Thematic area 6: *Glossina* control and eradication

By insecticides, by traps and targets, by insecticide-treated animals, by biological methods, by sequential aerosol technique (SAT), by other methods, effect of insecticide treatments on the environment.

Thematic area 7: Land use and Environment

Natural resources, community participation.

SECTION B - ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

15615. **Aksoy, S., 2011.** Sleeping sickness elimination in sight: time to celebrate and reflect, but not relax. *PLoS Neglected Tropical Diseases*, **5** (2): e1008.

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Sleeping sickness, also known as human African trypanosomiasis (HAT), is one of the neglected tropical diseases of sub-Saharan Africa that has plagued human health and agricultural development. In West Africa, infection with *Trypanosoma brucei gambiense* gives rise to a chronic disease that mainly affects humans. Infection with *T. b. gambiense* accounts for more than 90 percent of reported sleeping sickness cases. In East Africa, infection with *Trypanosoma bruci rhodesiense* generates acute disease in humans, and also circulates in a relatively unaffected livestock reservoir. Both Gambian and Rhodesian sleeping sickness are fatal if left untreated. The parasites are transmitted to the mammalian host through the bite of an infected tsetse fly. Since its discovery a century ago, several waves of HAT epidemics have plagued the continent. During the colonial regimes, it was possible to bring about a steady decline in the number of reported *gambiense* cases from the 1930s onwards with systematic screening, treatment, and patient follow-up in western and central Africa. However, during the post-independence period of the 1960s when HAT cases declined, control programmes within the endemic countries gradually were run down, resulting in a steep rise in incidence during the following 40 years. It has been difficult to estimate the true burden of HAT, as the disease affects the most neglected populations living in remote and rural settings where the majority of people affected are beyond the reach of health care systems and are not reported in any of the health metrics. The World Health Organization (WHO) Expert Committee on HAT control and surveillance estimated in 1995 that the true number of cases was at least 10 times more than that reported considering the huge uncertainties between the reported cases and the factual field situation. Thus, from the 30 000 reported cases annually, it was estimated that some 300 000 infected individuals remained infected in the field.
In this issue of *PLoS Neglected Tropical Diseases*, Pere P. Simarro and colleagues from WHO report that the number of new cases diagnosed with HAT in 2009 has dropped below 10,000 for the first time in 50 years, signalling a possible end to the latest epidemic cycle as a major public health problem. This decline was achieved through an ambitious campaign led by WHO, and many nongovernmental organizations (NGOs) and thanks to a public–private

partnership with Sanofi-Aventis and Bayer to donate and distribute the necessary drugs to WHO for use in affected countries. Without this generosity, patients would have no access to life-saving drugs, however unsatisfactory, given that many national health budgets are already stretched. A new nifurtimox/eflornithine combination treatment (NECT) was also developed recently that reduced both the cost of drugs and their delivery. Also crucial in HAT control was the recognition of the problem by African heads of state and governments during the African Union Summit in Lomé in 2000 where the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) was initiated with the objective to render Africa a tsetse- and trypanosomiasis-free continent. While the news of reaching a probable elimination phase for HAT is most welcome, sustainable management of disease control now poses a formidable challenge to endemic countries and health ministries that have to juggle a multitude of diseases when faced with shortages of funds. If the decline in the reported HAT cases is taken at face value, it could signal African governments to abandon their local control efforts and for funding agencies to modify their disease research priorities. Should this happen, it is most certain that epidemics will likely flare up in the near future as has happened in the past. Given that HAT epidemiology varies significantly for *rhodesiense* and *gambiense* disease, the measures adopted will have to vary in different epidemiological settings. Nevertheless, since the distribution of sleeping sickness is limited to ancient and in many cases well-recognized foci, vigilant monitoring through surveillance at these foci should be continued for both forms of the disease. Towards this end, in February 2008 WHO launched the initiative of the Atlas of HAT to map all reported cases for the period 2000–2009 at the village level, which should be continued and updated. Many reports have highlighted the feared merger of the *gambiense* and *rhodesiense* disease belts in Uganda, which would cause havoc given the different diagnostics and treatment regimes required for two diseases. Continued monitoring of diseases emerging in the potential merger zone has to remain a high priority for Uganda. For the *rhodesiense* disease with documented animal reservoirs, stringent implementation of animal treatments at livestock markets should continue to be a priority, especially in Uganda where cattle movements have been suggested to result in the continued spread of *rhodesiense* disease into new territories.

Despite intensive research into the biology of the trypanosomes and tsetse, to date the toolbox for diagnostics and treatment of sleeping sickness has remained extremely small and plagued with difficulties. This is largely due to the complexity of the parasite's biology where an antigenic variation mechanism has hampered the development of mammalian vaccines. There are no prophylactic drugs. Furthermore, clinical tests applicable in the field for staging of disease have been difficult to develop. However, the basic knowledge accumulated on parasite and tsetse biology and the unprecedented technological advancements we are witnessing in science at this time provide the impetus for continued future research where the prospects for translational science are excellent. You can find a sample of the research that has been published in *PLoS Neglected Tropical Diseases* highlighting such promising discoveries in the special collection presented in this issue. It is most likely that innovative and interdisciplinary cutting-edge research will lead the way for improved and effective tools to monitor and prevent the next epidemic.

One promising area is in the development of DNA-based diagnostic tools such as the use of the loop-mediated isothermal amplification (LAMP) method for rapid detection of parasites in the field setting. This approach can also be applied to detect the circulating infectious parasites in natural tsetse populations or in reservoir animals to monitor the potential risk for human disease emergence during endemic periods. It has also been suggested that piggybacking HAT diagnostic efforts on the platforms already used for other diseases, such as tuberculosis as well as malaria and HIV, can lead to more sustainable surveillance efforts. One immediate

application is the use of a light-emitting diode (LED)–based fluorescence microscope developed for tuberculosis, which has been found to be highly sensitive for trypanosome diagnostics. Use of mini anion exchange centrifugation technique (mAECT) has improved diagnosis of *T. b. gambiense* infections, which are hard to detect due to low parasite densities in patient blood. Continued research into safe, effective, and easy-to-use treatments are essential such as the recently rediscovered compound fexinidazole, which has entered into clinical development for the treatment of sleeping sickness.

A most effective means of preventing disease transmission is to remove tsetse flies, either at a local level (e.g., a group of villages) or regionally (covering large parts of a country or region). However, a major problem has been the cost and logistical difficulty of implementing such control programmes. New research into variations of target design now indicate that catch efficiencies of the major human disease vector species *Glossina fuscipes fuscipes* can be improved by at least 10-fold, resulting in a considerable (more than 6-fold) cost saving for control programmes. Tsetse population genetics information has repeatedly identified extensive structuring in the field. Closer communication between the scientific community and vector control programmes should be encouraged, as vector genetics information can benefit the on-going control activities in the field and improve the sustainability of tsetse reduction efforts. Eco-epidemiology is another growing discipline that can benefit HAT control efforts through the identification of potential disease loci and tsetse breeding sites for targeted control efforts as well as any potential impact of the anticipated climate change on disease patterns.

Finally, the genomes of the human host and the infectious trypanosomes have been sequenced. Technological advancements have made it possible to determine the transcriptome of the trypanosome parasite at a single nucleotide level, opening up prospects for future studies where discoveries into disease stage–specific transcriptomes or proteomes will surely identify novel molecules for diagnostics and therapies. An international consortium of scientists brought together by WHO/TDR has recently announced that the tsetse vector genome (*Glossina morsitans morsitans*) is in the final stages of annotation. Furthermore, the United States National Institutes of Health has recently approved a comprehensive project to obtain the genome sequence of five additional tsetse species along with extensive transcriptome analysis. An immediate application of genomics discoveries could be on vector olfactory biology and could speed up investigations for the development of species-specific and cost-effective chemicals such as attractants to lure tsetse to targets and traps to reduce population densities. Lack of such chemicals for human disease–transmitting tsetse species has discouraged extensive use of vector control. Tsetse–trypanosome interactions can also now be investigated at the molecular level, and this has the potential to lead to pathways or targets to block the parasite's transmission ability in the vector.

In conclusion, it is time to celebrate the efforts of the HAT community that has succeeded in curbing the latest epidemic. However, it is important to remember that there are still 10 000 cases out there that need our attention. It is also important to remember that countries must put in place mechanisms and health personnel who can recognize and report any potential HAT cases in endemic areas to prevent the re-emergence of disease. Finally, this is not the time to abandon research on tsetse and trypanosomes, but rather to exploit the accumulating knowledge in light of the technological advancements and build the toolbox by moving discoveries from bench to field for more effective diagnostics, therapies, and vector control methods.

15616. **Astelbauer, F. & Walochnik, J., 2011.** Antiprotozoal compounds: state of the art and new developments. *International Journal of Antimicrobial Agents,* **38**(2): 118-124.

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Protozoa can cause severe diseases, including malaria, leishmaniasis, Chagas disease, sleeping sickness and amoebiasis, all being responsible for morbidity and mortality particularly in tropical countries. To date there are no protective vaccines against any of these diseases, and many of the available drugs are old or elicit serious adverse reactions. Moreover, parasite resistance to existing drugs has become a serious problem. Owing to lack of financial returns, research in this field is of limited interest to pharmaceutical companies and largely depends on funding by public authorities. This article aims to provide a concise overview of the state-of-the-art treatment for the most important tropical protozoal infections as well as new approaches.

15617. **Bentivoglio, M., Mariotti, R. & Bertini, G., 2011.** Neuroinflammation and brain infections: historical context and current perspectives. *Brain Research Reviews*, **66** (1-2): 152-173.

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An overview of current concepts on neuroinflammation and on the dialogue between neurons and non-neuronal cells in three important infections of the central nervous system (rabies, cerebral malaria, and human African trypanosomiasis or sleeping sickness) is presented. Large numbers of cases affected by these diseases are currently reported. In the context of an issue dedicated to Camillo Golgi, historical notes on seminal discoveries on these diseases are also presented. Neuroinflammation is currently closely associated with pathogenetic mechanisms of chronic neurodegenerative diseases. Neuroinflammatory signaling in brain infections is instead relatively neglected in the neuroscience community, despite the fact that the above infections provide paradigmatic examples of alterations of the intercellular crosstalk between neurons and non-neuronal cells. In rabies, strategies of immune evasion of the host lead to silencing neuroinflammatory signaling. In the intravascular pathology which characterizes cerebral malaria, leukocytes and *Plasmodium* do not enter the brain parenchyma. In sleeping sickness, leukocytes and African trypanosomes invade the brain parenchyma at an advanced stage of infection. Both the latter pathologies leave open many questions on the targeting of neuronal functions and on the pathogenetic role of non-neuronal cells, and in particular astrocytes and microglia, in these diseases. All three infections are hallmarked by very severe clinical pictures and relative sparing of neuronal structure. Multidisciplinary approaches and a concerted action by the neuroscience community are needed to shed light on intercellular crosstalk in these dreadful brain diseases. Such effort could also lead to new knowledge on non-neuronal mechanisms which determine neuronal death or survival.

15618. **Boelaert, M., Meheus, F., Robays, J. & Lutumba, P., 2010.** Socio-economic aspects of neglected diseases: sleeping sickness and visceral leishmaniasis. *Annals of Tropical Medicine & Parasitology*, **104** (7): 535-542.

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Several tropical diseases that are essentially poverty-related have recently gained more attention under the label of "neglected tropical diseases" or NTD. It is estimated that over 1 000 million people currently suffer from one or more NTD. Here, the socio-economic aspects of two NTD - human African trypanosomiasis and human visceral leishmaniasis - are reviewed. Both of these diseases affect the poorest of the poor in endemic countries, cause considerable direct and indirect costs (even though the national control programmes tend to provide free care) and push affected households deeper into poverty.

15619. **Chatelain, E. & Ioset, J. R., 2011.** Drug discovery and development for neglected diseases: the DNDi model. *Drug Design, Development and Therapy*, **5**: 175-181.

Drugs for Neglected Diseases Initiative (DND i), Geneva, Switzerland.

New models of drug discovery have been developed to overcome the lack of modern and effective drugs for neglected diseases such as human African trypanosomiasis (HAT; sleeping sickness), leishmaniasis, and Chagas disease, which have no financial viability for the pharmaceutical industry. With the purpose of combining the skills and research capacity in academia, pharmaceutical industry, and contract researchers, public-private partnerships or product development partnerships aim to create focused research consortia that address all aspects of drug discovery and development. These consortia not only emulate the projects within pharmaceutical and biotechnology industries, e.g. identification and screening of libraries, medicinal chemistry, pharmacology and pharmacodynamics, formulation development, and manufacturing, but also use and strengthen existing capacity in disease-endemic countries, particularly for the conduct of clinical trials. The Drugs for Neglected Diseases initiative (DNDi) has adopted a model closely related to that of a virtual biotechnology company for the identification and optimization of drug leads. The application of this model to the development of drug candidates for the kinetoplastid infections of HAT, Chagas disease, and leishmaniasis has already led to the identification of new candidates issued from DNDi's own discovery pipeline. This demonstrates that the model DNDi has been implementing is working but its sustainability remains to be proven.

15620. **Corbel, V. & Henry, M. C., 2011.** Prevention and control of malaria and sleeping sickness in Africa: where are we and where are we going? *Parasites & Vectors*, **4**: 37.

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The International Symposium on Malaria and Human African Trypanosomiasis: New Strategies for their Prevention & Control was held 7-8 October, 2010 in Cotonou, Benin with about 250 participants from 20 countries. This scientific event aimed at identifying the gaps and research priorities for the prevention and control of malaria and sleeping sickness in Africa and to promote exchange between North and South in the fields of medical entomology, epidemiology, immunology and parasitology. A broad range of influential partners from academia (scientists), stakeholders, public health workers and industry attended the meeting

and about 40 oral communications and 20 posters were presented by PhD students and internationally-recognized scientists from the North and the South. Finally, a special award ceremony was held to recognize efforts in pioneer work conducted by staff involved in the diagnostic of the Sleeping illness in West Africa with partnership and assistance from WHO and the Sanofi-Aventis group.

