a.j.ferguson@dundee.ac.uk].

The protozoan parasite *Trypanosoma brucei* is the causative agent of African sleeping sickness, and there is an urgent unmet need for improved treatments. Parasite protein kinases are attractive drug targets, provided that the host and parasite kinomes are sufficiently divergent to allow specific inhibition to be achieved. Current drug discovery efforts are hampered by the fact that comprehensive assay panels for parasite targets have not yet been developed. Here, we employ a kinase-focused chemoproteomics strategy that enables the simultaneous profiling of kinase inhibitor potencies against more than 50 endogenously expressed *T. brucei* kinases in parasite cell extracts. The data reveal that *T. brucei* kinases are sensitive to typical kinase inhibitors with nanomolar potency and demonstrate the potential for the development of species-specific inhibitors.

16434. **Vodnala, S., Lundback, T., Sjoberg, B., Svensson, R., Rottenberg, M. E. & Hammarstrom, L. G., 2012.** *In vitro* and i*n vivo* activity of 2-aminopyrazines/2-aminopyridines in experimental models of human African trypanosomiasis.

Antimicrobial Agents & Chemotherapy. **E Publication ahead of print, 17 December.**

Department of Microbiology, Tumor- and Cell Biology, Karolinska Institutet, Stockholm, Sweden.

New drugs for the treatment of human African trypanosomiasis are urgently needed. A number of 2-aminopyrazines/2-aminopyridines were identified as promising leads following a focused screen of 5 500 compounds for *Trypanosoma brucei brucei* viability. Described compounds are trypanotoxic in the sub-micromolar range and show comparably low cytotoxicity on representative mammalian cells lines. Specifically, 6-[(6-fluoro-3, 4-dihydro-2H-1-benzopyran-4-yl)oxy]-N-(piperidin-4-yl)pyrazin-2-amine (CBK201352) is trypanotoxic for *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense*, is non-toxic to mammalian cells lines, and *in vitro* preclinical assays predict promising pharmacokinetic parameters. Mice inoculated i.p. with 25 mg/kg CBK201352 twice daily for 10 days, starting on the day of infection with *T. b. brucei*, show complete clearance of parasites for more than 90 days. Thus, CBK201352 and related analogues are promising leads for the development of novel treatments for human African trypanosomiasis.

16435. Wolkmer, P., da Silva, C. B., Paim, F. C., Duarte, M. M., Castro, V., Palma, H. E., Franca, R. T., Felin, D. V., Siqueira, L. C., Lopes, S. T., Schetinger, M. R., Monteiro, S. G. & Mazzanti, C. M., 2012. Pre-treatment with curcumin modulates acetylcholinesterase activity and proinflammatory cytokines in rats infected with *Trypanosoma evansi*. *Parasitology International,* **6** (2):144-149.

Department of Chemistry, Universidade Federal de Santa Maria, Brazil. [patiwol@hotmail.com].

The potent activity against trypanosomes and health beneficial effects of curcumin (Cur) have been demonstrated in various experimental models. In this study, we evaluated the *in vivo* effect of Cur as a trypanocide and as a potential anti-inflammatory agent through the evaluation of immunomodulatory mechanisms in rats infected with *Trypanosoma evansi*. Oral Cur was administered daily at doses of 0, 20 or 60 mg/kg as preventive treatment (30 and 15 days pre-infection) and as treatment (post-infection). The treatment of the groups continued until the day of euthanasia. Fifteen days after inoculation, parasitaemia, plasma proinflammatory cytokines (IFN-gamma, TNF-alpha, IL-1, IL-6), anti-inflammatory cytokines (IL-10) and blood acetylcholinesterase activity (AChE) were analysed. Pre-treatment with Cur reduced parasitaemia and lethality. Cur inhibited AChE activity and improved the proinflammatory immunological response by cytokines during *T. evansi* infection. We found that Cur is more important as immunomodulator agent. These findings reveal that the preventive use of Cur stimulates anti-inflammatory mechanisms, reducing an excessive inflammatory response.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

16436. **Kuriakose, S., Muleme, H. M., Onyilagha, C., Singh, R., Jia, P. & Uzonna, J. E.,**

2012. Diminazene aceturate (Berenil) modulates the host cellular and inflammatory responses to *Trypanosoma congolense* infection. *PLoS One*, **7** (11): e48696.

Department of Immunology, University of Manitoba, Winnipeg, Manitoba, Canada. [uzonna@cc.umanitoba.ca].

Trypanosoma congolense are extracellular and intravascular blood parasites that cause debilitating acute or chronic disease in cattle and other domestic animals. Diminazene aceturate (Berenil) has been widely used as a chemotherapeutic agent for trypanosomiasis in livestock since 1955. As in livestock, treatment of infected highly susceptible BALB/c mice with Berenil leads to rapid control of parasitaemia and survival from an otherwise lethal infection. The molecular and biochemical mechanisms of action of Berenil are still not very well defined and its effect on the host immune system has remained relatively unstudied. Here, we investigated whether Berenil has, in addition to its trypanolytic effect, a modulatory effect on the host immune response to *Trypanosoma congolense*. BALB/c and C57BL/6 mice were infected intraperitoneally with *T. congolense*, treated with Berenil and the expression of CD25 and FoxP3 on splenic cells was assessed directly *ex vivo*. In addition, serum levels and spontaneous and LPS-induced production of pro-inflammatory cytokines by splenic and hepatic $CD11b^+$ cells were determined by ELISA. Berenil treatment significantly reduced the percentages of $CD25^+$ cells, produced a concomitant reduction in the percentage of regulatory ($CD4^+Foxp3^+$) T cells and a striking reduction in serum levels of disease exacerbating pro-inflammatory cytokines including IL-6, IL-12, TNF and IFN-gamma. Furthermore, Berenil treatment significantly suppressed spontaneous and LPS-induced production of inflammatory cytokines by splenic and liver macrophages and significantly ameliorated LPS-induced septic shock and the associated cytokine storm. Collectively, these results provide evidence that in addition to its direct trypanolytic effect, Berenil also modulates the host immune response to the parasite in a manner that dampen excessive immune activation and production of pathology-promoting pro-inflammatory cytokines, suggesting that this drug may also be beneficial for treatment of disease conditions caused by excessive production of inflammatory cytokines.

16437**. Pourjafar, M., Badiei, K., Sharifiyazdi, H., Chalmeh, A., Naghib, M., Babazadeh, M., Mootabi Alavi, A. & Hosseini Joshani-Zadeh, N., 2012.** Genetic characterization and phylogenetic analysis of *Trypanosoma evansi* in Iranian dromedary camels. *Parasitology Research*. **E Publication ahead of print, September 25.**

Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, P.O. box 71345, Shiraz, Iran. [dmp4m@yahoo.com].

Whole blood samples were collected from 117 male clinically healthy *Camelus dromedarius* aged between 6 months to 18 years from several farms in Yazd Province of Iran. *Trypanosoma evansi*-affected camels were detected by Giemsa-stained blood smears, and the positive blood samples (4 out of 117) were submitted for PCR examination and phylogenetic analysis. Basic local alignment search tool data of the complete internal transcribed spacer (ITS) sequences obtained revealed that they corresponded to those of *T. evansi*, Thailand cattle isolate (AY912276) with the homology of 99 percent. Both phylogenetic trees generated by ITS1 and complete ITS were unable to clearly show inter- and intra-specific

genetic diversity of *Trypanosoma* spp. isolates. The phylogenetic tree inferred from the ITS2 nucleotide sequences (569 bp) clearly showed the genetic diversity of the parasites. Phylogenetic and molecular analyses of this region showed that two distinct genotypes of *T. evansi* in Iranian dromedary camels are present. In contrast to the ITS1 and ITS2 regions, multiple alignment of the nucleotide sequence of the 5.8S rRNA showed a high degree of sequence conservation during evolution in various *Trypanosoma* spp.

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

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

16438**. Adung'a, V. O. & Field, M. C., 2012.** TbFRP, a novel FYVE-domain containing phosphoinositide-binding Ras-like GTPase from trypanosomes. *Experimental Parasitology*. **E Publication ahead of print, December 3.**

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK. [mcf34@cam.ac.uk].

Ras-like small GTPases are regulatory proteins that control multiple aspects of cellular function, and are particularly prevalent in vesicular transport. A proportion of GTPase paralogues appear restricted to certain eukaryote lineages, suggesting roles specific to a restricted lineage, and hence potentially reflecting adaptation to individual lifestyles or ecological niches. Here we describe the role of a GTPase, TbFRP, a FYVE domain N-terminally fused to a Ras-like GTPase, originally identified in *Trypanosoma brucei*. As FYVE-domains specifically bind phosphoinositol 3-phosphate (PI3P), which associates with endosomes, we suggest that TbFRP may unite phosphoinositide and small G protein endosomal signalling in Trypanosomatids. TbFRP orthologues are present throughout the Euglenazoa suggesting that FRP has functions throughout the group. We show that the FYVE domain of TbFRP is functional in PI3P-dependent membrane targeting and localizes at the endosomal region. Further, while TbFRP is apparently non-essential, knockdown and immunochemical evidence indicate that TbFRP is rapidly cleaved upon synthesis, releasing the GTPase and FYVE-domains. Finally, TbFRP expression at both mRNA and protein levels is cell density-dependent. Together, these data suggest that TbFRP is an endocytic GTPase with a highly unusual mechanism of action that involves proteolysis of the nascent protein and membrane targeting via PI3P.

16439. **Ali, J. A., Creek, D. J., Burgess, K., Allison, H. C., Field, M. C., Maser, P. & De Koning, H. P., 2012.** Pyrimidine salvage in *Trypanosoma* b*rucei* bloodstream forms and the trypanocidal action of halogenated pyrimidines. *Molecular Pharmacology*. **Published on November 27, 2012 as doi:10.1124/mol.112.082321**

Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK. [Harry.de-Koning@glasgow.ac.uk].

African trypanosomes are capable of both pyrimidine biosynthesis and salvage of pre-formed pyrimidines from the host. However, uptake of pyrimidines in bloodstream form trypanosomes has not been investigated, making it difficult to judge the relative importance of

salvage and synthesis, or to design a pyrimidine-based chemotherapy. Detailed characterization of pyrimidine transport activities in bloodstream form *Trypanosoma brucei brucei* found that these cells express a high affinity uracil transporter (designated TbU3) that is clearly distinct from the procyclic pyrimidine transporters. This transporter had low affinity for uridine and 2'deoxyuridine and was the sole pyrimidine transporter expressed in these cells. In addition, thymidine was taken up inefficiently through a P1-type nucleoside transporter. Importantly, the anti-cancer drug 5-fluorouracil was an excellent substrate for TbU3 and several 5-fluoropyrimidine analogues were investigated for uptake and trypanocidal activity; 5F-orotic acid, 5F-2'deoxyuridine displayed activity in the low µM range. The metabolism and mode of action of these analogues were determined using metabolomic assessments of *T. brucei* clonal lines adapted to high levels of these pyrimidine analogues, as well as of the sensitive parental strains. The analysis showed that 5-fluorouracil is incorporated into a large number of metabolites but likely exerts toxicity through incorporation into RNA. 5F-2'dUrd and 5F-2'dCtd are not incorporated into nucleic acids but act as pro-drugs by inhibiting thymidylate synthase as 5F-dUMP. We present the most complete model of pyrimidine salvage in *Trypanosoma brucei* to date, supported by genome-wide profiling of the predicted pyrimidine biosynthesis and conversion enzymes.

16440. **Alsford, S., duBois, K., Horn, D. & Field, M. C., 2012.** Epigenetic mechanisms, nuclear architecture and the control of gene expression in trypanosomes. *Expert Reviews in Molecular Medicine*, **14**: e13.

London School of Hygiene and Tropical Medicine, London, UK. [david.horn@lshtm.ac.uk].

The control of gene expression, and more significantly gene cohorts, requires tight transcriptional coordination and is an essential feature of probably all cells. In higher eukaryotes, the mechanisms used involve controlled modifications to both local and global DNA environments, principally through changes in chromatin structure as well as cis-element-driven mechanisms. Although the mechanisms regulating chromatin in terms of transcriptional permissiveness and the relation to developmental programmes and responses to the environment are becoming better understood for animal and fungal cells, it is only just beginning to become clear how these processes operate in other taxa, including the trypanosomatids. Recent advances are now illuminating how African trypanosomes regulate higher-order chromatin structure, and further, how these mechanisms impact on the expression of major surface antigens that are of fundamental importance to life-cycle progression. It is now apparent that several mechanisms are rather more similar between animal and fungal cells and trypanosomes than they originally appeared, but some aspects do involve gene products unique to trypanosomes. Therefore, both evolutionarily common and novel mechanisms cohabit in trypanosomes, offering both important biological insights and possible therapeutic opportunity.