15621. **Flohe, L., 2011.** The trypanothione system and the opportunities it offers to create drugs for the neglected kinetoplast diseases. *Biotechnology Advances.* **E publication ahead of print, May 19.**

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Parasitic trypanosomatids (Kinetoplastida) are the causative agents of devastating and hard-to-treat diseases such as African sleeping sickness, Chagas disease and various forms of Leishmaniasis. Altogether they affect >30 million patients, account for half a million fatalities p.a. and cause substantial economic problems in the Third World due to human morbidity and livestock losses. The design of efficacious and safe drugs is expected from inhibition of metabolic pathways that are unique and essential to the parasite and absent in the host. In this respect, the trypanothione system first detected in the insect-pathogenic trypanosomatid *Crithidia fasciculata* qualified as an attractive drug target area. The existence of the system in pathogenic relatives was established by homology cloning and PCR. The vital importance of the system was verified in *Trypanosoma brucei* by dsRNA technology or knock-out in other trypanosomatids, respectively, and is explained by its pivotal role in the parasite's antioxidant defence and DNA synthesis. The key system component is the bis-glutathionyl derivative of spermidine, trypanothione. It is the proximal reductant of tryparedoxin which substitutes for thioredoxin-, glutaredoxin- and glutathione-dependent reactions. Heterologous expression, functional characterization and crystallization of recombinant system components finally enable structure-based rational inhibitor design.

15622. **Geiger, A., Simo, G., Grebaut, P., Peltier, J. B., Cuny, G. & Holzmuller, P., 2011.** Transcriptomics and proteomics in human African trypanosomiasis: Current status and perspectives. *Journal of Proteomics*. **In press, corrected proof.**

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Human African trypanosomiasis, or sleeping sickness, is a neglected vector-borne parasitic disease caused by protozoa of the species *Trypanosoma brucei*. Within this complex species, *T. b. gambiense* is responsible for the chronic form of sleeping sickness in Western and Central Africa, whereas *T. b. rhodesiense* causes the acute form of the disease in East Africa. Presently, 1.5 million disability-adjusted life years (DALYs) per year are lost due to sleeping sickness. In addition, on the basis of the mortality, the disease is ranked ninth out of 25 human infectious and parasitic diseases in Africa. Diagnosis is complex and needs the intervention of a specialized skilled staff; treatment is difficult and expensive and has potentially life-threatening side effects. The use of transcriptomic and proteomic technologies, currently in rapid development and increasing in sensitivity and discriminating power, is already generating a large panel of promising results. The objective of these technologies is to

significantly increase our knowledge of the molecular mechanisms governing the parasite establishment in its vector, the development cycle of the parasite during the parasite's intra-vector life, its interactions with the fly and the other microbial inhabitants of the gut, and finally human host-trypanosome interactions. Such fundamental investigations are expected to provide opportunities to identify key molecular events that would constitute accurate targets for further development of tools dedicated to field work for early, sensitive, and stage-discriminant diagnosis, epidemiology, new chemotherapy, and potentially vaccine development, all of which will contribute to fighting the disease. The present review highlights the contributions of the transcriptomic and proteomic analyses developed thus far in order to identify potential targets (genes or proteins) and biological pathways that may constitute a critical step in the identification of new targets for the development of new tools for diagnostic and therapeutic purposes.

15623. **Gilbert, I. H., Leroy, D. & Frearson, J. A., 2011.** Finding new hits in neglected disease projects: target or phenotypic based screening? *Current Topics in Medicinal Chemistry*, **11** (10): 1284-1291.

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In this article, we discuss the merits of both target-based and phenotypic screening strategies to find starting points for drug discovery projects in neglected tropical disease including: human African trypanosomiasis, Chagas disease, leishmaniasis and malaria. Technological advances now mean that it is possible to undertake high quality screens against isolated molecular targets at considerable scale. However, target selection is a minefield of potential issues and often molecules identified and developed as potent inhibitors of targets do not translate into actives against the whole parasite. The potential for rapid resistance development is also a key issue when tackling individual molecular targets. In phenotypic screening, compounds are screened against the whole organism, looking for activity without *a priori* knowledge of the target(s) being modulated. This approach brings the benefits of increased chances of efficacy and potentially slowed resistance development of a successful medicine but the lack of knowledge of the molecular target can make the optimisation process more challenging. Advances in screening technologies has now brought phenotypic approaches up to the scale attained by target-based approaches and we discuss opportunities for advances in this arena, concluding that a robust drug discovery portfolio for such diseases should include both phenotypic and target-based approaches.

15624. **Gonzalez, M. & Cerecetto, H., 2011.** Novel compounds to combat trypanosomatid infections: a medicinal chemical perspective. *Expert Opinion on Therapeutic Patents*, **21** (5): 699-715.

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The current therapeutic arsenal against the kinetoplastids *Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania* spp. is clearly inadequate and underscores the urgent need

to develop new effective, safe and cost-effective drugs. Accordingly, this review of patented products and processes using anti-kinetoplastid agents provides insight into the identification of novel or more refined drugs. In this review, we describe products developed in recent years for the treatment of human African trypanosomiasis, American trypanosomiasis and leishmaniasis from a medicinal chemical perspective. Applications so far have looked only superficially for candidate anti-trypanosomatid drugs and are deficient in the final stages of drug development studies, i.e. tolerance/safety, selectivity, drug-resistance, scaling-up, pharmacokinetic and pharmacodynamic assays. The ultimate goal for production of agents with anti-HAT activity has been the development of dicationic agents with parasite DNA-binding activity. Another goal for control of human African trypanosomiasis as well as for Chagas disease and leishmaniasis is the development of protease inhibitors. It should also be noted that several recent studies describing promising targets and compounds have not yet been patented. An effort should be made by foundations, international health organizations and pharmaceutical corporations to support analysis and development of promising new chemotherapeutic agents for controlling the trypanosomiasis and leishmaniasis.

15625. **Hannaert, V., 2011.** Sleeping sickness pathogen (*Trypanosoma brucei*) and natural products: therapeutic targets and screening systems. *Planta Medica*, **77** (6): 586-597.

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Trypanosoma brucei is the causative agent of human African trypanosomiasis (sleeping sickness) which is fatal if left untreated. This disease occurs in 36 African countries, south of the Sahara, where 60 million people are at risk of acquiring infection. The current chemotherapy relies on only four drugs, three of which were developed more than 60 years ago. These drugs have many limitations, ranging from oral inabsorption, acute toxicities, short duration of action and the emergence of trypanosomal resistance. Despite decades of use of most of the current trypanocides, little is known about their mode of action. That being said, African trypanosomes continue to be among the most extensively studied parasitic protists to date. Many of their intriguing biological features have been well documented and can be viewed as attractive targets for antitrypanosomal chemotherapy. A considerable number of natural products with diverse molecular structures have revealed antiparasitic potency in the laboratory and represent interesting lead compounds for the development of new and urgently needed antiparasitics. The major validated drug targets in *T. brucei* are discussed with particular emphasis on those known to be attacked by natural compounds.

15626. **Harrington, J. M., 2011.** Antimicrobial peptide killing of African trypanosomes. *Parasite Immunology,* **31**(8*):* 461-469.

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The diseases caused by trypanosomes are medically and economically devastating to the population of sub-Saharan Africa. Parasites of the genus *Trypanosoma* infect both humans, causing African sleeping sickness, and livestock, causing nagana. The development of effective treatment strategies has suffered from the severe side effects of approved drugs,

resistance and major difficulties in delivering drugs. Antimicrobial peptides are ubiquitous components of immune defence and are being rigorously pursued as novel sources of new therapeutics for a variety of pathogens. Here we review the role of antimicrobial peptides in the innate immune response of the tsetse fly to African trypanosomes, catalogue trypanocidal antimicrobial peptides from diverse organisms and highlight the susceptibility of bloodstream form African trypanosomes to killing by unconventional toxic peptides.

15627. **Hotez, P., 2011.** A handful of "antipoverty" vaccines exist for neglected diseases, but the world's poorest billion people need more. *Health Affairs*, **30** (6): 1080-1087.

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So-called neglected tropical diseases are the most common infections of the world's poor. Almost all of the "bottom billion" - the 1.4 billion people who live below the poverty level defined by the World Bank - suffer from one or more neglected diseases including hookworm infection, sleeping sickness, or Chagas disease. These diseases are actually a cause of poverty because of their adverse effects on child growth and development and worker productivity. Vaccines to combat such diseases have come to be known as "antipoverty vaccines." Unfortunately, the recent surge in the development and delivery of vaccines to combat the major childhood killers such as pneumococcal pneumonia and measles has bypassed neglected diseases. Nevertheless, some vaccines for these neglected diseases are now entering the clinical pipeline. This article describes how some antipoverty vaccine development is proceeding and offers recommendations for stimulating further development such as through pooled funding for innovation, developing-country manufacturers, and public-private partnerships for product development.

15628. **Isaacs, D., 2010.** To sleep, perchance to dream. *Journal of Paediatric Child Health*, **46** (10): 553.

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No abstract available.

15629. **Kariuki, T., Phillips, R., Njenga, S., Olesen, O. F., Klatser, P. R., Porro, R., Lock, S., Cabral, M. H., Gliber, M. & Hanne, D., 2011** Research and capacity building for control of neglected tropical diseases: the need for a different approach. *PLoS Neglected Tropical Diseases*, **5**(5): e1020.

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The neglected tropical diseases (NTDs) are a group of chronic disabling infections affecting more than 1 billion people worldwide, mainly in Africa and mostly those living in remote rural areas, urban slums, or conflict zones. By considering the NTDs together, it is clear that they threaten the health of the poorest to a similar extent as HIV/AIDS, malaria, and tuberculosis (TB). Beyond their negative direct impact on health, NTDs also fuel the vicious circle of poverty and stigma that leaves people unable to work, go to school, or participate in family and community life. Whilst "the big three" infections have caught the world's attention, these other disabling and sometimes fatal infectious diseases in Africa have until very recently been receiving relatively little attention from donors, policymakers, and public health officials. Yet NTD control represents a largely untapped development opportunity to alleviate misery and poverty in the world's poorest populations, and therefore has a direct impact on the achievement of the Millennium Development Goals.

The impact of NTDs on health and economy is now increasingly discussed in international fora, e.g. recently in a series of articles in *The Lancet* at the beginning of 2010 (http://www.thelancet.com/series/neglected-tropical-diseases). However, the discussion is clearly dominated by scientists from the industrialized ("Northern") countries. While the African continent is particularly hard hit by NTDs, the African scientific community has so far been poorly represented during global priority setting for research on NTDs. Also, in most cases, the importance of scientific capacity building in endemic developing countries that would guarantee ownership, support, and sustainability of control programmes is neglected. In this article, we would therefore like to summarize the deliberations and recommendations of two workshops held in Bamako and Lisbon in 2008 and 2010, respectively, where about 50 researchers from sub-Saharan Africa discussed their views on research and capacity requirements for the control of NTDs. The workshops were organized under the framework of the European Foundation Initiative for African Research into Neglected Tropical Diseases (EFINTD, http://www.ntd-africa.net/), which consists of five European foundations, and aims to combat NTDs by offering funding for postdoctoral fellows from sub-Saharan Africa to pursue scientific careers in their home continent. The initiative also facilitates the creation of collaborative scientific networks linking researchers within Africa, and between Northern and African scientists.

It was interesting to observe that the workshop participants identified the same scientific challenges that must be overcome for control of NTDs as their colleagues from the North: the need for the development of new diagnostic tools, vaccines, and drugs; the development of efficient drug delivery systems; detailed epidemiological investigations; and community-based implementation research. All these might reflect the intensive networking between the scientists and general agreement on the urgent imperative to investigate on these topics, but it might also be the result of the general domination of Northern scientists in these discussions. At the same time, there were some distinct differences expressed by the African scholars: the need to focus more strongly on the short-term applicability of research and its relevance to national and regional health problems in Africa, as well as its benefits to the local population. Instead of concentrating on pure basic research and scientific impact factors, more research efforts should be dedicated to operational research and better application of existing tools in the health system. In addition, the African scholars highlighted the lack of sufficient funds from individual funding organizations available for African institutions (for infrastructure support as well as project funding) to enable them to work efficiently in their home countries. Indeed, this was noted to be a major factor hindering African scholars trained abroad from returning to their home countries to pursue careers in health research.

In addition to the paucity of financial resources for conducting research, the African scientists were unanimous in supporting the institution of career development schemes (e.g. mentorship programmes, project management courses, proposal-writing workshops, language training, and networking opportunities, such as workshops and conferences). The African researchers reiterated that the burden of supporting research for NTDs was not the sole purview of Northern donors, but that the governments of their home countries should also take some responsibility for providing adequate infrastructure and job opportunities. The EFINTD was seen as a promising and novel contribution to long-term capacity development, and as a platform for initiation of networking schemes. However, it was suggested that better, faster, and more focused outcomes could be achieved if a coordinated approach involving other funding organizations was in place; this could provide much needed support to large research infrastructures or permit extensions of programmes, which would help to sustain research activities in sub-Saharan Africa. The selection process adopted by the EFINTD was seen as very positive: it was one of the few opportunities for junior researchers to apply for their own funding, and the intense selection through a final conference with presentation and interviews was seen as most appropriate, because it was one of the very few occasions where they received direct feedback on their ideas from a panel of internationally recognized experts.

The deliberations raised above pose a serious question to research funders in general: what is the role of funding organizations when trying to promote careers of young scientists from developing countries? The traditional way of funding large cooperative research projects between partners in the North and partners in the South is not the only solution, because they are usually dominated by the Northern scholars and often result in brain drain from the South. At the same time, without the support of international experts, most junior scholars from sub-Saharan Africa would not be able to develop their careers, because they need their expertise, access to state-of-art facilities, and networks. For most African research institutions, capacity building is also needed at the institutional level. Even when funding is granted directly to research institutions in the South, the scholars may not be able to exploit the full potential of the resources provided because the institutions may not have the critical scientific mass and equipment to execute the proposed programmes, or there may not be sufficient administrative capacity available to deal with these sometimes very large and complex projects. This lack of institutional administrative capacity has been a deterrent for funding organizations that prefer to lodge their finances with Northern partners. A further aspect of this is that funding organizations have their own rules for monitoring and evaluation which sometimes differ immensely (not to mention the sometimes very complicated application procedures). Combined with the usual high expectations on the side of the donors, there is accordingly an increased risk of failure compared to the situation in the North.

These thoughts and ideas are leading to two conclusions for organizations engaged in funding research in sub-Saharan Africa, which are usually overlooked: 1) funding organizations should communicate more amongst each other to really complement, coordinate, and harmonize efforts, without losing sight of accepted good practices or rigorous (peer) review of both the science and the instruments of financial administration. Ideally, they should standardize and simplify application and reporting procedures, and 2) they should be involved more strongly in the projects from the beginning, not in a way of patronizing or controlling, but rather through genuine partnerships. If funding organizations are willing to become partners rather than mere providers of funds, then they need to acquire the necessary knowledge of the scientific fields and regions they are investing in. It will require more investment in human resources in disease endemic countries, a focused approach to assist African institutions in developing their own

capacities, and a new mind-set and willingness to be much more than just donors and detached grant administrators.

15630. **Jacobs, R. T., Nare, B. & Phillips, M. A., 2011.** State of the art in African trypanosome drug discovery. *Current Topics in Medicinal Chemistry*, **11** (10): 1255-1274.

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African sleeping sickness is endemic in sub-Saharan Africa where the WHO estimates that 60 million people are at risk for the disease. Human African trypanosomiasis (HAT) is 100 percent fatal if untreated and the current drug therapies have significant limitations due to toxicity and difficult treatment regimes. No new chemical agents have been approved since eflornithine in 1990. The pentamidine analogue DB289, which was in late stage clinical trials for the treatment of early stage HAT recently failed due to toxicity issues. A new protocol for the treatment of late-stage *T. brucei gambiense* that uses combination nifurtomox/eflornithine (NECT) was recently shown to have better safety and efficacy than eflornithine alone, while being easier to administer. This breakthrough represents the only new therapy for HAT since the approval of eflornithine. A number of research programmes are on-going to exploit the unusual biochemical pathways in the parasite to identify new targets for target based drug discovery programmes. Efforts are also underway to discover new chemical entities through whole organism screening approaches. A number of inhibitors with anti-trypanosomal activity have been identified by both approaches, but none of the programmes are yet at the stage of identifying a preclinical candidate. This dire situation underscores the need for continued effort to identify new chemical agents for the treatment of HAT.

15631. **Kappagoda, S., Singh, U. & Blackburn, B. G., 2011.** Antiparasitic therapy. *Mayo Clinic Proceedings*, **86** (6): 561-583.

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Parasitic diseases affect more than two billion people globally and cause substantial morbidity and mortality, particularly among the world's poorest people. This overview focuses on the treatment of the major protozoan and helminth infections in humans. Recent developments in antiparasitic therapy include the expansion of artemisinin-based therapies for malaria, new drugs for soil-transmitted helminths and intestinal protozoa, expansion of the indications for antiparasitic drug treatment in patients with Chagas disease, and the use of combination therapy for leishmaniasis and human African trypanosomiasis.

15632. **Kroubi, M., Karembe, H. & Betbeder, D., 2011.** Drug delivery systems in the treatment of African trypanosomiasis infections. *Expert Opinion on Drug Delivery*, **8** (6): 735-747.

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Animal African trypanosomiasis (AT) is treated and controlled with homidium, isometamidium and diminazene, whereas human AT is treated with suramin, pentamidine, melarsoprol and eflornithine (DFMO), or a combination of DFMO and nifurtimox. Monotherapy can present serious side effects, for example, melarsoprol, the more frequently used drug that is effective for both haemolymphatic and meningoencephalic stages of the disease, is so toxic that it kills 5 percent of treated patients. These treatments are inefficient, have a narrow safety index and drug resistance is a growing concern. No new drug has been developed since the discovery of DFMO in the 1970s. There is a pressing need for an effective, safe drug for both stages of the disease, and recent research is focused on the development of new formulations in order to improve their therapeutic index. This review shows the potential interest of using nanoparticulate formulations of trypanocidal drugs to improve parasite targeting, efficacy and, potentially, safety while being cost-effective. The design of drug formulations relevant to the treatment of AT must include a combination of very specific properties. In summary, the drug delivery system must be compatible with the physicochemical properties of the drug (charge, lipophilicity and molecular mass) in order to allow high drug payloads while being biocompatible for the patient.

15633. **Matemba, L. E., Fevre, E. M., Kibona, S. N., Picozzi, K., Cleaveland, S., Shaw, A. P. & Welburn, S. C., 2010.** Quantifying the burden of *rhodesiense* sleeping sickness in Urambo District, Tanzania. *PLoS Neglected Tropical Diseases*, **4** (11): e868.

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Human African trypanosomiasis is a severely neglected vector-borne disease that is always fatal if untreated. In Tanzania it is highly focalised and of major socio-economic and public health importance in affected communities. This study aimed to estimate the public health burden of *rhodesiense* HAT in terms of DALYs and financial costs in a highly disease endemic area of Tanzania using hospital records. Data were obtained from 143 patients admitted in 2004 for treatment for HAT at Kaliua Health Centre, Urambo District. The direct medical and other indirect costs incurred by individual patients and by the health services were calculated. DALYs were estimated using methods recommended by the Global Burden of Disease Project as well as those used in previous *rhodesiense* HAT estimates assuming HAT under reporting of 45 percent, a figure specific for Tanzania. The DALY estimate for HAT in Urambo District with and without age-weighting were 215.7 (95 percent CI: 155.3-287.5) and 281.6 (95 percent CI: 209.1-362.6) respectively. When 45 percent under-reporting was included, the results were 622.5 (95 percent CI: 155.3-1098.9) and 978.9 (95 percent CI: 201.1-1870.8) respectively. The costs of treating 143 patients in terms of admission costs, diagnosis, hospitalization and sleeping sickness drugs were estimated at US$ 15 514, of which patients themselves paid US$ 3 673 and the health services US$ 11 841. The burden in terms of indirect non-medical costs for the 143 patients was estimated at US$ 9 781. This study shows that HAT imposes a considerable burden on affected rural communities in Tanzania and stresses the urgent need for location- and disease-specific burden estimates tailored to particular rural settings in countries like Tanzania where a considerable number of infectious diseases are prevalent and, due to their focal nature, are often concentrated in certain locations where they impose an especially high burden.

15634. **Matthews, K. R., 2011.** Controlling and coordinating development in vector-transmitted parasites. *Science*, **331** (6021): 1149-1153.

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Vector-borne parasites cause major human diseases of the developing world, including malaria, human African trypanosomiasis, Chagas disease, leishmaniasis, filariasis, and schistosomiasis. Although the life cycles of these parasites were defined over 100 years ago, the strategies they use to optimize their successful transmission are only now being understood in molecular terms. Parasites are now known to monitor their environment in both their host and vector and in response to other parasites. This allows them to adapt their developmental cycles and to counteract any unfavourable conditions they encounter. Here, the interactions that parasites engage in with their hosts and vectors to maximize their survival and spread are reviewed.

15635. **Pohlit, A. M., Rezende, A. R., Lopes Baldin, E. L., Lopes, N. P. & Neto, V. F., 2011.** Plant extracts, isolated phytochemicals, and plant-derived agents which are lethal to arthropod vectors of human tropical diseases-a review. *Planta Medica*, **77** (6): 618-630.

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The recent scientific literature on plant-derived agents with potential or effective use in the control of the arthropod vectors of human tropical diseases is reviewed. Arthropod-borne tropical diseases include: amebiasis, Chagas disease (American trypanosomiasis), cholera, cryptosporidiosis, dengue (haemorrhagic fever), epidemic typhus (Brill-Zinsser disease), filariasis (elephantiasis), giardia (giardiasis), human African trypanosomiasis (sleeping sickness), isosporiasis, leishmaniasis, Lyme disease (lyme borreliosis), malaria, onchocerciasis, plague, recurrent fever, sarcocystosis, scabies (mites as causal agents), spotted fever, toxoplasmosis, West Nile fever, and yellow fever. Thus, coverage was given to work describing plant-derived extracts, essential oils (EOs), and isolated chemicals with toxic or noxious effects on filth bugs (mechanical vectors), such as common houseflies (*Musca domestica* Linnaeus), American and German cockroaches (*Periplaneta americana* Linnaeus, *Blatella germanica* Linnaeus), and oriental latrine/blowflies (*Chrysomya megacephala* Fabricius) as well as biting, blood-sucking arthropods such as blackflies (*Simulium Latreille* spp.), fleas (*Xenopsylla cheopis* Rothschild), kissing bugs (*Rhodnius Stal* spp., *Triatoma infestans* Klug), body and head lice (*Pediculus humanus humanus* Linnaeus, *P. humanus capitis* De Geer), mosquitoes (*Aedes* Meigen, *Anopheles* Meigen, *Culex* L., and *Ochlerotatus* Lynch Arribalzaga spp.), sandflies *(Lutzomyia longipalpis* Lutz & Neiva, *Phlebotomus* Loew spp.), scabies mites (*Sarcoptes scabiei* De Geer, *S. scabiei var hominis, S. scabiei var canis, S. scabiei var suis*), and ticks (*Ixodes* Latreille, *Amblyomma* Koch, *Dermacentor* Koch, and *Rhipicephalus* Koch spp.). Examples of plant extracts, EOs, and isolated chemicals exhibiting noxious or toxic activity comparable or superior to the synthetic control agents of choice (pyrethroids, organophosphorous compounds, etc.) are provided in the text for many arthropod vectors of tropical diseases.

15636. **Simarro, P. P., Diarra, A., Ruiz Postigo, J. A., Franco, J. R. & Jannin, J. G., 2011.** The human African trypanosomiasis control and surveillance programme of the World Health Organization 2000-2009: the way forward. *PLoS Neglected Tropical Diseases*, **5** (2): e1007.

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By the end of the last century, WHO and its partners had developed a strong and successful advocacy programme to secure access to diagnosis and treatment, ensuring availability of funds and drugs to support DECs. As a result, during the first decade of the current century, great advances have been made in HAT control. In 2007, a WHO informal consultation of the heads of NSSCPs held in Geneva reached the conclusion that elimination of the disease as a public health problem was possible. This conclusion was based on the achievements obtained, on the current understanding of the epidemiology of the disease, and on the willingness of African heads of states and governments to eradicate tsetse and trypanosomiasis as stated when the PATTEC was established in 2000. The time has now come to sensitize stakeholders on the pertinence and ethical duty of embarking on the process of eliminating HAT as a public health problem despite the difficulties, obstacles, and threats that are expected in this process. Without such hammering approach, there is a risk of stagnation in control and surveillance as occurred in the late 1960s that ultimately led to the return of the disease.

Today, WHO and its partners are committed to reinforcing and coordinating actions towards a sustainable elimination process. While there are still technical aspects to be solved, the elimination of HAT as a public health problem will require social peace, institutional support, and adequate funding for its implementation. These last conditions are not exclusive to the control, elimination, and sustained surveillance of HAT but also for the overall development of DECs, which would contribute to the control of HAT as well.

When targeting the elimination of HAT as a public health problem, the goal should be recognized as a major achievement but must never be considered as an end point. Without appropriate discrimination, the use of the word “elimination” may lead to risky conclusions. The disease believed to “no longer exist” will reach oblivion, placing in the background the required pressing efforts for a sustained and effective surveillance. It must be kept in mind that "elimination" is not synonymous with “eradication”. Elimination is only a point in time in the control process of the disease, at which stage the classical vertical control intervention approaches are no longer cost effective. Thus, the national health system must take the ownership of sustaining elimination by integrating HAT surveillance in their services while maintaining the capacity to react rapidly according to the analytical results of the surveillance outcome.

Elimination should be considered as the beginning of a new process involving new actors. Therefore, elimination of HAT as a public health problem will require continuous efforts and innovative approaches. There is no doubt that new tools would facilitate the elimination process and the sustainability of results; thus, funding efforts for HAT control and research must continue based on public health objectives, and no longer on the burden of the disease.

15637. **Sternberg, J. M., Black, S. J. & Magez, S., 2010.** African trypanosomiasis: new insights for disease control. Preface. *Parasitology*, **137** (14): 1975.

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No abstract available

15638 **Tong, J., Valverde, O., Mahoudeau, C., Yun, O. & Chappuis, F., 2011.** Challenges of controlling sleeping sickness in areas of violent conflict: experience in the Democratic Republic of Congo. *Conflict &Health*, **5** (1): 7.

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Human African trypanosomiasis (HAT), or sleeping sickness, is a fatal neglected tropical disease if left untreated. HAT primarily affects people living in rural sub-Saharan Africa, often in regions afflicted by violent conflict. Screening and treatment of HAT are complex and resource-intensive, and especially difficult in insecure, resource-constrained settings. The country with the highest endemicity of HAT is the Democratic Republic of Congo (DRC), which has a number of foci of high disease prevalence. We present here the challenges of carrying out HAT control programmes in general and in a conflict-affected region of DRC. We discuss the difficulties of measuring disease burden, medical care complexities, waning international support, and research and development barriers for HAT. In 2007, Médecins Sans Frontières (MSF) began screening for HAT in the Haut-Uele and Bas-Uele districts of Orientale Province in northeastern DRC, an area of high prevalence affected by armed conflict. Through early 2009, an HAT prevalence rate of 3.4 percent was found, reaching 10 percent in some villages. More than 46 000 patients were screened and 1 570 treated for HAT during this time. In March 2009, two treatment centres were forced to close due to insecurity, disrupting patient treatment, follow-up, and transmission-control efforts. One project was reopened in December 2009 when the security situation improved, and another in late 2010 based on concerns that population displacement might reactivate historic foci. In all of 2010, 770 patients were treated at these sites, despite a limited geographical range of action for the mobile teams. It is concluded that in conflict settings where HAT is prevalent, targeted medical interventions are needed to provide care to the patients caught in these areas. Strategies of integrating care into existing health systems may be unfeasible since such infrastructure is often absent in resource-poor contexts. HAT care in conflict areas must balance logistical and medical capacity with security considerations, and community networks and international-response coordination should be maintained. Research and development for less complicated, field-adapted tools for diagnosis and treatment, and international support for funding and programme implementation, are urgently needed to facilitate HAT control in these remote and insecure areas.