16441. **Barquilla, A., Saldivia, M., Diaz, R., Bart, J. M., Vidal, I., Calvo, E., Hall, M. N. & Navarro, M., 2012.** Third target of rapamycin complex negatively regulates development of quiescence in *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences USA*, **109** (36): 14399-14404.

Instituto de Parasitologia y Biomedicina Lopez-Neyra, Consejo Superior de

Investigaciones Cientificas, 18100 Granada, Spain. [miguel.navarro@ipb.csic.es].

African trypanosomes are protozoan parasites transmitted by a tsetse fly vector to a mammalian host. The life cycle includes highly proliferative forms and quiescent forms, the latter being adapted to host transmission. The signalling pathways controlling the developmental switch between the two forms remain unknown. *Trypanosoma brucei* contains two targets of rapamycin (TOR) kinases, TbTOR1 and TbTOR2, and two TOR complexes, TbTORC1 and TbTORC2. Surprisingly, two additional TOR kinases are encoded in the *T. brucei* genome. We report that TbTOR4 associates with an Armadillo domain-containing protein (TbArmtor), a major vault protein, and LST8 to form a unique TOR complex, TbTORC4. Depletion of TbTOR4 caused irreversible differentiation of the parasite into the quiescent form. AMP and hydrolysable analogues of cAMP inhibited TbTOR4 expression and induced the stumpy quiescent form. Our results reveal unexpected complexity in TOR signalling and show that TbTORC4 negatively regulates differentiation of the proliferative form into the quiescent form.

16442**. Basu, S., Leonard, J. C., Desai, N., Mavridou, D. A., Tang, K. H., Goddard, A. D., Ginger, M. L., Lukes, J. & Allen, J. W., 2012.** Divergence of Erv1-associated mitochondrial import and export pathways in trypanosomes and anaerobic protists. *Eukaryotic Cell*. **E Publication ahead of print, December 21.**

Biology Centre, Institute of Parasitology, Czech Academy of Sciences and Faculty of Sciences, University of South Bohemia, 370 05 Ceske Budejovice (Budweis), Czech Republic. [m.ginger@lancaster.ac.uk.

16443**. Benmerzouga, I., Concepcion-Acevedo, J., Kim, H. S., Vandoros, A. V., Cross, G. A., Klingbeil, M. M. & Li, B., 2012.** *Trypanosoma brucei* Orc1 is essential for nuclear DNA replication and affects both VSG silencing and VSG switching. *Molecular Microbiology,* **87**(1): 196-210.

Center for Gene Regulation in Health & Disease, Department of Biological, Geological, and Environmental Sciences, Cleveland State University, 2121 Euclid Avenue, Cleveland, OH, 44115, USA. [b.li37@csuohio.edu].

Binding of the origin recognition complex (ORC) to replication origins is essential for initiation of DNA replication, but ORC has non-essential functions outside of DNA replication, including in heterochromatic gene silencing and telomere maintenance. *Trypanosoma brucei*, a protozoan parasite that causes human African trypanosomiasis, uses antigenic variation as a major virulence mechanism to evade the host's immune attack by expressing its major surface antigen, the variant surface glycoprotein (VSG), in a monoallelic manner. An Orc1/Cdc6 homologue has been identified in *T. brucei*, but its role in DNA replication has not been directly confirmed and its potential involvement in VSG repression or switching has not been thoroughly investigated. In this study, we show that TbOrc1 is essential for nuclear DNA replication in mammalian-infectious bloodstream and tsetse procyclic forms (BF and PF). Depletion of TbOrc1 resulted in de-repression of telomere-linked silent VSGs in both BF and PF, and increased VSG switching particularly through the *in situ* transcriptional switching mechanism. TbOrc1 associates with telomere repeats but appears to do so independently of two known *T. brucei* telomere proteins, TbRAP1 and

TbTRF. We conclude that TbOrc1 has conserved functions in DNA replication and is also required to control telomere-linked VSG expression and VSG switching.

16444. **Brasseur, A., Rotureau, B., Vermeersch, M., Blisnick, T., Salmon, D., Bastin, P., Pays, E., Vanhamme, L. & Perez-Morga, D., 2012.** *Trypanosoma brucei* FKBP12 differentially controls motility and cytokinesis in procyclic and bloodstream forms. *Eukaryotic Cell*. **E Publication ahead of print, October 26.**

Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, 12 rue des Professeurs Jeener et Brachet, B-6041 Gosselies, Belgium.

FKBP12 proteins are able to inhibit TOR kinases or calcineurine phosphatases upon binding of rapamycin and FK506 drugs respectively. The *Trypanosoma brucei* homologue TbFKBP12 was found to be a cytoskeleton-associated protein with specific localization in the flagellar pocket area of the bloodstream form. In the insect procyclic form, RNA interference-mediated knockdown of TbFKBP12 affected motility. In bloodstream cells, depletion of TbFKBP12 affected cytokinesis and cytoskeleton architecture. These last effects were associated with the presence of internal translucent cavities limited by an inside-out configuration of the normal cell surface, with a luminal VSG coat lined up by microtubules. These cavities, which recreated the streamlined shape of the normal trypanosome cytoskeleton, might represent unsuccessful attempts for cell abscission. We propose that TbFKBP12 differentially affects stage-specific processes through association with the cytoskeleton.

16445. **Buisson, J., Chenouard, N., Lagache, T., Blisnick, T., Olivo-Marin, J. C. & Bastin, P., 2012.** Intraflagellar transport proteins cycle between the flagellum and its base. *Journal of Cell Science*. **E Publication ahead of print, September 19.**

Institut Pasteur International, Département de Parasitologie et Mycologie de Paris, Paris, France. [philippe.bastin@pasteur.fr].

16446. **Butter, F., Bucerius, F., Michel, M., Cicova, Z., Mann, M. & Janzen, C. J., 2013.** Comparative proteomics of two life cycle stages of stable isotope-labelled *Trypanosoma brucei* reveals novel components of the parasite's host adaptation machinery. *Molecular & Cellular Proteomics,* **12**: 172-179.

Max Planck Institute for Biochemistry, Germany; [mmann@biochem.mpg.de].

Trypanosoma brucei developed a sophisticated life cycle to adapt to different host environments. Although developmental differentiation of *T. brucei* has been the topic of intensive research for decades, the mechanisms responsible for adaptation to different host environments are not well understood. We developed stable isotope labelling by amino acids in cell culture (SILAC) in trypanosomes to compare the proteomes of two different life cycle stages. Quantitative comparison of 4 364 protein groups identified many proteins previously not known to be stage-specifically expressed. The identification of stage-specific proteins helps to understand how parasites adapt to different hosts and provides new insights into differences in metabolism, gene regulation and cell architecture. A DEAD-box RNA helicase,

which is highly up-regulated in the blood stream form of this parasite and which is essential for viability and proper cell cycle progression in this stage is described as an example.

16447**. Carnes, J., Schnaufer, A., McDermott, S. M., Domingo, G., Proff, R., Steinberg, A. G., Kurtz, I. & Stuart, K., 2012.** Mutational analysis of *Trypanosoma brucei* editosome proteins KREPB4 and KREPB5 reveals domains critical for function. *RNA*, **18** (10): 1897-1909.

Seattle Biomedical Research Institute, Seattle, Washington 98109, USA.

The transcriptome of kinetoplastid mitochondria undergoes extensive RNA editing that inserts and deletes uridine residues (U's) to produce mature mRNAs. The editosome is a multiprotein complex that provides endonuclease, TUTase, exonuclease, and ligase activities required for RNA editing. The editosome's KREPB4 and KREPB5 proteins are essential for editosome integrity and parasite viability and contain semi-conserved motifs corresponding to zinc finger, RNase III, and PUF domains, but to date no functional analysis of these domains has been reported. We show here that various point mutations to KREPB4 and KREPB5 identify essential domains, and suggest that these proteins do not themselves perform RNase III catalysis. The zinc finger of KREPB4 but not KREPB5 is essential for editosome integrity and parasite viability, and mutation of the RNase III signature motif in KREPB5 prevents integration into editosomes, which is lethal. Isolated TAP-tagged KREPB4 and KREPB5 complexes preferentially associate with components of the deletion subcomplex, providing additional insights into editosome architecture. A new alignment of editosome RNase III sequences from several kinetoplastid species implies that KREPB4 and KREPB5 lack catalytic activity and reveals that the PUF motif is present in the editing endonucleases KREN1, KREN2, and KREN3. The data presented here are consistent with the hypothesis that KREPB4 and KREPB5 form intermolecular heterodimers with the catalytically active editing endonucleases, which is unprecedented among known RNase III proteins.

16448. **Castillo-Acosta, V. M., Aguilar-Pereyra, F., Bart, J. M., Navarro, M., Ruiz-Perez, L. M., Vidal, A. E. & Gonzalez-Pacanowska, D., 2012.** Increased uracil insertion in DNA is cytotoxic and increases the frequency of mutation, double strand break formation and VSG switching in *Trypanosoma brucei*. *DNA Repair (Amst)*, **11** (12): 986-995.

Instituto de Parasitologia y Biomedicina "Lopez-Neyra", Consejo Superior de Investigaciones Cientificas, Parque Tecnologico de Ciencias de la Salud, Avenida del Conocimiento, s/n 18100-Armilla, Granada, Spain. [avidal@ipb.csic.es].

Deoxyuridine 5'-triphosphate pyrophosphatase (dUTPase) and uracil-DNA glycosylase (UNG) are key enzymes involved in the control of the presence of uracil in DNA. While dUTPase prevents uracil mis-incorporation by removing dUTP from the deoxynucleotide pool, UNG excises uracil from DNA as a first step of the base excision repair pathway (BER). Here, we report that strong down-regulation of dUTPase in UNG-deficient *Trypanosoma brucei* cells greatly impairs cell viability in both bloodstream and procyclic forms, underscoring the extreme sensitivity of trypanosomes to uracil in DNA. Depletion of dUTPase activity in the absence of UNG provoked cell cycle alterations, massive dUTP misincorporation into DNA and chromosomal fragmentation. Overall, trypanosomatid cells that lack dUTPase and UNG activities exhibited greater proliferation defects and DNA

damage than cells deficient in only one of these activities. To determine the mutagenic consequences of uracil in DNA, mutation rates and spectra were analysed in dUTPase-depleted cells in the presence of UNG activity. These cells displayed a spontaneous mutation rate nine-fold higher than the parental cell line. Base substitutions at A:T base pairs and deletion frequencies were both significantly enhanced which is consistent with the generation of mutagenic AP sites and DNA strand breaks. The increase in strand breaks conveyed a concomitant increase in VSG switching *in vitro*. The low tolerance of *T. brucei* to uracil in DNA emphasizes the importance of uracil removal and regulation of intracellular dUTP pool levels in cell viability and genetic stability and suggests potential strategies to compromise parasite survival.

16449**. Castillo-Acosta, V. M., Aguilar-Pereyra, F., Garcia-Caballero, D., Vidal, A. E., Ruiz-Perez, L. M. & Gonzalez-Pacanowska, D., 2012.** Pyrimidine requirements in deoxyuridine triphosphate nucleotidohydrolase deficient *Trypanosoma brucei* mutants. *Molecular & Biochemical Parasitology*, **187** (1): 9-13.

Instituto de Parasitologia y Biomedicina "Lopez-Neyra", Consejo Superior de Investigaciones Cientificas, Parque Tecnologico de Ciencias de la Salud, Avenida del Conocimiento, s/n 18100-Armilla (Granada), Spain. [avidal@ipb.csic.es].

16450**. Comini, M. A., Krauth-Siegel, R. L. & Bellanda, M., 2012.** Mono- and dithiol glutaredoxins in the trypanothione-based redox metabolism of pathogenic trypanosomes. *Antioxidants & Redox Signaling*. **E Publication ahead of print, October 25.**

Laboratory Redox Biology of Trypanosomes, Institut Pasteur de Montevideo, Montevideo, Uruguay.

16451. **Creek, D. J., Chokkathukalam, A., Jankevics, A., Burgess, K. E., Breitling, R. & Barrett, M. P., 2012.** Stable isotope-assisted metabolomics for network-wide metabolic pathway elucidation. *Analytical Chemistry*, **84** (20): 8442-8447.