15639. **Wastling, S. L. & Welburn, S. C., 2011.** Diagnosis of human sleeping sickness: sense and sensitivity. *Trends in Parasitology*. **E publication ahead of print, June 17.**

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In 1997 the World Health Organization (WHO) advocated increased access to diagnosis and treatment, as well as reinforcement of surveillance, for the control of sleeping sickness (human African trypanosomiasis, HAT). This coincided with the end of decades of civil conflicts in several endemic regions and negotiation of a sustainable supply of "free" curative drugs and, as a result, HAT is at its lowest level in 50 years. However, reported cases underestimate prevalence and downplay HAT when compared with data generated by advanced diagnostic capacity for human immunodeficiency virus (HIV), tuberculosis (TB) and malaria, and, because HAT case numbers fall between epidemics, diagnostics become less commercially appealing. Here, recent trends in the development of diagnostics for sleeping sickness are considered and progress towards a much-needed sensitive, specific and affordable point-of-care diagnostic is assessed.

15640. **Zucca, M. & Savoia, D., 2011.** Current developments in the therapy of protozoan infections. *Open Medicinal Chemistry Journal*, **5**: 4-10.

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Protozoan parasites cause serious human and zoonotic infections, including life-threatening diseases such as malaria, African and American trypanosomiasis, and leishmaniasis. These diseases are no longer common in the developed world, but together they still threaten about 40 percent of the world population (WHO estimates). Mortality and morbidity are high in developing countries, and the lack of vaccines makes chemotherapy the only suitable option. However, available antiparasitic drugs are hampered by more or less marked toxic side effects and by the emergence of drug resistance. As the main prevalence of parasitic diseases occurs in the poorest areas of the world, the interest of the pharmaceutical companies in the development of new drugs has been traditionally scarce. The establishment of public-private partnerships focused on tropical diseases is changing this situation, allowing the exploitation of the technological advances that took place during the past decade related to genomics, proteomics, and *in silico* drug discovery approaches. These techniques allowed the identification of new molecular targets that in some cases are shared by different parasites. This review outlines the recent developments in the fields of protease and topoisomerase inhibitors, antimicrobial and cell-penetrating peptides, and RNA interference. It also describes the rapidly developing field of new vectors (micro and nano particles, mesoporous materials) that in some cases can cross host or parasite natural barriers and, by selectively delivering new or already used drugs to the target site, minimize dosage and side effects.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

15641. **Vreysen, M. J., Saleh, K. M., Lancelot, R. & Bouyer, J., 2011.** Factory tsetse flies must behave like wild flies: a prerequisite for the sterile insect technique. *PLoS Neglected Tropical Diseases*, **5** (2): e907.

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Tsetse flies are the vectors of human and animal African trypanosomoses, the former a major neglected disease, and the latter considered among the greatest constraints to livestock production in sub-Saharan Africa. To date, the disease is mainly contained through the prophylactic and curative treatment of livestock with trypanocidal drugs, which is not sustainable. The removal of the vector, the tsetse fly, would be the most efficient way of managing these diseases. A number of efficient tsetse control tactics are available that can be combined and applied following area-wide integrated pest management (AW-IPM) principles. The concept entails (1) the integration of various control tactics, preferably combining those methods that are effective at high population densities with those that are effective at low population densities to obtain maximal efficiency, and (2) the control effort is directed against an entire tsetse population within a delimited area. This is particularly relevant in case eradication is the strategy of choice. Genetic control tactics such as the sterile insect technique (SIT) show great potential for integration in such AW-IPM programmes because they are very efficient for controlling low-density populations, which is not the case for most other techniques. Sterile male insects are reared and, after sterilization with ionizing radiation, sequentially released in large quantities to outnumber the wild male flies. A mating of a sterile male with a virgin wild female fly results in no offspring. Recently, transgenic and paratransgenic techniques have been proposed to sterilize male insects or to make strains refractory to disease parasites in the case of vectors. However, to ensure the success of these control methods, factory-reared tsetse flies must be competitive with their wild counterparts and must exhibit a similar behaviour in a natural environment.

The SIT as part of an AW-IPM approach is a robust technique, which has proven to be very efficient in eradicating, suppressing, or containing dipteran pests such as *Cochliomyia hominivorax* (New World screwworm) in Central America, Mexico, the United States, and Libya. *Ceratitis capitata* (Mediterranean fruit fly) in Argentina, Chile, Israel, Mexico, Peru, Spain, and the US, *Bactrocera cucurbitae* (melon fly) in the Okinawa archipelago of Japan, and lepidopteran pests such as *Cydia pomonella* (codling moth) in Canada, Australian painted apple moth (*Teia anartoides*) in New Zealand, and *Cactoblastic cactorum* (cactus moth) and *Pectinophora gossypiella* (pink bollworm) in Mexico and the US. Similarly, the SIT has been successfully integrated with other control tactics against several tsetse species, i.e., with aerial spraying of insecticides against *Glossina morsitans morsitans* in Tanzania, with insecticide-impregnated targets and traps against *Glossina palpalis gambiensis* and *Glossina tachinoides* in Burkina Faso and *G. palpalis palpalis* in Nigeria, and with the live-bait technique against *Glossina austeni* on Unguja Island (Zanzibar). These programmes showed that the SIT against tsetse is feasible, but with the exception of the programme on Unguja Island, they proved to be unsustainable.

Some have thus questioned whether competitive sterile male tsetse flies can be produced, especially since learning mechanisms like site- or host-fidelity might influence their behaviour and prevent them from feeding efficiently on wild hosts after being reared on artificial membranes in a laboratory environment. Understanding all the factors that contributed to the success on Unguja Island would be useful for future eradication programmes.

Earlier work has already demonstrated that laboratory reared and released sterile tsetse flies were able to feed on wild hosts: (1) recaptured sterile male flies often had residues of blood meals in their digestive tract, (2) a mean lifespan in nature of 11-17 days, which was similar to

captured, marked, and released wild males, and (3) released sterile male tsetse that received too low a dose of isomethamidium chloride in their blood meal before release were found infected with trypanosomes after release in a natural environment, which would have been impossible in the absence of a blood meal on wild hosts.

Adequate survival, dispersal, dispersion, mobility, and mating compatibility are critical factors influencing sexual competitiveness of the released sterile male flies. An analysis of the data collected during the AW-IPM programme against *G. austeni* on the Island of Unguja indicated that the sterile males did not disperse randomly but showed the same spatial distribution as their wild counterparts. The most detailed data sets were available from the primary Jozani Forest Reserve (now part of the Jozani-Chwaka Bay National Park) (6°15′S and 39°25′E), where the vegetation was very homogeneous and where >300 sterile male flies were released per week per km^2. All sterile male flies were marked with fluorescent dye, irradiated with 120 Gy, packaged in carton release containers, and released by air at an altitude of 250 m. The release of the sterile male flies by air ensured their random distribution on the island. The release cartons were dropped at very regular intervals and opened upon contact with the airstream that forced the flies out of the carton box. By the time the flies reached the vegetation, they were not clustered anymore but occupied a certain area of surface in a random way. The flies were sampled with 12 royal blue panels with white legs made sticky with the non-setting adhesive Temoocid. Data sets from week 40, 1994 (start of operational release) to week 26, 1995 were used for the analysis. Thereafter, the number of wild *G. austeni* flies was too low to be meaningful for the analysis. The sticky panels were checked every weekday. The vegetation around each sticky panel was cleared within a radius of 3 m to ensure homogeneous trap efficiency. After collection, all flies were transferred to the laboratory at the Zanzibar Commission of Agriculture and Livestock to examine the head capsules for fluorescence under a UV microscope to distinguish sterile from wild flies.

The main purpose of the statistical analysis was to assess similarities or differences in the spatial pattern of apparent densities of wild and sterile male flies using capture records from the sticky panels. Firstly, we tested the existence of a spatial trend in wild male counts (after log transformation), and we subtracted this trend from log-counts before investigating the independence of trap locations and wild male fly abundance. This was achieved with a Monte Carlo test for marked point processes: the point process being the set of trap locations, and the marks being the wild male fly counts. Secondly, we used a χ^2 test to assess the spatial heterogeneity in wild male fly abundance, and correlation tests to assess the independence of wild males and females, and sterile males. To plot the data, we transformed fly counts into standardized contributions. For each fly category i (wild male or female, sterile male) and trap j ($j = 1,\ldots J$), each observed trap count $n_{i,j}$ was divided by the total observed count N_i for this fly category to give the observed relative contribution of each trap $o_{i,j} = n_{i,j}/N_i$. The expected relative contribution of trap j under the assumption of homogeneous spatial distribution ($e_{i,j} = 1/J$) was then subtracted to $o_{i,j}$ and the result was divided by $e_{i,j}$, thus providing the standardized contribution $c_{i,j} = (o_{i,j}-e_{i,j})/e_{i,j} = J\, n_{i,j}/N_i - 1$. A total of 422 wild female, 679 wild male, and 3 318 sterile male *G. austeni* were trapped in the 12 monitoring sites over this 10-month period. Wild male fly trap catches were higher in the northern part of the forest (linear trend, $R^2 = 0.38$, $p = 0.03$). A similar trend was observed for wild female ($R^2 = 0.72$, $p = 5.10^{-4}$) and sterile male flies ($R^2 = 0.46$, $p = 0.02$). These spatial trends were removed from the data sets for further analyses. The marked point process analysis showed that wild male fly counts were independent from trap locations (Monte Carlo test, $p>0.05$), i.e. no interaction was detected between trap locations and fly counts. The linear spatial trend showed that the observed heterogeneous distribution among trap positions cannot be explained by differences in trap efficiency, but by an aggregation in certain preferred

sites. Barclay has shown the importance of insect aggregation in pest control, especially when using the SIT or any other genetic control method. Even in this fairly homogeneous primary forest habitat on Unguja Island, the distribution of wild *G. austeni* was heterogeneous and thus aggregated, as was observed in South Africa. The ability of sterile males to aggregate (and thus locate) those areas preferred by the wild males is of primary importance to ensure adequate sterile-to-wild male ratios everywhere and was therefore an important factor contributing to the success of the programme. It would be important to reconfirm this observation in other programmes that have a sterile insect component where it should be included as a quality control measure. In addition, the present data suggest that tsetse fly dispersal cannot be solely considered as a homogeneous diffusion process, as often assumed. It confirms that mass-reared and gamma-sterilized male *G. austeni* were able to respond to environmental cues and to aggregate in the preferred sites of the wild population. Their dispersal behaviour was therefore similar to that of wild flies, which confirms that tsetse flies are very good candidates for genetic control.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

15642. **Abd-Alla, A. M., Salem, T. Z., Parker, A. G., Wang, Y., Jehle, J. A., Vreysen, M. J. & Boucias, D., 2011.** Universal primers for rapid detection of hytrosaviruses. *Journal of Virological Methods*, **171** (1): 280-283.

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Hytrosaviridae is a proposed virus family encompassing viruses that cause salivary gland hypertrophy (SGH) syndrome in infected insects and reduce the fertility in their dipteran insect hosts. They contain a large, double stranded DNA genome of 120-190 kbp. To date, these viruses have been detected only in adult Diptera. These include hytrosaviruses detected in various tsetse fly species (*Glossina* spp.), the narcissus bulb fly *Merodon equestris* and the house fly *Musca domestica*. The limited number of hytrosaviruses reported to date may be a reflection of the frequent absence of external symptoms in infected adult flies and the fact that the virus does not cause rapid mortality. Based on the complete genome sequence of *Glossinia pallidipes* (GpSGHV) and *Musca domestica* (MdSGHV) salivary gland hypertrophy viruses, a PCR based methodology was developed to detect the viruses in these species. To be able to detect hytrosaviruses in other Diptera, five degenerate primer pairs were designed and tested on GpSGHV and MdSGHV DNA using gradient PCR with annealing temperatures from 37 to 61 ^{0}C. Two pairs of primers were selected from p74, two pairs from PIF-1 and one pair from ODV-e66 homologous proteins. Four primer pairs generated a virus specific PCR product on both MdSGHV and GpSGHV at all tested annealing temperatures, while the ODV-e66 based primers did not generate a virus specific product with annealing temperatures higher that 47 ^{0}C. No non-specific PCR product was found when using genomic DNA of infected flies as template DNA. These results offer new sets of primers that could be used to detect hytrosaviruses in other insects.

15643. **Basson, C. H. & Terblanche, J. S., 2011.** Respiratory pattern transitions in three species of *Glossina* (Diptera, Glossinidae). *Journal of Insect Physioliology*, **57** (4): 433-443.

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Glossina exhibit cyclic ^{CYC}GE or continuous gas exchange ^{CON}GE patterns at rest. However, the factors influencing the transition from one pattern to another are not well understood for these or other insect species. This study examines which factors could aid in predicting the presence or absence of ^{CYC}GE in adults of three *Glossina* species: *G. palpalis, G. brevipalpis* and *G. austeni*. We report the results of temperature effects on VCO_2, pattern type and the proportion of a population showing ^{CYC}GE, and the prediction of ^{CYC}GE versus ^{CON}GE in *Glossina*. First, we investigated the influence of temperature on VCO_2 and found significant elevation in resting metabolic rate (RMR) with higher temperature in all three species ($p<0.001$). Temperature-induced increases in VCO_2 were modulated by increased burst volume and by cycle frequency, except in *G. brevipalpis* which only appeared to modulate burst volume. These results are largely in keeping with VCO_2 modulation reported for other *Glossina* species previously. Second, elevating temperature resulted in significantly reduced numbers of individuals showing ^{CYC}GE ($p<0.001$ for all three species) contrary to previous reports for other *Glossina* species. Finally, we examined a range of variables as potential predictors of presence or absence of ^{CYC}GE in these three species. Using an information theoretic approach (Akaike weights) to select the best explanatory combination of variables which predicts likelihood of ^{CYC}GE, we found that results varied among species. When species were pooled, the simplest, best-fit model (DeltaAIC<2 from the best model, 44.4 percent probability of being the best model) for predicting pattern type variation was RMR. Overall these results suggest that RMR is a key variable driving pattern type and that elevated temperature reduces the number of individuals showing cyclic patterns through elevation of RMR in these species. This study supports the idea that an interaction between cellular metabolic demand, morphological features of the gas exchange system (e.g. tracheal and spiracular conductances), and CO_2 buffer capacity likely determine gas exchange pattern variation over short time-scales.