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

The combination of high-resolution LC-MS-based untargeted metabolomics with stable isotope tracing provides a global overview of the cellular fate of precursor metabolites. This methodology enables detection of putative metabolites from biological samples and simultaneous quantification of the pattern and extent of isotope labelling. Labelling of *Trypanosoma brucei* cell cultures with 50 percent uniformly ^{13}C-labelled glucose demonstrated incorporation of glucose-derived carbon into 187 of 588 putatively identified metabolites in diverse pathways including carbohydrate, nucleotide, lipid, and amino acid metabolism. Labelling patterns confirmed the metabolic pathways responsible for the biosynthesis of many detected metabolites, and labelling was detected in unexpected metabolites, including two higher sugar phosphates annotated as octulose phosphate and nonulose phosphate. This untargeted approach to stable isotope tracing facilitates the biochemical analysis of known pathways and yields rapid identification of previously

unexplored areas of metabolism.

16452. **Das, A., Morales, R., Banday, M., Garcia, S., Hao, L., Cross, G. A., Estevez, A. M. & Bellofatto, V., 2012.** The essential polysome-associated RNA-binding protein RBP42 targets mRNAs involved in *Trypanosoma brucei* energy metabolism. *RNA*, **18** (11): 1968-1983.

Department of Microbiology and Molecular Genetics, UMDNJ-NJ Medical School, Newark, New Jersey 07103, USA.

RNA-binding proteins that target mRNA coding regions are emerging as regulators of post-transcriptional processes in eukaryotes. Here we describe a newly identified RNA-binding protein, RBP42, which targets the coding region of mRNAs in the insect form of the African trypanosome, *Trypanosoma brucei*. RBP42 is an essential protein and associates with polysome-bound mRNAs in the cytoplasm. A global survey of RBP42-bound mRNAs was performed by applying HITS-CLIP technology, which captures protein-RNA interactions *in vivo* using UV light. Specific RBP42-mRNA interactions, as well as mRNA interactions with a known RNA-binding protein, were purified using specific antibodies. Target RNA sequences were identified and quantified using high-throughput RNA sequencing. Analysis revealed that RBP42 bound mainly within the coding region of mRNAs that encode proteins involved in cellular energy metabolism. Although the mechanism of RBP42's function is unclear at present, we speculate that RBP42 plays a critical role in modulating *T. brucei* energy metabolism.

16453. **Desy, S., Schneider, A. & Mani, J., 2012.** *Trypanosoma brucei* has a canonical mitochondrial processing peptidase. *Molecular & Biochemical Parasitology*, **185** (2): 161-164.

Department of Chemistry and Biochemistry, University of Bern, Freiestr. 3, CH-3012 Bern, Switzerland. [jan.mani@dcb.unibe.ch].

16454. **Duncan, M. R., Fullerton, M. & Chaudhuri, M., 2012.** Tim50 in *Trypanosoma brucei* possesses a dual-specificity phosphatase activity and is critical for mitochondrial protein import. *Journal of Biological Chemistry*. **First Published December 4, doi: 10.1074/jbc.M112.436378**

Meharry Medical College, USA. [mchaudhuri@mmc.edu].

16455. **Eltschinger, S., Greganova, E., Heller, M., Butikofer, P. & Altmann, M., 2012.** Eukaryotic translation elongation factor 1A (eEF1A) domain I from *S. cerevisiae* is required but not sufficient for inter-species complementation. *PLoS One*, **7** (7): e42338.

Institute of Biochemistry & Molecular Medicine, University of Bern, Bern, Switzerland. [peter.buetikofer@ibmm.unibe.ch].

16456. **Erben, E. D., Nardelli, S. C., de Jesus, T. C., Schenkman, S. & Tellez-Inon, M. T., 2012.** Trypanosomatid Pin1-type peptidyl-prolyl isomerase is cytosolic and not

essential for cell proliferation. *Journal of Eukaryotic Microbiology,* **60** (1): 101-105.

Instituto de Investigaciones en Ingenieria Genetica y Biologia Molecular "Dr. Hector N. Torres" (INGEBI-CONICET), Vuelta de Obligado 2490, C1428ADN, Buenos Aires, Argentina. [e.erben@zmbh.uni-heidelberg.de].

Pin1-type peptidyl-prolyl cis/trans isomerases (PPIases) isomerise the peptide bond of specific phosphorylated (Ser/Thr)-Pro residues, regulating various cellular events. Previously, we reported a Pin1-type PPIase in *Trypanosoma cruzi,* but little is known about its function and subcellular localization. Immunofluorescence analysis revealed that in contrast with Pin1-like proteins from diverse organisms, TcPin1 mainly localized in the cytoplasm and was excluded from the nuclei. In addition, RNAi-mediated down regulation of TbPin1 in *Trypanosoma brucei* did not abolish cell proliferation. Using yeast two-hybrid assay, we identified a MORN domain-containing protein as putative Pin1-binding partner. These data suggest that Pin1-mediated signalling mechanism plays a different role in protozoan parasites.

16457. **Farine, L. & Butikofer, P., 2012.** The ins and outs of phosphatidylethanolamine synthesis in *Trypanosoma brucei*. *Biochimica Biophysica Acta*. **E Publication ahead of print, September 23.**

Institute of Biochemistry and Molecular Medicine, University of Bern, 3012 Bern, Switzerland. [luce.farine@ibmm.unibe.ch].

Phospholipids are not only major building blocks of biological membranes but fulfil a wide range of critical functions that are often widely unrecognized. In this review, we focus on phosphatidylethanolamine, a major glycerophospholipid class in eukaryotes and bacteria, which is involved in many unexpected biological processes. We describe (i) the ins, i.e. the substrate sources and biochemical reactions involved in phosphatidylethanolamine synthesis, and (ii) the outs, i.e. the different roles of phosphatidylethanolamine and its involvement in various cellular events. We discuss how the protozoan parasite, *Trypanosoma brucei*, has contributed and may contribute in the future as eukaryotic model organism to our understanding of phosphatidylethanolamine homeostasis.

16458. **Farr, H. & Gull, K., 2012.** Cytokinesis in trypanosomes. *Cytoskeleton (Hoboken)*, **69** (11): 931-941.

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK. [helen.farr@path.ox.ac.uk].

Cytokinesis is a crucial step in the cell division cycle whereby the cell membrane and underlying cortex are remodelled and drawn together to create two new daughter cells. While in many eukaryotic systems this process is accomplished by an actomyosin contractile ring, the protozoan parasite *Trypanosoma brucei* displays an unusual mechanism for cytokinesis, with an increased reliance on microtubules. There are a number of crucial preparatory steps involving the replication and segregation of organelles that must be undertaken in order for cytokinesis to occur. In this review, we discuss the cellular architecture of the trypanosome and its importance within cytokinesis, and the recent progress in understanding the regulatory systems involved. Recent advances in three-dimensional imaging techniques have improved

our understanding of the mechanisms driving cytokinesis and are likely to yield further insights in the future.

16459**. Fernandez-Moya, S. M., Garcia-Perez, A., Kramer, S., Carrington, M. & Estevez, A. M., 2012.** Alterations in DRBD3 ribonucleoprotein complexes in response to stress in *Trypanosoma brucei*. *PLoS One*, **7** (11): e48870.

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

Regulation of RNA polymerase II transcription initiation is apparently absent in trypanosomes. Instead, these eukaryotes control gene expression mainly at the post-transcriptional level. Regulation is exerted through the action of numerous RNA-binding proteins that modulate mRNA processing, turnover, translation and localization. In this work we show that the RNA-binding protein DRBD3 resides in the cytoplasm, but localizes to the nucleus upon oxidative challenge and to stress granules under starvation conditions. DRBD3 associates with other proteins to form a complex, the composition of which is altered by cellular stress. Interestingly, target mRNAs remain bound to DRBD3 under stress conditions. Our results suggest that DRBD3 transports regulated mRNAs within the cell in the form of ribonucleoprotein complexes that are remodelled in response to environmental cues.

16460. **Fisk, J. C., Li, J., Wang, H., Aletta, J. M., Qu, J. & Read, L. K., 2012.** Proteomic analysis reveals diverse classes of arginine methylproteins in mitochondria of typanosomes. *Molecular & Cell Proteomics*. **E Publication ahead of print, November 14.**

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

16461**. Foda, B. M., Downey, K. M., Fisk, J. C. & Read, L. K., 2012.** Multifunctional G-rich and RRM-containing domains of TbRGG2 perform separate yet essential functions in trypanosome RNA editing. *Eukaryotic Cell*, **11** (9): 1119-1131.

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

Efficient editing of *Trypanosoma brucei* mitochondrial RNAs involves the actions of multiple accessory factors. *T. brucei* RGG2 (TbRGG2) is an essential protein crucial for initiation and 3'-to-5' progression of editing. TbRGG2 comprises an N-terminal G-rich region containing GWG and RG repeats and a C-terminal RNA recognition motif (RRM)-containing domain. Here, we perform *in vitro* and *in vivo* separation-of-function studies to interrogate the mechanism of TbRGG2 action in RNA editing. TbRGG2 preferentially binds pre-edited mRNA *in vitro* with high affinity attributable to its G-rich region. RNA-annealing and -melting activities are separable, carried out primarily by the G-rich and RRM domains, respectively. *In vivo*, the G-rich domain partially complements TbRGG2 knockdown, but the RRM domain is also required. Notably, TbRGG2's RNA-melting activity is dispensable for RNA editing *in vivo*. Interactions between TbRGG2 and MRB1 complex proteins are

mediated by both G-rich and RRM-containing domains, depending on the binding partner. Overall, our results are consistent with a model in which the high-affinity RNA binding and RNA-annealing activities of the G-rich domain are essential for RNA editing *in vivo*. The RRM domain may have key functions involving interactions with the MRB1 complex and/or regulation of the activities of the G-rich domain.

16462**. Gargantini, P. R., Lujan, H. D. & Pereira, C. A., 2012.** *In silico* analysis of trypanosomatids' helicases. *FEMS Microbiology Letters*, **335** (2): 123-129.

Laboratorio de Bioquimica y Biologia Molecular, Facultad de Medicina, Universidad Catolica de Cordoba, Cordoba, Argentina. [cpereira@retina.ar].

Trypanosomatids are unicellular protozoan parasites that cause many diseases in animals, including humans, and plants. These early divergent eukaryotes have many singular structures and processes, including the hyper-modified base J', a mitochondrial DNA network, RNA editing, and trans-splicing; all of these unique features involve a wide variety of specific DNA/RNA helicases. In this work, the genomes of trypanosomatids were analysed by data mining, searching for genes coding for DNA/RNA helicases. Specific motifs and full-length sequences from all families present in the helicase's super families (SFs) 1 and 2 were used as baits for genome analyses. A total of 328 putative helicases were identified; 204 genes were assigned to the SF2, 42 genes to the SF1, and 76 genes remain unclassified. Eight species-specific SF2 helicases were also found; *Trypanosoma cruzi* has three DEAD-box and one DEAH/RHA-specific helicases, while *Leishmania major* has three Swi2/Snf2 and *Trypanosoma brucei* has only one RigI helicase. Finally, to identify helicases that could be used as future therapeutic targets, all obtained genes were compared with those present in the human genome. Forty-two helicases underrepresented in the human genome were identified; constituting 16 orthologue groups from *L. major*, *T. brucei*, and *T. cruzi.*

16463. **Gassen, A., Brechtefeld, D., Schandry, N., Arteaga-Salas, J. M., Israel, L., Imhof, A. & Janzen, C. J., 2012.** DOT1A-dependent H3K76 methylation is required for replication regulation in *Trypanosoma brucei*. *Nucleic Acids Research*, **40** (20): 10302-10311.

Department of Biology I, University of Munich (LMU), Genetics, Grosshaderner Str. 2-4, 82152 Martinsried and Department of Molecular Biology, University of Munich (LMU), Adolf Butenandt Institute, Schillerstr. 44, 80336 Munich, Germany.

Cell-cycle progression requires careful regulation to ensure accurate propagation of genetic material to the daughter cells. Although many cell-cycle regulators are evolutionarily conserved in the protozoan parasite *Trypanosoma brucei*, novel regulatory mechanisms seem to have evolved. Here, we analyse the function of the histone methyltransferase DOT1A during cell-cycle progression. Over-expression of DOT1A generates a population of cells with aneuploid nuclei as well as enucleated cells. Detailed analysis shows that DOT1A over-expression causes continuous replication of the nuclear DNA. In contrast, depletion of DOT1A by RNAi abolishes replication but does not prevent karyokinesis. As histone H3K76 methylation has never been associated with replication control in eukaryotes before, we have discovered a novel function of DOT1 enzymes, which might not be unique to trypanosomes.

16464. **Ghozlane, A., Bringaud, F., Soueidan, H., Dutour, I., Jourdan, F. & Thebault, P., 2012.** Flux analysis of the *Trypanosoma brucei* glycolysis based on a multiobjective-criteria bioinformatic approach. *Advances in Bioinformatics*, **2012**: 159423.

Laboratoire Bordelais de Recherche en Informatique, UMR CNRS 5800, Université Bordeaux, 351 Cours de la Libération, 33405 Talence Cedex, France; and Centre de Bioinformatique de Bordeaux, Université Bordeaux Segalen, 142 Rue Leo Saignat, 33076 Bordeaux Cedex, France.

16465**. Ginger, M. L., Sam, K. A. & Allen, J. W., 2012.** Probing why trypanosomes assemble atypical cytochrome c with an AxxCH haem-binding motif instead of CxxCH. *Biochemical Journal*, **448** (2): 253-260.

Faculty of Health and Medicine, Division of Biomedical and Life Sciences, Lancaster University, Lancaster LA1 4YQ, U.K. [jwaallen1@gmail.com].

Mitochondrial cytochromes c and c1 are core components of the respiratory chain of all oxygen-respiring eukaryotes. These proteins contain haem, covalently bound to the polypeptide in a catalysed post-translational modification. In all eukaryotes, except members of the protist phylum Euglenozoa, haem attachment is to the cysteine residues of a CxxCH haem-binding motif. In the Euglenozoa, which include medically relevant trypanosomatid parasites, haem attachment is to a single cysteine residue in an AxxCH haem-binding motif. Moreover, genes encoding known c-type cytochrome biogenesis machineries are all absent from trypanosomatid genomes, indicating the presence of a novel biosynthetic apparatus. In the present study, we investigate expression and maturation of cytochrome c with a typical CxxCH haem-binding motif in the trypanosomatids *Crithidia fasciculata* and *Trypanosoma brucei*. Haem became attached to both cysteine residues of the haem-binding motif, indicating that, in contrast with previous hypotheses, nothing prevents formation of a CxxCH cytochrome c in euglenozoan mitochondria. The cytochrome variant was also able to replace the function of wild-type cytochrome c in *T. brucei*. However, the haem attachment to protein was not via the stereospecifically conserved linkage universally observed in natural c-type cytochromes, suggesting that the trypanosome cytochrome c biogenesis machinery recognized and processed only the wild-type single-cysteine haem-binding motif. Moreover, the presence of the CxxCH cytochrome c resulted in a fitness cost in respiration. The level of cytochrome c biogenesis in trypanosomatids was also found to be limited, with the cells operating at close to maximum capacity.

16466**. Gjini, E., Haydon, D. T., Barry, J. D. & Cobbold, C. A., 2012.** The impact of mutation and gene conversion on the local diversification of antigen genes in African trypanosomes. *Molecular Biology & Evolution*, **29** (11): 3321-3331.

School of Mathematics and Statistics, College of Science and Engineering, University of Glasgow, Glasgow, UK. [egjini@igc.gulbenkian.pt].

Patterns of genetic diversity in parasite antigen gene families hold important information about their potential to generate antigenic variation within and between hosts. The evolution of such gene families is typically driven by gene duplication, followed by point mutation and gene conversion. There is great interest in estimating the rates of these processes from

molecular sequences for understanding the evolution of the pathogen and its significance for infection processes. In this study, a series of models are constructed to investigate hypotheses about the nucleotide diversity patterns between closely related gene sequences from the antigen gene archive of the African trypanosome, the protozoan parasite causative of human sleeping sickness in sub-Saharan Africa. We use a hidden Markov model approach to identify two scales of diversification: clustering of sequence mismatches, a putative indicator of gene conversion events with other lower-identity donor genes in the archive, and at a sparser scale, isolated mismatches, likely arising from independent point mutations. In addition to quantifying the respective probabilities of occurrence of these two processes, our approach yields estimates for the gene conversion tract length distribution and the average diversity contributed locally by conversion events. Model fitting is conducted using a Bayesian framework. We find that diversifying gene conversion events with lower-identity partners occur at least five times less frequently than point mutations on variant surface glycoprotein (VSG) pairs, and the average imported conversion tract is between 14 and 25 nucleotides long. However, because of the high diversity introduced by gene conversion, the two processes have almost equal impact on the per-nucleotide rate of sequence diversification between VSG subfamily members. We are able to disentangle the most likely locations of point mutations and conversions on each aligned gene pair.

16467**. Goringer, H. U., 2012. "**Gestalt", composition and function of the *Trypanosoma brucei* editosome. *Annual Review of Microbiology*, **66**: 65-82.

Department of Genetics, Darmstadt University of Technology, Germany. [goringer@hrzpub.tu-darmstadt.de].

RNA editing describes a chemically diverse set of biomolecular reactions in which the nucleotide sequence of RNA molecules is altered. Editing reactions have been identified in many organisms and frequently contribute to the maturation of organellar transcripts. A special editing reaction has evolved within the mitochondria of the kinetoplastid protozoa. The process is characterized by the insertion and deletion of uridine nucleotides into otherwise non-translatable messenger RNAs. Kinetoplastid RNA editing involves an exclusive class of small, noncoding RNAs known as guide RNAs. Furthermore, a unique molecular machinery, the editosome, catalyses the process. Editosomes are megadalton multienzyme assemblies that provide a catalytic surface for the individual steps of the reaction cycle. Here, the current mechanistic understanding and molecular inventory of kinetoplastid RNA editing and the editosome machinery are reviewed. Special emphasis is placed on the molecular morphology of the editing complex in order to correlate structural features with functional characteristics.

16468**. Gualdron-Lopez, M., Brennand, A., Avilan, L. & Michels, P. A., 2013.** Translocation of solutes and proteins across the glycosomal membrane of trypanosomes: possibilities and limitations for targeting with trypanocidal drugs. *Parasitology*, **140**(1):1-20.

Research Unit for Tropical Diseases, de Duve Institute, Université catholique de Louvain, Avenue Hippocrate 74, Postal Box B1.74.01, B-1200 Brussels, Belgium.

Glycosomes are specialized peroxisomes found in all kinetoplastid organisms. The

organelles are unique in harbouring most enzymes of the glycolytic pathway. Matrix proteins, synthesized in the cytosol, cofactors and metabolites have to be transported across the membrane. Recent research on *Trypanosoma brucei* has provided insight into how these translocations across the membrane occur, although many details remain to be elucidated. Proteins are imported by a cascade of reactions performed by specialized proteins, called peroxins, in which a cytosolic receptor with bound matrix protein inserts itself in the membrane to deliver its cargo into the organelle and is subsequently retrieved from the glycosome to perform further rounds of import. Bulky solutes, such as cofactors and acyl-CoAs, seem to be translocated by specific transporter molecules, whereas smaller solutes such as glycolytic intermediates probably cross the membrane through pore-forming channels. The presence of such channels is in apparent contradiction with previous results that suggested a low permeability of the glycosomal membrane. We propose three possible, not mutually exclusive, solutions for this paradox. Glycosomal glycolytic enzymes have been validated as drug targets against trypanosomatid-borne diseases. We discuss the possible implications of the new data for the design of drugs to be delivered into glycosomes.

16469. **Gualdron-Lopez, M. & Michels, P. A., 2012.** Processing of the glycosomal matrix-protein import receptor PEX5 of *Trypanosoma brucei*. *Biochemical & Biophysical Research Communications*. **E Publication ahead of print, December 22.**

Research Unit for Tropical Diseases, de Duve Institute, Université catholique de Louvain, Brussels, Belgium. [paul.michels@uclouvain.be].

16470. **Gunasekera, K., Wuthrich, D., Braga-Lagache, S., Heller, M. & Ochsenreiter, T., 2012.** Proteome remodelling during development from blood to insect-form *Trypanosoma brucei* quantified by SILAC and mass spectrometry. *BMC Genomics*, **13** (1): 556.

Institute for Cell Biology, University of Bern, Bern, Switzerland. [torsten.ochsenreiter@izb.unibe].

Trypanosoma brucei is the causative agent of human African sleeping sickness and nagana in cattle. In addition to being an important pathogen *T. brucei* has developed into a model system in cell biology. Using stable isotope labelling of amino acids in cell culture (SILAC) in combination with mass spectrometry we determined the abundance of >1 600 proteins in the long slender (LS), short stumpy (SS) mammalian bloodstream form stages relative to the procyclic (PC) insect-form stage. In total we identified 2 645 proteins, corresponding to ~30 percent of the total proteome and for the first time present a comprehensive overview of relative protein levels in three life stages of the parasite. We show the extent of pre-adaptation in the SS cells, especially at the level of the mitochondrial proteome. The comparison with a previously published report on monomorphic *in vitro* grown bloodstream and procyclic *T. brucei* indicates a loss of stringent regulation particularly of mitochondrial proteins in these cells when compared with the pleomorphic *in vivo* situation. In order to better understand the different levels of gene expression regulation in this organism we compared mRNA steady state abundance with the relative protein abundance-changes and detected moderate but significant correlation indicating that trypanosomes possess a significant repertoire of translational and posttranslational mechanisms to regulate protein abundance.

16471. **Hovel-Miner, G. A., Boothroyd, C. E., Mugnier, M., Dreesen, O., Cross, G. A. & Papavasiliou, F. N., 2012.** Telomere length affects the frequency and mechanism of antigenic variation in *Trypanosoma brucei*. *PLoS Pathogens*, **8** (8): e1002900.

Laboratory of Lymphocyte Biology, The Rockefeller University, New York, USA. [papavasiliou@rockefeller.edu].

Trypanosoma brucei is a master of antigenic variation and immune response evasion. Utilizing a genomic repertoire of more than 1 000 variant surface glycoprotein-encoding genes (VSGs), *T. brucei* can change its protein coat by "switching" from the expression of one VSG to another. Each active VSG is monoallelically expressed from only one of approximately 15 subtelomeric sites. Switching VSG expression occurs by three predominant mechanisms, arguably the most significant of which is the non-reciprocal exchange of VSG containing DNA by duplicative gene conversion (GC). How *T. brucei* orchestrates its complex switching mechanisms remains to be elucidated. Recent work has demonstrated that an exogenous DNA break in the active site could initiate a GC based switch, yet the source of the switch-initiating DNA lesion under natural conditions is still unknown. Here we investigated the hypothesis that telomere length directly affects VSG switching. We demonstrate that telomerase deficient strains with short telomeres switch more frequently than genetically identical strains with long telomeres and that, when the telomere is short, switching preferentially occurs by GC. Our data support the hypothesis that a short telomere at the active VSG expression site results in an increase in subtelomeric DNA breaks, which can initiate GC based switching. In addition to their significance for *T. brucei* and telomere biology, the findings presented here have implications for the many diverse pathogens that organize their antigenic genes in subtelomeric regions.

16472. **Jones, D. C., Alphey, M. S., Wyllie, S. & Fairlamb, A. H., 2012.** Chemical, genetic and structural assessment of pyridoxal kinase as a drug target in the African trypanosome. *Molecular Microbiology*, **86** (1): 51-64.

Division of Biological Chemistry & Drug Discovery, College of Life Sciences, University of Dundee, Dundee, UK. [a.h.fairlamb@dundee.ac.uk].

Pyridoxal-5'-phosphate (vitamin B_6) is an essential cofactor for many important enzymatic reactions such as transamination and decarboxylation. African trypanosomes are unable to synthesise vitamin B_6 *de novo* and rely on uptake of B_6 vitamers such as pyridoxal and pyridoxamine from their hosts, which are subsequently phosphorylated by pyridoxal kinase (PdxK). A conditional null mutant of PdxK was generated in *Trypanosoma brucei* bloodstream forms showing that this enzyme is essential for growth of the parasite *in vitro* and for infectivity in mice. Activity of recombinant *T. brucei* PdxK was comparable to previously published work having a specific activity of 327 +/- 13 μ mg^{-1} and a K_{mapp} with respect to pyridoxal of 29.6 +/- 3.9 μM. A coupled assay was developed demonstrating that the enzyme has equivalent catalytic efficiency with pyridoxal, pyridoxamine and pyridoxine, and that ginkgotoxin is an effective pseudo substrate. A high resolution structure of PdxK in complex with ATP revealed important structural differences with the human enzyme. These findings suggest that pyridoxal kinase is an essential and druggable target that could lead to much needed alternative treatments for this devastating disease.