15644. **De Vooght, L., Caljon, G., Coosemans, M. & Van den Abbeele, J., 2011.** Functional analysis of the twin-arginine translocation pathway in *Sodalis glossinidius*, a bacterial symbiont of the tsetse fly. *Applied &Environmental Microbiology*, **77** (3): 1132-1134.

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This study demonstrates a functional twin-arginine (Tat) translocation pathway present in the tsetse fly symbiont *Sodalis glossinidius* and its potential to export active heterologous proteins to the periplasm. Functionality was demonstrated using green fluorescent protein (GFP) fused to the Tat signal peptide of *Escherichia coli* trimethylamine N-oxide reductase (TorA).

15645. **Farikou, O., Njiokou, F., Cuny, G. & Geiger, A., 2011.** Microsatellite genotyping reveals diversity within populations of *Sodalis glossinidius*, the secondary symbiont of tsetse flies. *Veterinary Microbiology*, **150** (1-2): 207-210.

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The aim of this study was to develop a PCR-based microsatellite genotyping method for identifying genetic diversity in *Sodalis glossinidius*, a symbiont associated with tsetse fly infection by trypanosomes causing human and animal trypanosomiasis. Allelic polymorphism at three loci, investigated on 40 fly gut extracts, evidenced eight alleles and the existence of five genotypes. This novel approach was shown to be efficient and suitable for routine large-scale genotyping of *S. glossinidius* present in the biologically complex tsetse fly extracts; it could favour progress in the fields of diagnosis, epidemiology, population genetics, and fly/symbiont/trypanosome interactions.

15646. **Heller, K., 2011.** Tsetse flies rely on symbiotic *Wigglesworthia* for immune system development. *PLoS Biology*, **9** (5): e1001070.

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When we even bother to think about them, we usually regard the bacteria and other microbes that live in our guts and on our skin with disgust. However, recent discoveries about the roles the trillions of microbes living in the average person's gut play in obesity, cardiovascular disease, and immunity have led us to look upon our omnipresent bacterial symbionts with a less jaundiced eye. Mutually beneficial partnerships with bacteria are widespread throughout the animal kingdom: some types of squid have bioluminescent bacteria that help them to escape detection, while marine tubeworms harbour bacteria that provide their hosts with nutrients.

A particularly fascinating example of symbiosis occurs between the tsetse fly *Glossina morsitans* and the bacterium *Wigglesworthia glossinidia* (named after Sir Vincent Wigglesworth, the entomologist with the best name ever). Tsetse flies are the sole vectors for the trypanosomes that cause about 10,000 new cases of sleeping sickness in Africa each year. *Wigglesworthia*, which can only survive inside the gut of tsetse flies, has a minimal genome, since it lost much of its DNA as it coevolved with its tsetse host over the last 50–80 million years. These bacteria hang out exclusively within tsetse flies; the set-up provides the *Wigglesworthia* with protection and an energy source. In return, the bacteria perform a variety of services for their host, including vitamin synthesis and resistance to energetically costly trypanosome infections, both of which may be important for tsetse fly fertility.

Previously, researcher Serap Aksoy and her colleagues discovered that tsetse flies that lack *Wigglesworthia* are more susceptible to infection with trypanosomes. In a new study described in this issue of *PLoS Biology*, Brian Weiss, Jingwen Wang, and Serap Aksoy explored how *Wigglesworthia* might affect the immune responses of tsetse flies. To do this, they produced larvae that lack *Wigglesworthia* (Gmm^{Wgm-}) by feeding the antibiotic ampicillin to pregnant female flies (ampicillin selectively kills *Wigglesworthia* without affecting other kinds of bacteria living in the flies). As a handy challenge to the flies' immune system, they injected the flies with *E. coli* K12 bacteria. While mature adult (8-day-old) wild-type (WT) tsetse flies are resistant to infection with *E. coli,* young (3-day-old) flies are quite susceptible. Compared with mature adult WT tsetse, after injection with *E. coli,* the age-matched Gmm^{Wgm-} dropped like . . . flies.

To figure out the basis of the Gmm^{Wgm-} flies' compromised immunity, the authors examined the expression of genes known to be involved in immune responses. They found that expression of

these genes was virtually the same in uninfected WT and *Gmm*$^{Wgm-}$ adults. However, when the flies were injected with *E. coli*, expression increased dramatically in WT flies compared to *Gmm*$^{Wgm-}$. Most striking was the difference in expression of genes involved in cellular immunity processes such as phagocytosis (engulfment of pathogens by host haemocytes) and melanisation (laying down of melanin to form a clot at wound sites).

This led the authors to question whether adult WT flies would become more susceptible to infection when phagocytosis was blocked, so they injected tiny beads directly into the circulatory system of mature WT flies in order to divert the haemocytes and make them unavailable for phagocytosis. The bead-injected WT flies turned out to be highly susceptible to *E. coli* infection, indicating that phagocytosis is an important component of the flies' immune response.

Next, to find out if *Wigglesworthia* is necessary for melanisation, the authors looked at the sites of *E. coli* injection. In *Gmm*$^{Wgm-}$, the wound was still oozing haemolymph (insect blood) 30 minutes after the injection and no melanin was observed. Meanwhile, in WT flies there was no haemolymph visible and a melanin clot had formed at the wound.

Haemocytes play a central important role in cellular immunity; not only do they phagocytose pathogens, but differentiated haemocytes called crystal cells also produce clot-forming melanin. Counting circulating and sessile haemocytes revealed that adult *Gmm*$^{Wgm-}$ had far fewer haemocytes than their wild-type counterparts. The authors speculated that the absence of haemocytes in adult *Gmm*$^{Wgm-}$ flies reflects a lack of blood cell differentiation during development. This was borne out by the drastically decreased expression in *Gmm*$^{Wgm-}$ of two transcription factors known to be involved in haemocyte differentiation in *Drosophila.*

Together, these results show that *Wigglesworthia* must be present in immature tsetse flies so that the immune system can develop and function properly in adults. Thus, reminiscent of the relationship between humans and the bacteria in their guts, *Wigglesworthia* and tsetse flies have coevolved to the point where they can't really survive without each other. The *Wigglesworthia*–tsetse fly association is a great model system for studying the effect of symbionts on host immunity, because of the short generation times of the flies, which are easy and inexpensive to rear. Furthermore, the authors' findings could lead to new ways of modulating tsetse flies' immune response to trypanosomes, hopefully making them more resistant to infection and therefore less efficient vectors for these deadly pathogens.

15647. **Kariithi, H. M., Ince, I. A., Boeren, S., Vervoort, J., Bergoin, M., van Oers, M. M., Abd-Alla, A. M. & Vlak, J. M., 2010.** Proteomic analysis of *Glossina pallidipes* salivary gland hypertrophy virus virions for immune intervention in tsetse fly colonies. *Journal of General Virology*, **91** (Pt 12): 3065-3074.

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Many species of tsetse flies (Diptera: Glossinidae) can be infected by a virus that causes salivary gland hypertrophy (SGH). The genomes of viruses isolated from *Glossina pallidipes* (GpSGHV) and *Musca domestica* (MdSGHV) have recently been sequenced. Tsetse flies with SGH have reduced fecundity and fertility which cause a serious problem for mass rearing in the frame of sterile insect technique (SIT) programmes to control and eradicate tsetse populations in the wild. A potential intervention strategy to mitigate viral infections in fly colonies is neutralizing of the GpSGHV infection with specific antibodies against virion proteins. Two major GpSGHV virion proteins of about 130 and 50kDa respectively, were identified by

Western analysis using a polyclonal rabbit antibody raised against whole GpSHGV virions. The proteome of GpSGHV, containing the antigens responsible for the immune response, was investigated by liquid chromatography tandem mass spectrometry and 61 virion proteins were identified by comparison with the genome sequence. Specific antibodies were produced in rabbits against seven candidate proteins, including the ORF10/C-terminal fragment, ORF47 and ORF96 as well as proteins involved in peroral infectivity PIF-1 (ORF102), PIF-2 (ORF53), PIF-3 (ORF76) and P74 (ORF1). Antiserum against ORF10 specifically reacted to the 130kDa protein in a Western blot analysis and to the envelope protein of GpSGHV, detected by using immunogold-electron microscopy. This result suggests that immune intervention of viral infections in colonies of *G. pallidipes* is a realistic option.

15648. **Lindh, J. M. & Lehane, M. J., 2011.** The tsetse fly *Glossina fuscipes fuscipes* (Diptera: Glossina) harbours a surprising diversity of bacteria other than symbionts. *Antonie Van Leeuwenhoek*, **99** (3): 711-720.

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Three different bacterial species are regularly described from tsetse flies. However, no broad screens have been performed to investigate the existence of other bacteria in this medically and agriculturally important vector insect. Utilising both culture dependent and independent methods we show that Kenyan populations of *Glossina fuscipes fuscipes* harbour a surprising diversity of bacteria. Bacteria were isolated from 72 percent of flies with 23 different bacterial species identified. The Firmicutes phylum dominated with 16 species of which seven belong to the genus *Bacillus*. The tsetse fly primary symbiont, *Wigglesworthia glossinidia*, was identified by the culture independent pathway. However, neither the secondary symbiont *Sodalis* nor *Wolbachia* was detected with either of the methods used. Two other bacterial species were identified with the DNA based method, *Bacillus subtilis* and *Serratia marcescens*. Further studies are needed to determine how tsetse flies, which only ever feed on vertebrate blood, pick up bacteria and to investigate the possible impact of these bacteria on *Glossina* longevity and vector competence.

15649. **Mwangi, S., Murungi, E., Jonas, M. & Christoffels, A., 2011.** Evolutionary genomics of *Glossina morsitans* immune-related CLIP domain serine proteases and serine protease inhibitors. *Infection, Genetics & Evolution*, **11** (4): 740-745.

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Several species of haematophagous tsetse flies (genus *Glossina*) are vectors for trypanosomes, the parasitic protozoans that cause human African trypanosomiasis (HAT). Although there was a reduced incidence of HAT in the mid-1960s, decreased disease surveillance has led to a resurgence of HAT in sub-Saharan Africa. Despite being efficient vectors for HAT transmission, the prevalence of *G. morsitans* infection by trypanosomes in the wild is surprisingly minimal. The precise mechanisms by which *G. morsitans* remain refractory to trypanosome infection are largely unknown although it has been demonstrated that *G. morsitans* mounts a strong immune response to invading pathogens. This study

identifies *G. morsitans* immune-related CLIP domain serine proteases and their inhibitors, serine protease inhibitors (serpin) genes. It further establishes their evolutionary relationships with counterparts in *Drosophila melanogaster*, *Anopheles gambiae, Bombyx mori, Manduca sexta* and *Culex quinquefasciatus*. Multiple sequence alignments show conservation of most secondary structure elements for both CLIPs and serpins. Amino acid composition of the serpin reactive site loop (RSL) indicates that the *G. morsitans* serpins act through an inhibitory mechanism to the target serine protease. Similar to *D. melanogaster* and unlike *A. gambiae*, the transcriptome data suggest that *G. morsitans* does not contain gene expansions in their CLIP-domain serine protease and serpin families. The presence of alternatively spliced variants in the *G. morsitans* serpins transcriptome data mirrors that of the *D. melanogaster* transcriptome.

15650. **Pellegrini, A., Bigliardi, E., Bechi, N., Paulesu, L., Lehane, M. J. & Avanzati, A. M., 2011.** Fine structure of the female reproductive system in a viviparous insect, *Glossina morsitans morsitans* (Diptera, Glossinidae). *Tissue Cell*, **43** (1): 1-7.

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The female reproductive system of the tsetse fly *Glossina morsitans morsitans* is analysed by scanning electron microscopy (SEM). The study focuses in particular on the choriothete, a peculiar uterine structure involved in the viviparous mode of reproduction of *Glossina morsitans morsitans*. Under light microscopy, the choriothete appears formed by numerous tongue-like folds projecting towards the uterine lumen and lined by a thin cuticle. SEM analysis highlights for the first time a distinctive new feature that is not visible by traditional histological methods. That is a cuticular covering of the choriothete, which shows numerous thorns in the form of crest-like structures arranged in nearly parallel lines. The role of the choriothete in pregnancy and in larval nourishment is discussed.

15651. **Saccone, G., Salvemini, M. & Polito, L. C., 2011.** The transformer gene of *Ceratitis capitata:* a paradigm for a conserved epigenetic master regulator of sex determination in insects. *Genetica*, **139** (1): 99-111.

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The transformer gene in *Ceratitis capitata* (Cctra(ep)) is the founding member of a family of related SR genes that appear to act as the master epigenetic switch in sex determination in insects. A functional protein seems to be produced only in individuals with a female XX karyotype where it is required to maintain the productive mode of expression through a positive feedback loop and to direct female development by instructing the downstream target genes accordingly. When zygotic activation of this loop is prevented, male development follows. Recently, tra(ep) orthologues were isolated in more distantly related dipteran species including *Musca domestica, Glossina morsitans* and *Lucilia cuprina* and in the Hymenopterans *Apis mellifera* and *Nasonia vitripennis*. All of these tra(ep) orthologues seem to act as binary switches that govern all aspects of sexual development. Transient silencing leads to complete masculinization of individuals with a female karyotype.

Reciprocally, in some systems it has been shown that transient expression of the functional TRA product is sufficient to transactivate the endogenous gene and implement female development in individuals with a male karyotype. Hence, a mechanism based on tra(ep) epigenetic autoregulation seems to represent a common and presumably ancestral single principle of sex determination in Insecta. The results of these studies will not only be important for understanding divergent evolution of basic developmental processes but also for designing new strategies to improve genetic sexing in different insect species of economical or medical importance.

15652. **Snyder, A. K., McMillen, C. M., Wallenhorst, P. & Rio, R. V., 2011.** The phylogeny of *Sodalis*-like symbionts as reconstructed using surface-encoding loci. *FEMS Microbiology Letters*, **317** (2): 143-151.