16473. **Kafkova, L., Ammerman, M. L., Faktorova, D., Fisk, J. C., Zimmer, S. L., Sobotka, R., Read, L. K., Lukes, J. & Hashimi, H., 2012.** Functional characterization of two paralogues that are novel RNA binding proteins influencing mitochondrial transcripts of *Trypanosoma brucei*. *RNA*, **18** (10): 1846-1861.

Biology Center, Institute of Parasitology, Czech Academy of Sciences, University of South Bohemia, 370 05 Ceske Budejovice (Budweis), Czech Republic. [hassan@paru.cas.cz].

A majority of *Trypanosoma brucei* proteins have unknown functions, a consequence of its independent evolutionary history within the order Kinetoplastida that allowed for the emergence of several unique biological properties. Among these is RNA editing, needed for expression of mitochondrial-encoded genes. The recently discovered mitochondrial RNA binding complex 1 (MRB1) is composed of proteins with several functions in processing organellar RNA. We characterize two MRB1 subunits, referred to herein as MRB8170 and MRB4160, which are paralogues arising from a large chromosome duplication occurring only in *T. brucei*. As with many other MRB1 proteins, both have no recognizable domains, motifs, or orthologues outside the order. We show that they are both novel RNA binding proteins, possibly representing a new class of these proteins. They associate with a similar subset of MRB1 subunits but not directly with each other. We generated cell lines that either individually or simultaneously target the mRNAs encoding both proteins using RNAi. Their dual silencing results in a differential effect on moderately and pan-edited RNAs, suggesting a possible functional separation of the two proteins. Cell growth persists upon RNAi silencing of each protein individually in contrast to the dual knockdown. Yet, their apparent redundancy in terms of cell viability is at odds with the finding that only one of these knockdowns results in the general degradation of pan-edited RNAs. While MRB8170 and MRB4160 share a considerable degree of conservation, our results suggest that their recent sequence divergence has led to them influencing mitochondrial mRNAs to differing degrees.

16474. **Koumandou, V. L., Boehm, C., Horder, K. A. & Field, M. C., 2012.** Evidence for recycling of invariant surface trans-membrane domain proteins in African trypanosomes. *Eukaryotic Cell*. **E Publication ahead of print, December 21.**

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QT, UK. [mcf34@cam.ac.uk].

Intracellular trafficking is a vital component of both virulence mechanisms and drug interactions in *Trypanosoma brucei*, the causative agent of human African trypanosomiasis and nagana of cattle. Both maintaining the surface proteome composition within a life stage and remodelling the composition when progressing between life stages are important features of immune evasion and development for trypanosomes. Our recent work implicates the abundant trans-membrane invariant surface glycoproteins (ISGs) in the uptake of first line therapeutic suramin, suggesting a potential therapeutic route into the cell. RME-8 is a mediator of recycling pathways in higher eukaryotes and is one of a small cohort of intracellular transport gene products up-regulated in mammalian-infective trypanosomes, suggesting a role in controlling the copy number of surface proteins in trypanosomes. Here we investigate RME-8 function and its contribution to intracellular trafficking and stability of

ISGs. RME-8 is a highly conserved protein and is broadly distributed across multiple endocytic compartments. By knockdown we find that RME-8 is essential and mediates delivery of endocytic probes to late endosomal compartments. Further, we find ISG accumulation within endosomes, but that RME-8 knockdown also increases ISG turnover; when combined with previous data, this suggests that it is most probable that ISGs are recycled, and that RME-8 is required to support recycling.

16475**. Li, Z., 2012.** Regulation of the cell division cycle in *Trypanosoma brucei*. *Eukaryotic Cell*, **11** (10): 1180-1190.

Department of Microbiology & Molecular Genetics, University of Texas Medical School at Houston, TX, USA. [Ziyin.Li@uth.tmc.edu].

The cell division cycle is tightly regulated by the activation and inactivation of a series of proteins that control the replication and segregation of organelles to the daughter cells. During the past decade, we have witnessed significant advances in our understanding of the cell cycle in *Trypanosoma brucei* and how the cycle is regulated by various regulatory proteins. However, many other regulators, especially those unique to trypanosomes, remain to be identified, and we are just beginning to delineate the signalling pathways that drive the transitions through different cell cycle stages, such as the G_1/S transition, G_2/M transition, and mitosis-cytokinesis transition. Trypanosomes appear to employ both evolutionarily conserved and trypanosome-specific molecules to regulate the various stages of its cell cycle, including DNA replication initiation, spindle assembly, chromosome segregation, and cytokinesis initiation and completion. Strikingly, trypanosomes lack some crucial regulators that are well conserved across evolution, such as Cdc6 and Cdt1, which are involved in DNA replication licensing, the spindle motor kinesin-5 which is required for spindle assembly, the central spindlin complex which has been implicated in cytokinesis initiation, and the actomyosin contractile ring which is located at the cleavage furrow. Conversely, trypanosomes possess certain regulators such as cyclins, cyclin-dependent kinases, and mitotic centromere-associated kinesins, that are greatly expanded and likely play diverse cellular functions. Overall, trypanosomes apparently have integrated unique regulators into the evolutionarily conserved pathways to compensate for the absence of those conserved molecules and, additionally, have evolved certain cell cycle regulatory pathways that are either different from its human host or distinct between its own life cycle forms.

16476. **Liu, W., Das, A., Morales, R., Banday, M., Aris, V., Lukac, D. M., Soteropoulos, P., Wah, D. A., Palenchar, J. & Bellofatto, V., 2012.** Chromatin immunoprecipitation and microarray analysis reveal that TFIIB occupies the SL RNA gene promoter region in *Trypanosoma brucei* chromosomes. *Molecular & Biochemical Parasitology*, **186** (2): 139-142.

Department of Microbiology and Molecular Genetics, University of Medicine and Dentistry-New Jersey Medical School, Newark, NJ 07103, USA; and VaxInnate Corporation, Cranbury, NJ 08512, USA. [bellofat@umdnj.edu].

16477. **Mach, J., Tachezy, J. & Sutak, R., 2012.** Efficient iron uptake via a reductive mechanism in procyclic *Trypanosoma brucei*. *Journal of Parasitology*. **E Publication ahead of print, August 27.**

Charles University in Prague, Department of Parasitology, Prague, Czech Republic.

The bloodstream form of *T. brucei* acquires iron from transferrin via receptor-mediated endocytosis. However, it is unknown how procyclic forms that cannot bind transferrin acquire iron. In this study, we show that the procyclic form of *T. brucei* efficiently takes up iron from ferric complexes via a reductive mechanism and that iron obtained using this mechanism is transported to and used in the mitochondria. The affinity of the transport system is comparable to that of *Saccharomyces cerevisiae*, with an apparent K_m of 0.85 µM.

16478. **Manta, B., Pavan, C., Sturlese, M., Medeiros, A., Crispo, M., Berndt, C., Krauth-Siegel, L., Bellanda, M. & Comini, M. A., 2012.** Iron-sulphur cluster (ISC) binding by mitochondrial monothiol glutaredoxin-1 of *Trypanosoma brucei*: molecular basis of ISC coordination and relevance for parasite infectivity. *Antioxidants & Redox Signaling*. **E Publication ahead of print, December 24.**

Institut Pasteur de Montevideo, Laboratory Redox Biology of Trypanosomes, Montevideo, Uruguay.[bmanta@pasteur.edu.uy].

Monothiol glutaredoxins (1-C-Grxs) are small proteins linked to cellular iron and redox metabolism. *Trypanosoma brucei brucei*, a model organism for human African trypanosomiasis, expresses three 1-C-Grxs. 1-C-Grx1 is a highly abundant mitochondrial protein capable of binding an iron sulphur cluster (ISC) *in vitro* using glutathione as cofactor. We report here on the functional and structural analysis of 1-C-Grx1 in relation to its ISC-binding properties. An N-terminal extension unique to 1-C-Grx1 from trypanosomatids affects the oligomeric structure and ISC-binding capacity of the protein. The active site Cys104 is essential for ISC binding and the parasite specific glutathionylspermidine and trypanothione can replace glutathione as ligands of the ISC. Interestingly, trypanothione forms stable protein-free ISC species that *in vitro* are incorporated into the dithiol *T. brucei* 2-C-Grx1 but not 1-C-Grx1. Overexpression of the C104S mutant of 1-C-Grx1 impairs disease progression in a mouse model. The structure of the Grx-domain of 1-C-Grx1 was solved by NMR spectroscopy. Despite the fact that several residues – which in other 1-C-Grxs are involved in non-covalent glutathione binding – are conserved, different physicochemical approaches did not reveal any specific interaction between 1-C-Grx1 and free thiol ligands. Parasite Grxs are able to coordinate an ISC formed with trypanothione, suggesting a new mechanism of ISC-binding and a novel function for the parasite specific dithiol. The first 3D structure and *in vivo* relevance of a 1-C-Grx from a pathogenic protozoan are reported. It is concluded that *T. brucei* 1-C-Grx1 is indispensable for mammalian parasitism and utilizes a new mechanism for ISC-binding.

16479. **Mashiyama, S. T., Koupparis, K., Caffrey, C. R., McKerrow, J. H. & Babbitt, P. C., 2012.** A global comparison of the human and *T. brucei* degradomes gives insights about possible parasite drug targets. *PLoS Neglected Tropical Diseases*, **6** (12): e1942.

Department of Bioengineering and Therapeutic Sciences, California Institute for Quantitative Biomedical Research (QB3), University of California San Francisco, San Francisco, California, USA; Center for Discovery and Innovation in Parasitic Diseases, and Department of Pathology, QB3, University of California San

Francisco, San Francisco, California, USA. [jmck@cgl.ucsf.edu].

We performed a genome-level computational study of sequence and structure similarity, the latter using crystal structures and models, of the proteases of *Homo sapiens* and the human parasite *Trypanosoma brucei*. Using sequence and structure similarity networks to summarize the results, we constructed global views that show visually the relative abundance and variety of proteases in the degradome landscapes of these two species, and provide insights into evolutionary relationships between proteases. The results also indicate how broadly these sequence sets are covered by three-dimensional structures. These views facilitate cross-species comparisons and offer clues for drug design from knowledge about the sequences and structures of potential drug targets and their homologs. Two protease groups ("M32" and "C51") that are very different in sequence from human proteases are examined in structural detail, illustrating the application of this global approach in mining new pathogen genomes for potential drug targets. Based on our analyses, a human ACE2 inhibitor was selected for experimental testing on one of these parasite proteases, TbM32, and was shown to inhibit it. These sequence and structure data, along with interactive versions of the protein similarity networks generated in this study, are available at http://babbittlab.ucsf.edu/resources.html.

16480. **Morriswood, B., Havlicek, K., Demmel, L., Yavuz, S., Sealey-Cardona, M., Vidilaseris, K., Anrather, D., Kostan, J., Djinovic-Carugo, K., Roux, K. & Warren, G., 2012.** Novel bilobe components in *Trypanosoma brucei* identified using proximity-dependent biotinylation. *Eukaryotic Cell*. E-**Publication ahead of print, December 21.**

Max F. Perutz Laboratories, University of Vienna and Medical University of Vienna, Doktor Bohr-Gasse 9, 1030, Vienna, Austria. [brooke.morriswood@mfpl.ac.at].

16481. **Nguyen, T. N., Nguyen, B. N., Lee, J. H., Panigrahi, A. K. & Gunzl, A., 2012.** Characterization of a novel class I transcription factor A (CITFA) subunit that is indispensable for transcription by the multifunctional RNA polymerase I of *Trypanosoma brucei*. *Eukaryotic Cell*, **11** (12): 1573-1581.

Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, Connecticut, USA. [gunzl@uchc.edu].

Trypanosoma brucei is the only organism known to have evolved a multifunctional RNA polymerase I (pol I) system that is used to express the parasite's ribosomal RNAs, as well as its major cell surface antigens, namely, the variant surface glycoprotein (VSG) and procyclin, which are vital for establishing successful infections in the mammalian host and the tsetse vector, respectively. Thus far, biochemical analyses of the *T. brucei* RNA pol I transcription machinery have elucidated the subunit structure of the enzyme and identified the class I transcription factor A (CITFA). CITFA binds to RNA pol I promoters, and its CITFA-2 subunit was shown to be absolutely essential for RNA pol I transcription in the parasite. Tandem affinity purification (TAP) of CITFA revealed the subunits CITFA-1 to -6, which are conserved only among kinetoplastid organisms, plus the dynein light chain DYNLL1. Here, by tagging CITFA-6 instead of CITFA-2, a complex was purified that contained all known CITFA subunits, as well as a novel proline-rich protein. Functional studies carried out *in vivo* and *in vitro*, as well as a colocalization study, unequivocally demonstrated that this protein is

a *bona fide* CITFA subunit, essential for parasite viability and indispensable for RNA pol I transcription of ribosomal gene units and the active VSG expression site in the mammalian-infective life cycle stage of the parasite. Interestingly, CITFA-7 function appears to be species specific, because expression of an RNA interference (RNAi)-resistant CITFA-7 transgene from *Trypanosoma cruzi* could not rescue the lethal phenotype of silencing endogenous CITFA-7.

16482. **Niemann, M., Wiese, S., Mani, J., Chanfon, A., Jackson, C., Meisinger, C., Warscheid, B. & Schneider, A., 2012.** Mitochondrial outer membrane proteome of *Trypanosoma brucei* reveals novel factors required to maintain mitochondrial morphology. *Molecular & Cellular Proteomics*. **E Publication ahead of print, December 6.**

University of Bern, Department of Chemistry & Biochemistry, Bern, Switzerland. [andre.schneider@ibc.unibe.ch].

Trypanosoma brucei is a unicellular parasite that causes devastating diseases in humans and animals. It diverged from most other eukaryotes very early in evolution and as a consequence has an unusual mitochondrial biology. Moreover, mitochondrial functions and morphology are highly regulated throughout the life cycle of the parasite. The outer mitochondrial membrane defines the boundary of the organelle. Its properties are therefore key for the understanding of how the cytosol and mitochondria communicate and how the organelle is integrated into the metabolism of the whole cell. We have purified the mitochondrial outer membrane of *T. brucei* and characterized its proteome using label-free quantitative mass spectrometry for protein abundance profiling in combination with statistical analysis. Our results show that the trypanosomal outer membrane proteome consists of 82 proteins, two thirds of which have never been associated with mitochondria before. Forty proteins share homology with proteins of known functions. The function of 42 proteins, 33 of which are specific for trypanosomatids, remains unknown. Eleven proteins are essential for the disease-causing bloodstream form of *T. brucei* and therefore may be exploited as novel drug targets. A comparison with the outer membrane proteome of yeast defines a set of 17 common proteins that are likely present in the mitochondrial outer membrane of all eukaryotes. Known factors involved in the regulation of mitochondrial morphology are virtually absent in *T. brucei*. Interestingly, RNAi-mediated ablation of three outer membrane proteins of unknown function results in a collapse of the network-like mitochondrion of procyclic cells and for the first time identifies factors that control mitochondrial shape in *T. brucei*.

16483. **Orrling, K. M., Jansen, C., Vu, X. L., Balmer, V., Bregy, P., Shanmugham, A., England, P., Bailey, D., Cos, P., Maes, L., Adams, E., van den Bogaart, E., Chatelain, E., Ioset, J. R., van de Stolpe, A., Zorg, S., Veerman, J., Seebeck, T., Sterk, G. J., de Esch, I. J. & Leurs, R., 2012.** Catechol pyrazolinones as trypanocidals: fragment-based design, synthesis, and pharmacological evaluation of nanomolar inhibitors of trypanosomal phosphodiesterase B1. *Journal of Medicinal Chemistry*, **55** (20): 8745-8756.

Leiden/Amsterdam Centre of Drug Research-LACDR, Amsterdam Institute of Molecules, Medicines and Systems- AIMMS, Division of Medicinal Chemistry, VU University Amsterdam, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

[R.Leurs@vu.nl].

Trypanosomal phosphodiesterases B1 and B2 (TbrPDEB1 and TbrPDEB2) play an important role in the life cycle of *Trypanosoma brucei*, the causative parasite of human African trypanosomiasis (HAT), also known as African sleeping sickness. We used homology modelling and docking studies to guide fragment growing into the parasite-specific P-pocket in the enzyme binding site. The resulting catechol pyrazolinones act as potent TbrPDEB1 inhibitors with IC_{50} values down to 49 nM. The compounds also block parasite proliferation (e.g. VUF13525 (20b): *T. brucei rhodesiense* IC_{50} = 60 nM, *T. brucei brucei* IC_{50}= 520 nM, *T. cruzi* = 7.6 µM), inducing a typical multiple nuclei and kinetoplast phenotype without being generally cytotoxic. The mode of action of 20b was investigated with recombinantly engineered trypanosomes expressing a cAMP-sensitive FRET sensor, confirming a dose-response related increase of intracellular cAMP levels in trypanosomes. Our findings further validate the TbrPDEB family as anti trypanosomal target.

16484. **Pena-Diaz, P., Pelosi, L., Ebikeme, C., Colasante, C., Gao, F., Bringaud, F. & Voncken, F., 2012.** Functional characterization of TbMCP5, a conserved and essential ADP/ATP carrier present in the mitochondrion of the human pathogen *Trypanosoma brucei*. *Journal of Biological Chemistry*, **287** (50): 41861-41874.

Department of Biological Sciences and Hull York Medical School, University of Hull, Cottingham Road, HU6 7RX Hull, UK. [F.Voncken@hull.ac.uk].

Trypanosoma brucei is a kinetoplastid parasite of medical and veterinary importance. Its digenetic life cycle alternates between the bloodstream form in the mammalian host and the procyclic form (PCF) in the blood sucking insect vector, the tsetse fly. PCF trypanosomes rely in the glucose-depleted environment of the insect vector primarily on the mitochondrial oxidative phosphorylation of proline for their cellular ATP provision. We previously identified two *T. brucei* mitochondrial carrier family proteins, TbMCP5 and TbMCP15, with significant sequence similarity to functionally characterized ADP/ATP carriers from other eukaryotes. Comprehensive sequence analysis confirmed that TbMCP5 contains canonical ADP/ATP carrier sequence features, whereas they are not conserved in TbMCP15. Heterologous expression in the ANC-deficient yeast strain JL1Delta2Delta3u(-) revealed that only TbMCP5 was able to restore its growth on the non-fermentable carbon source lactate. Transport studies in yeast mitochondria showed that TbMCP5 has biochemical properties and ADP/ATP exchange kinetics similar to those of Anc2p, the prototypical ADP/ATP carrier of *S. cerevisiae*. Immunofluorescence microscopy and Western blot analysis confirmed that TbMCP5 is exclusively mitochondrial and is differentially expressed with 4.5-fold more TbMCP5 in the procyclic form of the parasite. Silencing of TbMCP5 expression in PCF *T. brucei* revealed that this ADP/ATP carrier is essential for parasite growth, particularly when depending on proline for energy generation. Moreover, ADP/ATP exchange in isolated *T. brucei* mitochondria was eliminated upon TbMCP5 depletion. These results confirmed that TbMCP5 functions as the main ADP/ATP carrier in the trypanosome mitochondrion. The important role of TbMCP5 in the *T. brucei* energy metabolism is further discussed.

16485. **Perez-Moreno, G., Sealey-Cardona, M., Rodrigues-Poveda, C., Gelb, M. H., Ruiz-Perez, L. M., Castillo-Acosta, V., Urbina, J. A. & Gonzalez-Pacanowska, D., 2012.** Endogenous sterol biosynthesis is important for mitochondrial function and cell

morphology in procyclic forms of *Trypanosoma brucei*. *International Journal of Parasitology*, **42** (11): 975-989.

Instituto de Parasitologia y Biomedicina Lopez-Neyra, Consejo Superior de Investigaciones Cientificas, Avda. del Conocimiento s/n, Armilla, Granada, Spain. [dgonzalez@ipb.csic.es].

Sterol biosynthesis inhibitors are promising entities for the treatment of trypanosomal diseases. Insect forms of *Trypanosoma brucei*, the causative agent of sleeping sickness, synthesize ergosterol and other 24-alkylated sterols, yet also incorporate cholesterol from the medium. While sterol function has been investigated by pharmacological manipulation of sterol biosynthesis, molecular mechanisms by which endogenous sterols influence cellular processes remain largely unknown in trypanosomes. Here we analyse by RNA interference, the effects of a perturbation of three specific steps of endogenous sterol biosynthesis in order to dissect the role of specific intermediates in proliferation, mitochondrial function and cellular morphology in procyclic cells. A decrease in the levels of squalene synthase and squalene epoxidase resulted in a depletion of cellular sterol intermediates and end products, impaired cell growth and led to aberrant morphologies, DNA fragmentation and a profound modification of mitochondrial structure and function. In contrast, cells deficient in sterol methyl transferase, the enzyme involved in 24-alkylation, exhibited a normal growth phenotype in spite of a complete abolition of the synthesis and content of 24-alkyl sterols. Thus, the data provided indicate that while the depletion of squalene and post-squalene endogenous sterol metabolites results in profound cellular defects, bulk 24-alkyl sterols are not strictly required to support growth in insect forms of *T. brucei in vitro*.

16486. **Phillips, M. A., 2012.** Stoking the drug target pipeline for human African trypanosomiasis. *Molecular Microbiology*, **86** (1): 10-14.

Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390-9041, USA. [margaret.phillips@UTSouthwestern.edu].

Trypanosoma brucei is the causative agent of African sleeping sickness, putting at risk up to 50 million people in sub-Saharan Africa. Current drug therapies are limited by toxicity and difficult treatment regimes and as the development of vaccines remains unlikely, the identification of better drugs to control this deadly disease is needed. Strategies for the identification of new lead compounds include phenotypic screening or target-based approaches. Implementation of the latter has been hampered by the lack of defined targets that are both essential and druggable. In this issue of *Molecular Microbiology*, Jones *et al.* (2012) report on the characterization of *T. brucei* pyridoxal kinase (PdxK), an enzyme required for the salvage of vitamin B_6, an essential enzymatic cofactor. Genetic knock-down and small molecule inhibitor studies were used to demonstrate that PdxK is essential for parasite growth both *in vitro* and in a mouse model, providing both genetic and chemical validation of the target. An enzyme assay compatible with high-throughput screening (HTS) was developed and the X-ray crystal structure solved, showing the potential for species selective inhibition. These studies add a greatly needed additional target into the drug discovery pipeline for this deadly parasitic infection.

16487. **Pinheiro, M. P., Emery, F. D. & Nonato, M. C., 2012.** Target sites for the design of

anti-trypanosomatid drugs based on the structure of dihydroorotate. *Current Pharmaceutical Design*. **E Publication ahead of print, October 31.**

Laboratorio de Cristalografia de Proteinas, Departamento de Fisica e Quimica, Faculdade de Ciencias Farmaceuticas de Ribeirao Preto, USP. Av. Cafe S/N, Monte Alegre, Ribeirao Preto, S.P. Brazil 14040-903. [cristy@fcfrp.usp.br].

Trypanosomatids consist of a large group of flagellated parasitic protozoa, including parasites from the genera *Leishmania* and *Trypanosoma*, responsible for causing infections in millions of humans worldwide and for which currently no appropriate therapy is available. The significance of pyrimidines in cellular metabolism makes their *de novo* and salvage pathways ideal druggable targets for pharmacological intervention and open an opportunity for pharmaceutical innovation. In the current review, we discuss the merits in targeting the enzyme dihydroorotate dehydrogenase (DHODH), a flavin-dependent enzyme that catalyzes the fourth and only redox step in pyrimidine *de novo* biosynthesis, as a strategy for the development of efficient therapeutic strategies for trypanosomatid-related diseases. We also describe the advances and perspectives from the structural biology point of view in order to unravel the structure-function relationship of trypanosomatid DHODHs, and to identify and validate target sites for drug development.

16488. **Religa, A. A. & Waters, A. P., 2012.** Sirtuins of parasitic protozoa: in search of function(s). *Molecular & Biochemical Parasitology*, **185** (2): 71-88.

Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow G12 8TA, UK. [Agnieszka.Religa@glasgow.ac.uk].