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Phylogenetic analyses of 16S rRNA support close relationships between the Gammaproteobacteria *Sodalis glossinidius*, a tsetse (Diptera: Glossinidae) symbiont, and bacteria infecting diverse insect orders. To further examine the evolutionary relationships of these *Sodalis*-like symbionts, phylogenetic trees were constructed for a subset of putative surface-encoding genes (i.e. ompA, spr, slyB, rcsF, ycfM, and ompC). The ompA and ompC loci were used toward examining the intra- and interspecific diversity of *Sodalis* within tsetse, respectively. Intraspecific analyses of ompA support elevated nonsynonymous (dN) polymorphism with an excess of singletons, indicating diversifying selection, specifically within the tsetse *Glossina morsitans*. Additionally, interspecific ompC comparisons between *Sodalis* and *Escherichia coli* demonstrate deviation from neutrality, with higher fixed dN observed at sites associated with extracellular loops. Surface-encoding genes varied in their phylogenetic resolution of *Sodalis* and related bacteria, suggesting conserved vs. host-specific roles. Moreover, *Sodalis* and its close relatives exhibit genetic divergence at the rcsF, ompA, and ompC loci, indicative of initial molecular divergence. The application of outer membrane genes as markers for further delineating the systematics of recently diverged bacteria is discussed. These results increase our understanding of insect symbiont evolution, while also identifying early genome alterations occurring upon integration of microorganisms with eukaryotic hosts.

15653. **Weiss, B. L., Wang, J. & Aksoy, S., 2011.** Tsetse immune system maturation requires the presence of obligate symbionts in larvae. *PLoS Biology*, **9** (5): e1000619.

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Beneficial microbial symbionts serve important functions within their hosts, including dietary supplementation and maintenance of immune system homeostasis. Little is known about the mechanisms that enable these bacteria to induce specific host phenotypes during development and into adulthood. Here we used the tsetse fly, *Glossina morsitans*, and its obligate mutualist, *Wigglesworthia glossinidia*, to investigate the co-evolutionary adaptations that influence the development of host physiological processes. *Wigglesworthia* is maternally

transmitted to tsetse's intrauterine larvae through milk gland secretions. We can produce flies that lack *Wigglesworthia* (GmmWgm $^-$ yet retain their other symbiotic microbes. Such offspring give rise to adults that exhibit a largely normal phenotype, with the exception being that they are reproductively sterile. Our results indicate that when reared under normal environmental conditions GmmWgm$^-$ adults are also immuno-compromised and highly susceptible to haemocoelic *E. coli* infections while age-matched wild-type individuals are refractory. Adults that lack *Wigglesworthia* during larval development exhibit exceptionally compromised cellular and humoral immune responses following microbial challenge, including reduced expression of genes that encode antimicrobial peptides (cecropin and attacin), haemocyte-mediated processes (thioester-containing proteins 2 and 4 and prophenoloxidase), and signal-mediating molecules (inducible nitric oxide synthase). Furthermore, GmmWgm$^-$ adults harbour a reduced population of sessile and circulating haemocytes, a phenomenon that likely results from a significant decrease in larval expression of serpent and lozenge, both of which are associated with the process of early haemocyte differentiation. Our results demonstrate that *Wigglesworthia* must be present during the development of immature progeny in order for the immune system to function properly in adult tsetse. This phenomenon provides evidence of yet another important physiological adaptation that further anchors the obligate symbiosis between tsetse and *Wigglesworthia*.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

15654. **Courtin, F., Rayaisse, J. B., Tamboura, I., Serdebeogo, O., Koudougou, Z., Solano, P. & Sidibe, I., 2010.** Updating the northern tsetse limit in Burkina Faso (1949-2009): impact of global change. *International Journal of Environmental Research & Public Health*, **7** (4): 1708-1719.

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The northern distribution limit of tsetse flies was updated in Burkina Faso and compared to previous limits to revise the existing map of these vectors of African trypanosomiases dating from several decades ago. From 1949 to 2009, a 25- to 150-km shift has appeared toward the south. Tsetse are now discontinuously distributed in Burkina Faso with a western and an eastern tsetse belt. This range shift can be explained by a combination of decreased rainfall and increased human density. Within a context of international control, this study provides a better understanding of the factors influencing the distribution of tsetse flies.

15655. **Echodu, R., Beadell, J. S., Okedi, L. M., Hyseni, C., Aksoy, S. & Caccone, A., 2011.** Temporal stability of *Glossina fuscipes fuscipes* populations in Uganda. *Parasites & Vectors*, **4** (1): 19.

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Glossina fuscipes, a riverine species of tsetse, is the major vector of human African trypanosomiasis (HAT) in sub-Saharan Africa. Understanding the population dynamics, and specifically the temporal stability, of *G. fuscipes* will be important for informing vector control

activities. We evaluated genetic changes over time in seven populations of the subspecies *G. f. fuscipes* distributed across south eastern Uganda, including a zone of contact between two historically isolated lineages. A total of 667 tsetse flies were genotyped at 16 microsatellite loci and at one mitochondrial locus. Results of an AMOVA indicated that time of sampling did not explain a significant proportion of the variance in allele frequencies observed across all samples. Estimates of differentiation between samples from a single population ranged from approximately 0 to 0.019, using Jost's DEST. Effective population size estimates using momentum-based and likelihood methods were generally large. We observed significant change in mitochondrial haplotype frequencies in just one population, located along the zone of contact. The change in haplotypes was not accompanied by changes in microsatellite frequencies, raising the possibility of asymmetric mating compatibility in this zone. Our results suggest that populations of *G. f. fuscipes* were stable over the 8-12 generations studied. Future studies should aim to reconcile these data with observed seasonal fluctuations in the apparent density of tsetse.

15656. **Hargrove, J. W., Ouifki, R. & Ameh, J. E., 2011.** A general model for mortality in adult tsetse (Glossina spp.). *Medical & Veterinary Entomology*. **Available online 17 March 2011.**

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Tsetse exhibit a U-shaped age-mortality curve, with high losses after eclosion and a well-marked ageing process, which is particularly dramatic in males. A three-parameter (k^1-k^3) model for age-dependent adult instantaneous mortality rates was constructed using mark-recapture data for the tsetse fly *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). Mortality changed linearly with k^1 over all ages; k^2 affected only losses in roughly the first week of adult life, and k^3 controlled the ageing rate. Mortality pooled over age was twice as sensitive to changes in k^3 as in k^1. Population growth rate was, however, similarly affected by these two parameters, reflecting the disproportionate effect of k^3 on mortality in the oldest flies that contribute least to the growth rate. Pooled-age mortality and growth rate were insensitive to changes in k^2. The same model also provided good fits to data for laboratory colonies of female *G. m. morsitans* and *Glossina austeni* Newstead and should be applicable to all tsetse of both sexes. The new model for tsetse mortality should be incorporated into models of tsetse and trypanosome population dynamics; it will also inform the estimation of adult female mortality from ovarian dissection data.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also **34:** 15641]

15657. **Barclay, H. J. & Vreysen, M. J. B.,** 2011. A dynamic population model for tsetse (Diptera: Glossinidae) area-wide integrated pest management. *Population Ecology*, **53**(1): 89-110.

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A spatial model of tsetse (*Glossina palpalis* ssp. and *G. pallidipes*) life cycle was created in FORTRAN, and four control measures [aerial spraying of non-residual insecticides, traps and targets, insecticide-treated livestock (ITL) and the sterile insect technique] were programmed into the model to assess how much of each of various combinations of these control tactics would be necessary to eradicate the population. The model included density-independent and -dependent mortality rates, temperature-dependent mortality, an age-dependent mortality, two mechanisms of dispersal and a component of aggregation. Sensitivity analyses assessed the importance of various life history features and indicated that female fertility and factors affecting survivorship had the greatest impact on the equilibrium of the female population. The female equilibrium was likewise reduced when dispersal and aggregation were acting together. Sensitivity analyses showed that basic female survivorship, age-dependent and temperature-dependent survivorship of adults, teneral-specific survivorship, daily female fertility, and mean temperature had the greatest effect on the four applied control measures. Time to eradication was reduced by initial knockdown of the population and due to the synergism of certain combinations of methods (e.g., traps-targets and sterile insect technique [SIT; ITL and SIT]). Competitive ability of the sterile males was an important parameter when sterile to wild male overflooding ratios were small. An aggregated wild population reduced the efficiency of the SIT, but increased it with increased dispersal. The model can be used interactively to facilitate decision making during the planning and implementation of operational area-wide integrated pest management programmes against tsetse.

15658. **Childs, S. J., 2011.** Theoretical levels of control as a function of mean temperature and spray efficacy in the aerial spraying of tsetse fly. *Acta Tropica*, **117** (3): 171-182.

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The hypothetical impact of aerial spraying on tsetse fly populations is investigated. Spray cycles are scheduled at intervals two days short of the first interlarval period and halted once the last of the female flies that originated from pre-spray-deposited pupae have been sprayed twice. The effect of temperature on the aerial spraying of tsetse, through its reproductive cycle and general population dynamics, is of particular interest, given that cooler weather is preferred for the settling of insecticidal droplets. Spray efficacy is found to come at a price due to the greater number of cycles necessitated by cooler weather. The extra cost is argued to be worthwhile. Pupae, still in the ground at the end of spraying, are identified as the main threat to a successful operation. They are slightly more vulnerable at the low temperature extreme of tsetse habitat (16 ^{0}C), when the cumulative, natural pupal mortality is high. One can otherwise base one's expectations on the closeness with which the time to the third last spray approaches one puparial duration. A disparity of anything close to the length of a spray cycle advocates caution, whereas one which comes close to vanishing should be interpreted as being auspicious. Three such key temperatures, just below which one can anticipate an improved outcome and just above which caution should be exercised, are 17.146 ^{0}C, 19.278 ^{0}C and 23.645 ^{0}C. A refinement of the existing formulae for the puparial duration and the first interlarval period might be prudent in the South African context of a sympatric *Glossina*

brevipalpis-Glossina austeni, tsetse population. The resulting aerial spraying strategy would then be formulated using a *G. brevipalpis* puparial duration and a *G. austeni* first interlarval period.

15659. **Hargrove, J., Torr, S. & Vale, G.,** 2011. Comment on Barclay and Vreysen: published dynamic population model for tsetse cannot fit field data. *Journal of Population Ecology*, **53**(2): 413-415.

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The structure, and assumed parameter values, of a recent dynamic population model for tsetse (Diptera: Glossinidae) render it unable to fit published data on tsetse control programmes using odour-baited targets, insecticide-treated cattle and the sterile insect technique (SIT). The underlying problem is a mismatch between the small size of the mapped cells (1 ha) and the long time-step, which allows flies to move only once every five days, and then only to an adjacent cell. Assumed rates of tsetse dispersal and killing by odour-baited targets are consequently at least an order of magnitude lower than observed in the field. Suggestions that *Glossina pallidipes* could be eradicated more rapidly with SIT, than using hundreds of targets per km^2, are contradicted both by the field data and by three other independent modelling studies.

15660. **Kagbadouno, M. S., Camara, M., Bouyer, J., Courtin, F., Onikoyamou, M. F., Schofield, C. J. & Solano, P., 2011.** Progress towards the eradication of tsetse from the Loos islands, Guinea. *Parasites & Vectors*, **4** (1): 18.

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The tsetse fly *Glossina palpalis gambiensis* is the main vector of sleeping sickness (human African trypanosomiasis - HAT) in West Africa, in particular in littoral Guinea where this disease is currently very active. The Loos islands constitute a small archipelago some 5 km from mainland Guinea, where *G. p. gambiensis* is well known as a nuisance and potential disease vector by inhabitants of the three main islands, Fotoba, Roume, and Kassa. The National Control Program against HAT of Guinea has decided to eradicate tsetse in Loos islands in order to sustainably protect humans and economic activities. After baseline data collection, tsetse control began on the islands in 2006. On each of the three islands a specific combination of control methods was implemented according to the entomological situation found. Starting densities before control operations were 10, 3 and 1 tsetse/trap/day in Kassa, Room and Fotoba respectively, but by July 2010, tsetse were no longer caught in any of the sentinel traps used for monitoring. The reduction rate was faster where several control methods were implemented as a combination (impregnated traps and targets ITT, selective ground spraying, epicutaneous insecticide treatment of pigs, and impregnated fences around pig pens), whereas it was slower when ITT were used as the only control method. This 100 percent suppression is a promising step in the eradication process, but *G. p. gambiensis* may still occur at very low, undetectable, densities on the archipelago. Next step will consist in assessing a 0.05 probability of tsetse absence to ascertain a provisional eradication status. Throughout these operations, a key factor has been the involvement of local teams and local communities

without whom such results would be impossible to obtain. Work will continue thanks to the partners involved until total eradication of the tsetse on Loos islands can be declared.

15661. **Sciarretta, A., Tikubet, G., Baumgartner, J., Girma, M. & Trematerra, P., 2010.** Spatial clustering and associations of two savannah tsetse species, *Glossina morsitans submorsitans* and *Glossina pallidipes* (Diptera: Glossinidae), for guiding interventions in an adaptive cattle health management framework. *Bulletin of Entomological Research*, **100** (6): 661-670.

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The paper deals with tsetse (family Glossinidae) control and aims at improving the methodology for precision targeting interventions in an adaptive pest management system. The spatio-temporal distribution of *Glossina morsitans submorsitans* Newstead, and *Glossina pallidipes* Austen, at Ethiopia's Keto pilot site, is analysed with the spatial analysis by distance indices (SADIE) methodology that focus on clustering and spatial associations between species and between sexes. Both species displayed an aggregated distribution characterised by two main patches in the south and an extended gap in the north. Spatial patterns were positively correlated and stable in most cases, with the exception of the early dry season and the short rainy season when there were differences between the species and sexes. For precision targeting interventions, the presented methods here are more effective than the previously used geostatistical analyses for identifying and delimiting hot spots on maps, measuring shapes and sizes of patches, and discarding areas with low tsetse density. Because of the improved knowledge on hot spot occurrences, the methods allow a better delimitation of the territory for control operations and a more precise computation of the number of the relatively expensive traps used for monitoring and control purposes.