The SIR2 family of NAD^+-dependent protein deacetylases, collectively called sirtuins, has been of central interest due to their proposed roles in lifespan regulation and ageing. Sirtuins are one group of environment sensors of a cell interpreting external information and orchestrating internal responses at the sub-cellular level, through participation in gene regulation mechanisms. Remarkably conserved across all kingdoms of life, SIR2 proteins in several protozoan parasites appear to have both conserved and intriguing unique functions. This review summarises our current knowledge of the members of the sirtuin families in Apicomplexa, including *Plasmodium*, and other protozoan parasites such as *Trypanosoma* and *Leishmania*. The wide diversity of processes regulated by SIR2 proteins makes them targets worthy of exploitation in anti-parasitic therapies.

16489. **Ruberto, I., Szoor, B., Clark, R. & Matthews, K. R., 2012.** Investigating mammalian tyrosine phosphatase inhibitors as potential "piggyback" leads to target *Trypanosoma brucei* transmission. *Chemical Biology & Drug Design*. **E Publication ahead of print, October 15.**

Centre for Immunity, Infection and Evolution, Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, King's Building, West Mains Road, Edinburgh EH9 3JT. UK; and Drug Discovery Portal, University of Strathclyde, SIPBS, 161 Cathedral Street, Glasgow, G4 0RE. UK [keith.matthews@ed.ac.uk].

African trypanosomiasis is a neglected tropical disease affecting humans and animals across 36 sub-Saharan African countries. We have investigated the potential to exploit a "piggyback" approach to inhibit *Trypanosoma brucei* transmission by targeting the key developmental regulator of transmission, *Trypanosoma brucei* protein tyrosine phosphatase 1 (TbPTP1). This strategy took advantage of the extensive investment in inhibitors for human PTP1B, a key target for pharmaceutical companies for the treatment of obesity and diabetes. Structural predictions for human and trypanosome tyrosine phosphatases revealed the overall conservation of important functional motifs, validating the potential for exploiting cross specific compounds. Thereafter, nineteen inhibitors were evaluated; seventeen from a PTP1B-targetted inhibitor library and two from literature analysis–oleanolic acid and suramin, the latter of which is a front line drug against African trypanosomiasis. The compounds tested displayed similar inhibitory activities against the human and trypanosome enzymes, mostly behaving as non-competitive inhibitors. However, their activity against *Trypanosoma brucei* in culture was low, necessitating further chemical modification to improve their efficacy and specificity. Nonetheless, the results validate the potential to explore a "piggyback" strategy targeting TbPTP1 through exploiting the large pharmacological investment in therapies for obesity targeting PTP1B.

16490. **Schwartz, K. J., Peck, R. F. & Bangs, J. D., 2012.** The intracellular trafficking and glycobiology of TbPDI2, a stage-specific protein disulphide isomerase in *Trypanosoma brucei*. *Eukaryotic Cell*. E **Publication ahead of print, November 16.**

Department of Medical Microbiology & Immunology, University of Wisconsin School of Medicine and Public Health, Microbial Sciences Building, 1550 Linden Drive, Madison WI 53706, USA. [jdbangs@wisc.edu].

Trypanosoma brucei protein disulphide isomerase 2 (TbPDI2) is a bloodstream stage specific lumenal ER glycoprotein. ER localization is dependent on the TbPDI2 C-terminal tetrapeptide (KQDL) and is mediated by TbERD2, an orthologue of the yeast ER retrieval receptor. Consistent with this function TbERD2 localizes prominently to ER exit sites, and RNAi knockdown results in specific secretion of a surrogate ER retention reporter, BiPN:KQDL. TbPDI2 is highly N-glycosylated and is reactive with tomato lectin, suggesting the presence of poly-N-acetyllactosamine modifications, which are common on lyso/endosomal proteins in trypanosomes but are inconsistent with ER localization. However, TbPDI2 is reactive with tomato lectin immediately following biosynthesis–far too rapidly for transport to the Golgi, the site of poly-N-acetyllactosamine addition. TbPDI2 also fails to react with *Erythrina cristagalli* lectin, confirming the absence of terminal N-acetyllactosamine units. We propose that tomato lectin binds the Manbeta1-4GlcNAcbeta1-4GlcNAc trisaccharide core of paucimannose glycans on both newly synthesized and mature TbPDI2. Consistent with this proposal, alpha-mannosidase treatment renders oligomannose N-glycans on the cathepsin L orthologue TbCatL reactive with tomato lectin. These findings resolve contradictory evidence on the location and glycobiology of TbPDI2 and provide a cautionary note on the use of tomato lectin as a poly-N-acetyllactosamine-specific reagent.

16491. **Selvapandiyan, A., Kumar, P., Salisbury, J. L., Wang, C. C. & Nakhasi, H. L., 2012.** Role of centrins 2 and 3 in organelle segregation and cytokinesis in *Trypanosoma brucei*. *PLoS One*, **7** (9): e45288.

Division of Emerging and Transfusion Transmitted Diseases, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland, USA. [selvapandiyan@immindia.org].

Centrins are calcium binding proteins involved in cell division in eukaryotes. Previously, we have shown that depletion of centrin1 in *Trypanosoma brucei* (*T. brucei*) displayed arrested organelle segregation resulting in loss of cytokinesis. In this study we analysed the role of *T. brucei* centrin2 (TbCen2) and *T. brucei* 3 (TbCen3) in the early events of the *T. brucei* procyclic cell cycle. Both the immunofluorescence assay and electron microscopy showed that TbCen2 and 3-deficient cells were enlarged in size with duplicated basal bodies, multinuclei and new flagella that are detached along the length of the cell body. In both TbCen2 and TbCen3 depleted cells, segregation of the organelles i.e. basal bodies, kinetoplast and nucleus was disrupted. Further analysis of the cells with defective organelle segregation identified three different sub configurations of organelle mis-segregations (Types 1-3). In addition, in majority of the TbCen2 depleted cells and in nearly half of the TbCen3 depleted cells, the kinetoplasts were enlarged and undivided. The abnormal segregations ultimately led to aborted cytokinesis and hence affected growth in these cells. Therefore, both centrin2 and 3 are involved in organelle segregation similar to centrin1 as was previously observed. In addition, we identified their role in kinetoplast division which may be also linked to overall mis-segregation.

16492**. Serricchio, M. & Butikofer, P., 2012.** Phosphatidylglycerophosphate synthase associates with a mitochondrial inner membrane complex and is essential for growth of *Trypanosoma brucei*. *Molecular Microbiology*. **E Publication ahead of print, December 11.**

Institute of Biochemistry & Molecular Medicine, University of Bern, Bern, Switzerland. [peter.buetikofer@ibmm.unibe.ch].

Maintenance of the lipid composition is important for proper function and homeostasis of the mitochondrion. In *Trypanosoma brucei*, the enzymes involved in the biosynthesis of the mitochondrial phospholipid, phosphatidylglycerol (PG), have not been studied experimentally. We now report the characterization of *T. brucei* phosphatidylglycerophosphate synthase (TbPgps), the rate-limiting enzyme in PG formation, which was identified based on its homology to other eukaryotic Pgps. Lipid quantification and metabolic labelling experiments show that TbPgps gene knock-down results in loss of PG and a reduction of another mitochondria-specific phospholipid, cardiolipin. Using immunohistochemistry and immunoblotting of digitonin-isolated mitochondria, we show that TbPgps localizes to the mitochondrion. Moreover, reduced TbPgps expression in *T. brucei* procyclic forms leads to alterations in mitochondrial morphology, reduction in the amounts of respiratory complexes III and IV and, ultimately, parasite death. Using native polyacrylamide gel electrophoresis we demonstrate for the first time in a eukaryotic organism that TbPgps is a component of a 720 kDa protein complex, co-migrating with *T. brucei* cardiolipin synthase and cytochrome c1, a protein of respiratory complex III.

16493. **Setzer, W. N. & Ogungbe, I. V., 2012.** *In-silico* investigation of antitrypanosomal phytochemicals from Nigerian medicinal plants. *PLoS Neglected Tropical Diseases*, **6** (7): e1727.

Department of Chemistry, University of Alabama in Huntsville, Huntsville, Alabama, USA. [wsetzer@chemistry.uah.edu].

Human African trypanosomiasis (HAT), a parasitic protozoal disease, is caused primarily by two subspecies of *Trypanosoma brucei*. HAT is a re-emerging disease and currently threatens millions of people in sub-Saharan Africa. Many affected people live in remote areas with limited access to health services and, therefore, rely on traditional herbal medicines for treatment. A molecular docking study was carried out on phytochemical agents that have been previously isolated and characterized from Nigerian medicinal plants, either known to be used ethnopharmacologically to treat parasitic infections or known to have *in vitro* antitrypanosomal activity. A total of 386 compounds from 19 species of medicinal plants were investigated using *in-silico* molecular docking with validated *Trypanosoma brucei* protein targets that were available from the protein data bank (PDB): Adenosine kinase (TbAK), pteridine reductase 1 (TbPTR1), dihydrofolate reductase (TbDHFR), trypanothione reductase (TbTR), cathepsin B (TbCatB), heat shock protein 90 (TbHSP90), sterol 14alpha-demethylase (TbCYP51), nucleoside hydrolase (TbNH), triose phosphate isomerase (TbTIM), nucleoside 2-deoxyribosyltransferase (TbNDRT), UDP-galactose 4' epimerase (TbUDPGE), and ornithine decarboxylase (TbODC). This study revealed that triterpenoid and steroid ligands were largely selective for sterol 14alpha-demethylase; anthraquinones, xanthones, and berberine alkaloids docked strongly to pteridine reductase 1 (TbPTR1); chromenes, pyrazole and pyridine alkaloids preferred docking to triose phosphate isomerase (TbTIM); and numerous indole alkaloids showed notable docking energies with UDP-galactose 4' epimerase (TbUDPGE). Polyphenolic compounds such as flavonoid gallates or flavonoid glycosides tended to be promiscuous docking agents, giving strong docking energies with most proteins. This *in silico* molecular docking study has identified potential biomolecular targets of phytochemical components of antitrypanosomal plants and has determined which phytochemical classes and structural manifolds likely target trypanosomal enzymes. The results could provide the framework for synthetic modification of bioactive phytochemicals, *de novo* synthesis of structural motifs, and lead to further phytochemical investigations.

16494**. Sousa Silva, M., Ferreira, A. E., Gomes, R., Tomas, A. M., Ponces Freire, A. & Cordeiro, C., 2012.** The glyoxalase pathway in protozoan parasites. *International Journal of Medical Microbiology*, **302** (4-5): 225-229.

Centro de Quimica e Bioquimica, Departamento de Quimica e Bioquimica, Faculdade de Ciencias da Universidade de Lisboa, Portugal. [cacordeiro@fc.ul.pt].

16495. **Steverding, D., Sexton, D. W., Chrysochoidi, N. & Cao, F., 2012.** *Trypanosoma brucei* transferrin receptor can bind C-lobe and N-lobe fragments of transferrin. *Molecular & Biochemical Parasitology*, **185** (2): 99-105.

Norwich Medical School, BioMedical Research Centre, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK. [dsteverding@hotmail.com].

Transferrin (Tf) is a dumbbell-shaped iron transport protein composed of two homologous lobes (C-lobe and N-lobe) and is an essential growth factor for the protozoan parasite *Trypanosoma brucei*. The trypanosomal receptor for Tf uptake (TbTfR) is a heterodimeric complex that bears no structural similarity with the human Tf receptor. As a first step in identifying the region of Tf involved in binding to the TbTfR, C-lobe and N-lobe fragments were assessed for their capability to interact with the receptor. Preparations of C-lobe and N-lobe fragments were obtained by digestion of iron-loaded bovine Tf with proteinase K-agarose. The individual fragments were then purified by concanavalin A affinity chromatography. Uptake experiments with bloodstream forms of *T. brucei* demonstrated that both C-lobe and N-lobe fragments were ingested by the parasites. The uptake of the isolated lobes could be inhibited by an excess of Tf and *vice versa*. Dot blot binding assays showed that both C-lobe and N-lobe fragments were capable of binding to the TbTfR. Both isolated lobes were also able to support the growth of bloodstream forms of *T. brucei* when cultured in Tf-depleted medium. However, the C-lobe fragment was more efficiently taken up and more potent in supporting parasite growth. The results indicate that the interaction of Tf with the TbTfR is different from that with the human Tf receptor. This difference may be exploited for the development of agents specifically interfering with the binding of Tf to the TbTfR.

16496. **Suganuma, K., Yamasaki, S., Asada, M., Kawazu, S. & Inoue, N., 2012.** The epimastigote stage-specific gene expression of CESP is tightly regulated by its 3' UTR. *Molecular & Biochemical Parasitology*, **186** (1): 77-80.

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido 080-8555, Japan. [kerokerokerozou@gmail.com].

It is known that gene expression in kinetoplastids is regulated post-transcriptionally. Although previous studies have shown that stage-specific gene expression in trypanosomes is regulated by cis-elements located in the 3' untranslated region (UTR) of mRNA and also by RNA binding proteins that use bloodstream and procyclic forms, no studies have been performed in the epimastigote form (EMF) of African trypanosomes. This study shows that the cis-elements of the *congolense* epimastigote-specific protein (cesp) gene regulate its EMF-specific expression. Four different egfp expression cassettes containing 5' and 3' UTRs derived from actin and cesp were integrated into the *Trypanosoma congolense* genome. EGFP expression was observed in EMF trypanosomes when the egfp cassette contained the cesp 3' UTR. These results indicate that the essential cis-element located in the cesp 3' UTR selectively stabilizes mRNA in the EMF stage and/or destabilized mRNA in other stages. This is the first report of post-transcriptional gene expression regulation in EMF African trypanosomes.

16497. **Sykes, S. E. & Hajduk, S. L., 2012.** Dual functions of alpha-ketoglutarate dehydrogenase E2 in the Krebs cycle and mitochondrial DNA inheritance in *Trypanosoma brucei*. *Eukaryotic Cell*. **E Publication ahead of print, November 2.**

Department of Cellular Biology, University of Georgia, Athens, GA 30602 USA. [shajduk@bmb.uga.edu].

The dihydrolipoyl succinyltransferase (E2) of the multi-subunit alpha-ketoglutarate

dehydrogenase complex (alpha-KD) is an essential Krebs cycle enzyme commonly found in the matrix of mitochondria. African trypanosomes developmentally regulate mitochondrial carbohydrate metabolism and lack a functional Krebs cycle in the bloodstream of the mammal. We found that despite the absence of a functional alpha-KD, bloodstream form (BF) trypanosomes express alpha-KDE2 that localized to the mitochondrial matrix and inner membrane. Furthermore, alpha-KDE2 fractionated with the mitochondrial genome, the kinetoplast DNA (kDNA), in a complex with the flagellum. A role for alpha-KDE2 in kDNA maintenance was revealed in alpha-KDE2 RNAi knockdowns. Following RNAi induction, bloodstream trypanosomes showed a pronounced growth reduction and often failed to equally distribute kDNA to daughter cells, resulting in accumulation of cells devoid of kDNA (dyskinetoplastic) or containing two kinetoplasts. Dyskinetoplastic trypanosomes lacked mitochondrial membrane potential and contained mitochondria of substantially reduced volume. These results indicate that alpha-KDE2 is bifunctional both as a metabolic enzyme and as a mitochondria inheritance factor necessary for the distribution of kDNA networks to daughter cells at cytokinesis.

16498. **Symula, R. E., Beadell, J. S., Sistrom, M., Agbebakun, K., Balmer, O., Gibson, W., Aksoy, S. & Caccone, A., 2012.** *Trypanosoma brucei gambiense* group 1 is distinguished by a unique amino acid substitution in the HpHb receptor implicated in human serum resistance. *PLoS Neglected Tropical Diseases*, **6** (7): e1728.

Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut, USA. [resymula@olemiss.edu].

Trypanosoma brucei rhodesiense (*Tbr*) and *T. b. gambiense* (*Tbg*), causative agents of human African trypanosomiasis (sleeping sickness), have evolved alternative mechanisms of resisting the activity of trypanosome lytic factors (TLFs), components of innate immunity in human serum that protect against infection by other African trypanosomes. In *Tbr*, lytic activity is suppressed by the *Tbr*-specific serum-resistance associated (SRA) protein. The mechanism in *Tbg* is less well understood but has been hypothesized to involve altered activity and expression of haptoglobin-haemoglobin receptor (HpHbR). HpHbR has been shown to facilitate internalization of TLF-1 in *T .b. brucei* (*Tbb*), a member of the *T. brucei* species complex that is susceptible to human serum. By evaluating the genetic variability of HpHbR in a comprehensive geographical and taxonomic context, we show that a single substitution that replaces leucine with serine at position 210 is conserved in the most widespread form of *Tbg* (*Tbg* group 1) and not found in related taxa, which are either human serum susceptible (*Tbb*) or known to resist lysis via an alternative mechanism (*Tbr* and *Tbg* group 2). We hypothesize that this single substitution contributes to reduced uptake of TLF and thus may play a key role in conferring serum resistance to *Tbg* group 1. In contrast, similarity in HpHbR sequence among isolates of *Tbg* group 2 and *Tbb/Tbr* provides further evidence that human serum resistance in *Tbg* group 2 is likely independent of HpHbR function.

16499. **Taschner, A., Weber, C., Buzet, A., Hartmann, R. K., Hartig, A. & Rossmanith, W., 2012.** Nuclear RNase P of *Trypanosoma brucei*: a single protein in place of the multicomponent RNA-protein complex. *Cell Reports*, **2** (1): 19-25.

Center for Anatomy & Cell Biology, Medical University of Vienna, Wahringer

Strasse 13, 1090 Vienna, Austria. [walter.rossmanith@meduniwien.ac.at].

16500. **Tiengwe, C., Marcello, L., Farr, H., Dickens, N., Kelly, S., Swiderski, M., Vaughan, D., Gull, K., Barry, J. D., Bell, S. D. & McCulloch, R., 2012.** Genome-wide analysis reveals extensive functional interaction between DNA replication initiation and transcription in the genome of *Trypanosoma brucei*. *Cell Reports*, **2** (1): 185-197.

The Wellcome Trust Centre for Molecular Parasitology, College of Medical, Veterinary and Life Sciences, Institute of Infection, Immunity and Inflammation, University of Glasgow, Sir Graeme Davies Building, 120 University Place, Glasgow G12 8TA, UK. [stephen.bell@path.ox.ac.uk].

Identification of replication initiation sites, termed origins, is a crucial step in understanding genome transmission in any organism. Transcription of the *Trypanosoma brucei* genome is highly unusual, with each chromosome comprising a few discrete transcription units. To understand how DNA replication occurs in the context of such organization, we have performed genome-wide mapping of the binding sites of the replication initiator ORC1/CDC6 and have identified replication origins, revealing that both localize to the boundaries of the transcription units. A remarkably small number of active origins is seen, whose spacing is greater than in any other eukaryote. We show that replication and transcription in *T. brucei* have a profound functional overlap, as reducing ORC1/CDC6 levels leads to genome-wide increases in mRNA levels arising from the boundaries of the transcription units. In addition, ORC1/CDC6 loss causes de-repression of silent variant surface glycoprotein genes, which are critical for host immune evasion.

16501. **Tonkin, M. L., Workman, S. D., Eyford, B. A., Loveless, B. C., Fudge, J. L., Pearson, T. W. & Boulanger, M. J., 2012.** Purification, crystallization and X-ray diffraction analysis of *Trypanosoma congolense* insect-stage surface antigen (TcCISSA). *Acta Crystallographia Section F Structural Biology & Crystalization Communications*, **68** (Pt 12): 1503-1506.

Department of Biochemistry and Microbiology, University of Victoria, Petch Building, 3800 Finnerty Road, Victoria, BC V8P 5C2, Canada.

16502. **Tschudi, C., Shi, H., Franklin, J. B. & Ullu, E., 2012.** Small interfering RNA-producing loci in the ancient parasitic eukaryote *Trypanosoma brucei*. *BMC Genomics*, **13**: 427.

Department of Internal Medicine, School of Medicine, Yale University, New Haven, CT 06536, USA. [elisabetta.ullu@yale.edu].

At the core of the RNA interference (RNAi) pathway in *Trypanosoma brucei* is a single Argonaute protein, TbAGO1, with an established role in controlling retroposon and repeat transcripts. Recent evidence from higher eukaryotes suggests that a variety of genomic sequences with the potential to produce double-stranded RNA are sources for small interfering RNAs (siRNAs). To test whether such endogenous siRNAs are present in *T. brucei* and to probe the individual role of the two Dicer-like enzymes, we affinity purified

TbAGO1 from wild-type procyclic trypanosomes, as well as from cells deficient in the cytoplasmic (TbDCL1) or nuclear (TbDCL2) Dicer, and subjected the bound RNAs to Illumina high-throughput sequencing. In wild-type cells the majority of reads originated from two classes of retroposons. We also considerably expanded the repertoire of trypanosome siRNAs to encompass a family of 147-bp satellite-like repeats, many of the regions where RNA polymerase II transcription converges, large inverted repeats and two pseudogenes. Production of these newly described siRNAs is strictly dependent on the nuclear DCL2. Notably, our data indicate that putative centromeric regions, excluding the CIR147 repeats, are not a significant source for endogenous siRNAs. Our data suggest that endogenous RNAi targets may be as evolutionarily old as the mechanism itself.

16503**. Verplaetse, E., Gualdron-Lopez, M., Chevalier, N. & Michels, P. A., 2012.** Studies on the organization of the docking complex involved in matrix protein import into glycosomes of *Trypanosoma brucei*. *Biochemical & Biophysical Research Communications*, **424** (4): 781-785.

Research Unit for Tropical Diseases, de Duve Institute, Laboratory of Biochemistry, Université catholique de Louvain, Brussels, Belgium. [paul.michels@uclouvain.be].

Trypanosoma brucei contains peroxisome-like organelles designated glycosomes because they sequester the major part of the glycolytic pathway. Import of proteins into the peroxisomal matrix involves a protein complex associated with the peroxisomal membrane of which PEX13 is a component. Two very different PEX13 isoforms have recently been identified in *T. brucei*. A striking feature of one of the isoforms, TbPEX13.1, is the presence of a C-terminal type 1 peroxisomal-targeting signal (PTS1), the tripeptide TKL, conserved in its orthologues in all members of the Trypanosomatidae family so far studied, but absent from TbPEX13.2 and the PEX13s in all other organisms. Despite their differences, both TbPEX13s function as part of a docking complex for cytosolic receptors with bound matrix proteins to be imported. We further characterized TbPEX13.1's function in glycosomal matrix-protein import. It provides a frame to anchor another docking complex component, PEX14, to the glycosomal membrane or information to correctly position it within the membrane. To investigate the possible function of the C-terminal TKL, we determined the topology of the C-terminal half of TbPEX13.1 in the membrane and show that its SH3 domain, located immediately adjacent to the PTS1, is at the cytosolic face.

16504**. Wolkmer, P., da Silva, C. B., Paim, F. C., Da Silva, A. S., Tavares, K. C., Lazzarotto, C. R., Palma, H. E., Thome, G. R., Miletti, L. C., Schetinger, M. R., Lopes, S. T. & Mazzanti, C. M., 2012.** Biochemistry detection of acetylcholinesterase activity in *Trypanosoma evansi* and possible functional correlations. *Experimental Parasitology*, **132** (4): 546-549.

Department of Chemistry, Universidade Federal de Santa Maria, Brazil. [patiwol@hotmail.com].

Several chemical and immunohistochemical techniques can be used for the detection of acetylcholinesterase (AChE) activity. In this experiment we aimed to detect AChE activity in *Trypanosoma evansi*. For this, the parasites were isolated from the blood of experimentally infected rats using a DEAE-cellulose column. Enzymatic activity was determined in

trypomastigote forms at 0, 0.2, 0.4, 0.8 and 1.2 mg/mL of protein concentrations by a standard biochemical protocol. At all concentrations tested, the study showed that *T. evansi* expresses the enzyme AChE and its activity was proportional to the concentration of protein, ranging between 0.64 and 2.70 µmol of AcSCh/h. Therefore, we concluded that it is possible to biochemically detect AChE in *T. evansi*, an enzyme that may be associated with vital functions of the parasite and also can be related to chemotherapy treatments, as further discussed in this article.

16505**. Xu, X., Olson, C. L., Engman, D. M. & Ames, J. B., 2012.** NMR structure of the calflagin Tb24 flagellar calcium binding protein of *Trypanosoma brucei*. *Protein Science*, **21** (12): 1942-1947.

Department of Chemistry, University of California, Davis, California 95616, USA. [ames@chem.ucdavis.edu].

16506**. Zinoviev, A., Akum, Y., Yahav, T. & Shapira, M., 2012.** Gene duplication in trypanosomatids - two DED1 paralogues are functionally redundant and differentially expressed during the life cycle. *Molecular & Biochemical Parasitology*, **185** (2): 127-136.

Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel. [shapiram@bgu.ac.il].

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