15662. **Torr, S. J., Chamisa, A., Vale, G. A., Lehane, M. J. & Lindh, J. M., 2011.** Responses of tsetse flies, *Glossina morsitans morsitans* and *Glossina pallidipes*, to baits of various size. *Medical &Veterinary Entomology*. Published online 17 March 2011.

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Recent studies of *palpalis* group tsetse [*Glossina fuscipes fuscipes* (Diptera: Glossinidae) in Kenya] suggest that small (0.25 x 0.25 m) insecticide-treated targets will be more cost-effective than the larger (>/=1.0 x 1.0 m) designs currently used to control tsetse. Studies were undertaken in Zimbabwe to assess whether small targets are also more cost-effective for the *morsitans* group tsetse, *Glossina morsitans morsitans* and *Glossina pallidipes*. Numbers of tsetse contacting targets of 0.25 x 0.25 m or 1.0 x 1.0 m, respectively, were estimated using arrangements of electrocuting grids which killed or stunned tsetse as they

contacted the target. Catches of *G. pallidipes* and *G. m. morsitans* at small (0.25 x 0.25 m) targets were, respectively, approximately 1 percent and approximately 6 percent of catches at large (1.0 x 1.0 m) targets. Hence, the tsetse killed per unit area of target was greater for the larger than the smaller target, suggesting that small targets are not cost-effective for use against *morsitans* group species. The results suggest that there is a fundamental difference in the host-orientated behaviour of *morsitans* and *palpalis* group tsetse and that the former are more responsive to host odours, whereas the latter seem highly responsive to visual stimuli.

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

[See also **34**: 15675, 15690, 15692, 15693]

15663. **Adam, Y., Marcotty, T., Cecchi, G., Mahama, C. I., Solano, P., Bengaly, Z. & Van den Bossche, P., 2011.** Bovine trypanosomosis in the Upper West Region of Ghana: Entomological, parasitological and serological cross-sectional surveys. *Research in Veterinary Science*. **In press, corrected proof.**

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Baseline surveys were conducted in the Upper West Region of Ghana to assess the distribution and densities of tsetse species, as well as the prevalence of bovine trypanosomosis. The entomological survey was designed to cover the suitable tsetse habitats along the three main rivers in the study area (i.e. Black Volta, Kulpawn and Sissili). Results indicated the presence of *Glossina tachinoides* in all three river basins, whilst *Glossina palpalis gambiensis* was only found close to the southern limit of the study area. A random sampling of 1 800 cattle of the West African Short Horn, Sanga and Zebu breeds from 36 randomly selected grid cells covering the study area showed substantial differences between parasitological and serological prevalences. The average parasitological prevalence was estimated at 2.5 percent (95 percent CI: 1.06-5.77) with the majority of the infections due to *Trypanosoma vivax*. Most of the infected cattle were found close to the major river systems. The serological prevalence, measured using the enzyme linked immunosorbent assay (ELISA) test was 19 percent (95 percent CI: 14.03-25.35). Cattle with anti-trypanosomal antibodies were also found throughout the study area.

15664. **Bucheton, B., Macleod, A. & Jamonneau, V., 2011.** Human host determinants influencing the outcome of *T. b. gambiense* infections. *Parasite Immunology,* **33** (8): 438-447.

Institut de Recherche pour le Développement (IRD), Unité Mixte de Recherche IRD-CIRAD 177, TA A-17/G, Campus International de Baillarguet, F-34398 Montpellier, France; Centre International de Recherche-Développement sur l'Elevage en zones Subhumides (CIRDES), Unité de recherches sur les bases biologiques de la lutte intégrée, 01 BP 454 Bobo-Dioulasso 01, Burkina Faso; Wellcome Trust Centre for Molecular Parasitology, College of Medical, Veterinary and Life Sciences, Sir Henry Wellcome Building for Comparative

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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.

15665. **Davis, S., Aksoy, S. & Galvani, A., 2011.** A global sensitivity analysis for African sleeping sickness. *Parasitology*, **138** (4): 516-526.

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African sleeping sickness is a parasitic disease transmitted through the bites of tsetse flies of the genus *Glossina*. We constructed mechanistic models for the basic reproduction number, R0, of *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, respectively the causative agents of West and East African human sleeping sickness. We present global sensitivity analyses of these models that rank the importance of the biological parameters that may explain variation in R0, using parameter ranges based on literature, field data and expertize out of Uganda. For West African sleeping sickness, our results indicate that the proportion of blood meals taken from humans by *Glossina fuscipes fuscipes* is the most important factor, suggesting that differences in the exposure of humans to tsetse are fundamental to the distribution of *T. b. gambiense*. The second ranked parameter for *T. b. gambiense* and the highest ranked for *T. b. rhodesiense* was the proportion of *Glossina* refractory to infection. This finding underlines the possible implications of recent work showing that nutritionally stressed tsetse are more susceptible to trypanosome infection, and provides broad support for control strategies in development that are aimed at increasing refractoriness in tsetse flies. We note though that for *T. b. rhodesiense* the population parameters for tsetse - species composition, survival and abundance - were ranked almost as highly as the proportion refractory, and that the model assumed regular treatment of livestock with trypanocides as an established practice in the areas of Uganda experiencing East African sleeping sickness.

15666. **Enyaru, J. C., Ouma, J. O., Malele, II, Matovu, E. & Masiga, D. K., 2010.** Landmarks in the evolution of technologies for identifying trypanosomes in tsetse flies. *Trends in Parasitology*, **26** (8): 388-394.

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Understanding what the trypanosome pathogens are, their vectors and mode of transmission

underpin efforts to control the disease they cause in both humans and livestock. The risk of transmission is estimated by determining what proportion of the vector population is carrying the infectious pathogens. This risk also depends on the infectivity of the trypanosomes to humans and livestock. Most livestock pathogens are not infective to humans, whereas the two sub-species that infect humans also infect livestock. As with other infectious diseases, we can therefore trace the foundation of many continuing disease control programmes for trypanosomiasis to the discovery of the pathogens and their vectors more than a century ago. Over this period, methods for detecting and identifying trypanosomes have evolved through various landmark discoveries. This review describes the evolution of methods for identifying African trypanosomes in their tsetse fly vectors.

15667. **Farikou, O., Njiokou, F., Simo, G., Asonganyi, T., Cuny, G. & Geiger, A., 2010.** Tsetse fly blood meal modification and trypanosome identification in two sleeping sickness foci in the forest of southern Cameroon. *Acta Tropica*, **116** (1): 81-88.

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The blood meal origins of 222 tsetse flies (213 *Glossina palpalis palpalis*, 7 *Glossina pallicera pallicera*, one *Glossina nigrofusca* and one *Glossina caliginea*) caught in 2008 in two human African trypanosomiasis foci (Bipindi and Campo) of south Cameroon were investigated. 88.7 percent of tsetse flies blood meals were identified using the heteroduplex method and the origin of the remaining blood meals (11.3 percent) was identified by sequencing the cytochrome B gene. Most of the meals were from humans (45.9 percent) and pigs (37.4 percent), 16.7 percent from wild animals. Interestingly, new tsetse fly hosts including turtle (*Trionyx* and *Kinixys*) and snake (*Python sebae*) were identified. Significant differences were recorded between Bipindi where the blood meals from pigs were predominant (66.7 percent vs 23.5 percent from humans) and Campo where blood meals from humans were predominant (62.9 percent vs 22.7 percent from pigs). Comparison with the data recorded in 2004 in the same foci (and with the same molecular approach) demonstrated significant modifications of the feeding patterns: increase in blood meals from pigs in Bipindi (66.7 percent in 2008 vs 44.8 percent in 2004) and in Campo (20.5 percent in 2008 vs 6.8 percent in 2004), decrease in that from human (significant in Bipindi only). 12.6 percent, 8.1 percent and 2.7 percent of the flies were, respectively, *Trypanosoma congolense* forest type, *Trypanosoma congolense* savannah type and *Trypanosoma brucei gambiense* infected. These results demonstrate that tsetse fly feeding patterns can be specific of a given area and can evolve rapidly with time. They show an active circulation of a variety of trypanosomes in sleeping sickness foci of southern Cameroon.

15668. **Geiger, A., Fardeau, M. L., Njiokou, F., Joseph, M., Asonganyi, T., Ollivier, B. & Cuny, G., 2011.** Bacterial diversity associated with populations of *Glossina* spp. from Cameroon and distribution within the Campo sleeping sickness focus. *Microbial Ecology*. **E Publication ahead of print, March 9.**

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Tsetse flies were sampled in three villages of the Campo sleeping sickness focus in South Cameroon. The aim of this study was to investigate the flies' gut bacterial composition using culture-dependent techniques. Out of the 32 flies analysed (27 *Glossina palpalis palpalis,* two

Glossina pallicera, one *Glossina nigrofusca*, and two *Glossina caliginea*), 17 were shown to be inhabited by diverse bacteria belonging to the Proteobacteria, the Firmicutes, or the Bacteroidetes phyla. Phylogenetic analysis based on 16S rRNA gene sequences indicated the presence of 16 bacteria belonging to the genera *Acinetobacter (*4), *Enterobacter* (4), *Enterococcus* (2), *Providencia* (1), *Sphingobacterium* (1), *Chryseobacterium* (1), *Lactococcus* (1), *Staphylococcus* (1), and *Pseudomonas* (1). Using identical bacterial isolation and identification processes, the diversity of the inhabiting bacteria analysed in tsetse flies sampled in Cameroon was much higher than the diversity found previously in flies collected in Angola. Furthermore, bacterial infection rates differed greatly between the flies from the three sampling areas (Akak, Campo Beach/Ipono, and Mabiogo). Last, the geographic distribution of the different bacteria was highly uneven; two of them identified as *Sphingobacterium* spp. and *Chryseobacterium* spp. were only found in Mabiogo. Among the bacteria identified, several are known for their capability to affect the survival of their insect hosts and/or insect vector competence. In some cases, bacteria belonging to a given genus were shown to cluster separately in phylogenetic trees; they could be novel species within their corresponding genus. Therefore, such investigations deserve to be pursued in expanded sampling areas within and outside Cameroon to provide greater insight into the diverse bacteria able to infect tsetse flies given the severe human and animal sickness they transmit.

15669. **Grady, S. C., Messina, J. P. & McCord, P. F., 2011.** Population vulnerability and disability in Kenya's tsetse fly habitats. *PLoS Neglected Tropical Diseases*, **5** (2): e957.

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Human African trypanosomiasis (HAT), also referred to as sleeping sickness, and African animal trypanosomaisis (AAT), known as nagana, are highly prevalent parasitic vector-borne diseases in sub-Saharan Africa. Humans acquire trypanosomiasis following the bite of a tsetse fly infected with the protozoa *Trypanosoma brucei* (T. b.) spp. -i.e., *T. b. gambiense* in West and Central Africa and *T. b. rhodesiense* in East and Southern Africa. Over the last decade HAT diagnostic capacity to estimate HAT prevalence has improved in active case-finding areas but enhanced passive surveillance programmes are still lacking in much of rural sub-Saharan Africa. This retrospective-cross-sectional study examined the use of national census data (1999) to estimate population vulnerability and disability in Kenya's 7 tsetse belts to assess the potential of HAT-acquired infection in those areas. A multilevel study design estimated the likelihood of disability in individuals, nested within households, nested within tsetse fly habitats of varying levels of poverty. Residents and recent migrants of working age were studied. The impact of tsetse flies on disability was conceptualised via two exposure pathways: directly from the bite of a pathogenic tsetse fly resulting in HAT infection or indirectly, as the potential for AAT takes land out of agricultural production and diseased livestock leads to livestock morbidity and mortality, contributing to nutritional deficiencies and poverty. Tsetse belts that were significantly associated with increased disability prevalence were identified and the direct and indirect exposure pathways were evaluated. Incorporating reports on disability from the national census is a promising surveillance tool that may enhance future HAT surveillance programmes in sub-Saharan Africa. The combined burdens of HAT and AAT and the opportunity costs of agricultural production in AAT areas are likely contributors to disability within tsetse-infested areas. Future research will assess changes in the spatial relationships between high tsetse infestation and human disability following the release

of the Kenya 2009 census at the local level.

15670. **Kohagne, T. L., M'Eyi M, P., Mimpfoundi, R. & Louis, J. F., 2010.** Entomological patterns in the human African trypanosomiasis focus of Komo Mondah, Gabon. *African Health Science*, **10** (4): 341-348.

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The incidence of sleeping sickness is still considerable in the Komo Mondah focus, in spite of case-detection strategy. A combined strategy of mass screening and vector control is effective for the control of the disease. In the perspective of a targeted vector control in main transmission sites, we have carried out an entomological survey in the epicentre of the focus. This study set out to determine tsetse fly distribution, human-fly contact point and eventually risk factors for acquisition of the disease. "Vavoua" traps were set for *Glossina* in four biotopes selected after an interview of HAT patients concerning their working places. Tsetse were captured and dissected. DNA from organs was analysed by PCR for trypanosome infections. The origin of blood meals was determined by ELISA. Results obtained showed that the focus is infested by three species of *Glossina: G. palpalis palpalis* (1 149: 91.85 percent) found in all biotopes; *G. fuscipes fuscipes* (85: 6.79 percent) and *G. caliginea* (17: 1.36 percent) found in water spots and landing stages. They are infected by three subgenera of trypanosomes and only *G. palpalis palpalis* is infected by human trypanosomes. *G. fuscipes fuscipes* is infected by *T. brucei* sl and *G. caliginea* is not infected. Flies are absent at the periphery of houses except in one village. Only 29.20 percent of blood meals were from humans. Landing stages built in swamp mangrove are presenting the higher index of epidemiological risk and populations are exposed to the disease when they go to the area for taking their fishing boats. It is concluded that swamp mangrove should be targeted as a priority during a vector control campaign.

15671. **Kone, N., N'Goran E, K., Sidibe, I., Kombassere, A. W. & Bouyer, J., 2011.** Spatio-temporal distribution of tsetse and other biting flies in the Mouhoun River basin, Burkina Faso. *Medical &Veterinary Entomology*, **25** (2): 156-168.

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In the Mouhoun River basin, Burkina Faso, the main vectors of African animal trypanosomoses are *Glossina palpalis gambiensis* Vanderplank and *Glossina tachinoides* Westwood (Diptera: Glossinidae), both of which are riverine tsetse species. The aim of our

study was to understand the impact of landscape anthropogenic changes on the seasonal dynamics of vectors and associated trypanosomosis risk. Three sites were selected on the basis of the level of disturbance of tsetse habitats and predominant tsetse species: disturbed (Boromo, for *G. tachinoides*) and half-disturbed (Douroula for *G. tachinoides* and Kadomba for *G. p. gambiensis*). At each of these sites, seasonal variations in the apparent densities of tsetse and mechanical vectors and tsetse infection rates were monitored over 17 months. Tsetse densities differed significantly between sites and seasons. Of 5 613 captured tsetse, 1 897 were dissected; 34 of these were found to be infected with trypanosomes. The most frequent infection was *Trypanosoma vivax* (1.4 percent), followed by *Trypanosoma congolense* (0.3 percent) and *Trypanosoma brucei* (0.05 percent). The mean physiological age of 703 tsetse females was investigated to better characterize the transmission risk. Despite the environmental changes, it appeared that tsetse lived long enough to transmit trypanosomes, especially in half-disturbed landscapes. A total of 3 021 other biting flies from 15 species (mainly Tabanidae and Stomoxyinae) were also caught: their densities also differed significantly among sites and seasons. Their relative importance regarding trypanosome transmission is discussed; the trypanosomosis risk in cattle was similar at all sites despite very low tsetse densities (but high mechanical vector densities) in one of them.

15672. **Muturi, C. N., Ouma, J. O., Malele, II, Ngure, R. M., Rutto, J. J., Mithofer, K. M., Enyaru, J. & Masiga, D. K., 2011.** Tracking the feeding patterns of tsetse flies (*Glossina* genus) by analysis of blood meals using mitochondrial cytochromes genes. *PloS One*, **6** (2): e17284.

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Tsetse flies are notoriously difficult to observe in nature, particularly when populations densities are low. It is therefore difficult to observe them on their hosts in nature; hence their vertebrate species can very often only be determined indirectly by analysis of their gut contents. This knowledge is a critical component of the information on which control tactics can be developed. The objective of this study was to determine the sources of tsetse blood meals, hence investigate their feeding preferences. We used mitochondrial cytochrome c oxidase 1 (COI) and cytochrome b (cytb) gene sequences for identification of tsetse fly blood meals, in order to provide a foundation for rational decisions to guide control of trypanosomiasis, and their vectors. *Glossina swynnertoni* were sampled from Serengeti (Tanzania) and *G. pallidipes* from Kenya (Nguruman and Busia), and Uganda. Sequences were used to query public databases, and the percentage identities obtained used to identify hosts. An initial assay showed that the feeds were from single sources. Hosts identified from blood fed flies collected in Serengeti ecosystem, included buffaloes (25/40), giraffes (8/40), warthogs (3/40), elephants (3/40) and one spotted hyena. In Nguruman, where *G. pallidipes* flies were analyzed, the feeds were from elephants (6/13) and warthogs (5/13), while buffaloes and baboons accounted for one blood meal each. Only cattle blood was detected in flies caught in Busia and Uganda. Out of four flies tested in Mbita Point, Suba District in western Kenya, one had fed on cattle, the other three on the Nile monitor lizard. These results demonstrate that cattle will form an integral part of a control strategy for trypanosomiasis in Busia and Uganda, while different approaches are required for Serengeti and Nguruman ecosystems, where wildlife abound and are the major component of the tsetse fly food source.

15673. **Salim, B., Bakheit, M. A., Kamau, J., Nakamura, I. & Sugimoto, C., 2011.** Molecular epidemiology of camel trypanosomiasis based on ITS1 rDNA and RoTat 1.2 VSG gene in the Sudan. *Parasites & Vectors*, **4**: 31.

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Internal transcribed spacer one (ITS1) of the ribosomal DNA is known to be a suitable target for PCR-based detection of trypanosomes. The analysis of this region provides a multi-species-specific diagnosis by a single PCR. Using ITS1 primer-based PCR, a cross sectional study was carried out in the period from September to November 2009 on samples collected from 687 camels from geographically distinct zones in the Sudan to detect all possible African trypanosomes, which can infect camels. The results showed that all PCR-positive camels were infected with a single parasite species: *Trypanosoma evansi.* The highest prevalence, 57.1 percent (117/205), was observed in the Butana plains of mid-eastern Sudan and the lowest, 6.0 percent (4/67), was in the Umshadeeda eastern part of White Nile State. In another experiment, the RoTat 1.2 gene encoding the variable surface glycoprotein (VSG) of *T. evansi* was analysed for its presence or absence by a polymerase chain reaction (PCR) using *T. evansi* species-specific primers. The study showed that the RoTat 1.2 VSG gene was absent in thirteen out of thirty *T. evansi*-positive samples. It is concluded that camel trypanosomiasis in Sudan is apparently caused by a single parasite species *T. evansi* and there were no other typanosomes species detected. In addition, the disease is highly prevalent in the country, which strengthens the need to change control policies and institute measures that help prevent the spread of the parasite. To our knowledge, this is the first molecular diagnosis report, which gives a picture of camel trypanosomiasis covering large geographical areas in Sudan.

15674. **Simo, G., Njitchouang, G. R., Njiokou, F., Cuny, G. & Asonganyi, T., 2011.** *Trypanosoma brucei* s.l.: Microsatellite markers revealed high level of multiple genotypes in the mid-guts of wild tsetse flies of the Fontem sleeping sickness focus of Cameroon. *Experimental Parasitology*, **128** (3): 272-278.

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To identify *Trypanosoma brucei* genotypes which are potentially transmitted in a sleeping sickness focus, microsatellite markers were used to characterize *T. brucei* found in the mid-guts of wild tsetse flies of the Fontem sleeping sickness focus in Cameroon. For this study, two entomological surveys were performed during which 2 685 tsetse flies were collected and 1 596 (59.2 percent) were dissected. Microscopic examination revealed 1.19 percent (19/1 596) mid-gut infections with trypanosomes; the PCR method identified 4.7 percent (75/1 596) infections with *T. brucei* in the mid-guts. Of these 75 trypanosomes identified in the mid-guts, *Trypanosoma brucei gambiense* represented 0.81 percent (13/1 596) of them, confirming the circulation of human infective parasite in the Fontem focus. Genetic characterization of the 75 *T. brucei* samples using five microsatellite markers revealed not only multiple *T. brucei* genotypes (47 percent), but also single genotypes (53 percent) in the mid-guts of the wild tsetse flies. These results show that there is a wide range of trypanosome genotypes circulating in the

mid-guts of wild tsetse flies from the Fontem sleeping sickness focus. They open new avenues to undertake investigations on the maturation of multiple infections observed in the tsetse fly mid-guts. Such investigations may allow to understand how the multiple infections evolve from the tsetse flies mid-guts to the salivary glands and also to understand the consequence of these evolutions on the dynamic (which genotype is transmitted to mammals) of trypanosomes transmission.

15675. **Van den Bossche, P. & Delespaux, V., 2011.** Options for the control of tsetse-transmitted livestock trypanosomosis. An epidemiological perspective. *Veterinary Parasitology*. **In press, corrected proof.**

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Tsetse-transmitted livestock trypanosomosis affects livestock in large parts of sub-Saharan Africa. In southern Africa two epidemiological situations can be distinguished. The disease can have an endemic nature with high morbidity and low mortality in the livestock population. Endemic livestock trypanosomosis is found mainly in areas where cattle constitute the main host of tsetse and reservoirs of trypanosomes. Epidemic trypanosomosis, with high morbidity and high mortality is found in areas where wildlife persist as main reservoir and where livestock come into contact with tsetse flies transmitting trypanosomes from the sylvatic reservoir. Based on the differences in impact of the disease on livestock health in these two epidemiological settings, the appropriateness of the available trypanosomosis control tools differs. In trypanosomosis endemic areas, trypanocidal drug use could be the most suitable approach. Possible problems associated with the development of resistance in trypanosomes to the drugs need to be investigated further. In epidemic situations, vector control seems the most appropriate long-term solution.

5. HUMAN TRYPANOSOMOSIS

(a) SURVEILLANCE

[See also **34:** 15633, 15636, 15638]

15676. **Berrang-Ford, L., Berke, O., Sweeney, S. & Abdelrahman, L., 2010.** Sleeping sickness in southeastern Uganda: a spatio-temporal analysis of disease risk, 1970-2003. *Vector Borne & Zoonotic Diseases*, **10** (10): 977-988.

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Sleeping sickness is a major threat to human health in sub-Saharan Africa. South eastern Uganda has experienced a number of significant epidemics in the past 100 years, most recently from 1976 to 1989. Recent and continued spread of the disease has highlighted gaps in the ability of current research to explain and predict the distribution of infection. Vegetation cover and changes in vegetation may be important determinants of transmission and disease risk because of the habitat preferences of the tsetse fly vector. This study examines the

determinants of sleeping sickness distribution and incidence in south eastern Uganda from 1970 to 2003, spanning the full epidemic region and cycle, and focusing in particular on vegetation cover and change. Sleeping sickness data were collected from records of the Ugandan Ministry of Health, individual sleeping sickness treatment centres, and interviews with public health officials. Vegetation data were acquired from satellite imagery for four dates spanning the epidemic period, 1973, 1986, 1995, and 2001. Zero-inflated regression models were used to model predictors of disease presence and magnitude. Correlations between disease incidence and the normalized difference vegetation index (NDVI) at the subcounty level were evaluated. Results indicate that sleeping sickness infection is predominantly associated with proximity to water and spatial location, while disease incidence is highest in subcounties with moderate to high NDVI. The vegetation density (NDVI) at which sleeping sickness incidence peaked differed throughout the study period. The optimal vegetation density capable of supporting sleeping sickness transmission may be lower than indicated by data from endemic regions, indicating increased potential for disease spread under suitable conditions.

15677. **Berrang-Ford, L., Lundine, J. & Breau, S., 2011.** Conflict and human African trypanosomiasis. *Social Science & Medicine*, **72** (3): 398-407.

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Human African Trypanosomiasis (HAT) has re-emerged in sub-Saharan Africa as a disease of major public health importance. The success of HAT elimination in sub-Saharan Africa is subject to the feasibility of controlling, eliminating, or mitigating the determinants of incidence in affected countries. Conflict has been widely recognized and cited as a contributing factor to the resurgence of HAT in many countries, as well as to continuing HAT incidence in politically unstable and resource-poor regions. Despite extensive anecdotal and qualitative recognition of the role of conflict, there has been no quantitative research of this topic at the population level in affected African countries. We characterize the qualitative and quantitative associations between HAT incidence and conflict-related processes in HAT-affected African countries over the past 30 years. HAT and conflict-related data were collected for 35 affected countries in sub-Saharan Africa for the years 1976-2004. Descriptive and univariate inferential statistics, as well as negative binomial regression modelling, are used to assess the associations between HAT and conflict. A space-time scan statistic is used to identify significant incidence clusters. Clusters of HAT incidence over the past 30 years have predominantly coincided with periods of conflict or socio-political instability. HAT cases occurred significantly more often in countries and during years with conflict, high political terror, and internationalized civil war. The results indicate a lag period between the start of conflict events and a peak in incidence of approximately 10 years. We recommend explicit consideration and quantification of socio-political measures such as conflict and terror indices in GIS (geographic information systems)-based risk assessments for HAT policy and intervention.

15678. **Courtin, F., Jamonneau, V., Kambire, R. & Solano, P., 2010.** Ivory Coast uprising and returning Burkinabe immigrants: evaluation of the risk for re-emergence of sleeping sickness in Burkina Faso. *Médecine Tropical (Mars)*, **70** (5-6): 490-496.

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Following the socio-political unrest that occurred in Ivory Coast in 2002, 360 000 Burkinabe immigrants returned to Burkina Faso that was the epicentre of sleeping sickness last century and is now thought to be free of autochthonous transmission. The purpose of this study was to determine if the massive return of immigrants from human African trypanosomiasis (HAT) endemic areas of Ivory Coast to areas in Burkina Faso where the vector (tsetse fly) is currently present could lead to re-emergence of the disease. Risk areas for re-emergence were identified taking into account the number of returning immigrants, history of the disease, and presence of tsetse flies. Based on these criteria, study was focused on two villages, i.e. Folonzo and Gbalara, located in southern Burkina Faso near the Ivory Coast border. Study in these two villages consisted of characterization of the population (repatriates or not, origin,..) and medical surveys to assess the presence/absence of the disease. Departure of some returning immigrants from areas including sleeping sickness foci in Ivory Coast (e.g. centre west) confirmed the potential risk of re-emergence of the disease. Although no case of sleeping sickness was diagnosed, several serologically positive people were identified and will be followed up. This study failed to demonstrate a clear-cut correlation between massive population movements due to war and re-emergence of sleeping sickness. However, this study may have been timed too soon after the return of immigrants to detect re-emergence of HAT that could require several years.

15679. **Hasker, E., Lumbala, C., Mbo, F., Mpanya, A., Kande, V., Lutumba, P. & Boelaert, M., 2011.** Health care-seeking behaviour and diagnostic delays for human African trypanosomiasis in the Democratic Republic of the Congo. *Tropical Medicine & International Health,* **16**(7): 869-874.

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About half of the patients with human African trypanosomiasis (HAT) reported in the Democratic Republic of the Congo (DRC) are currently detected by fixed health facilities and not by mobile teams. Given the recent policy to integrate HAT control into general health services, we studied health-seeking behaviour in these spontaneously presenting patients. We took a random sample from all patients diagnosed with a first-time HAT episode through passive case finding between 1 October 2008 and 30 September 2009 in the two most endemic provinces of the DRC. Patients were approached at their homes for a structured interview. We documented patient delay (i.e. time between onset of symptoms and contacting a health centre) and health system delay (i.e. time between first contact and correct diagnosis of HAT). Median patient delay was four months (IQR 1-10 months, n = 66); median health system delay was three months (IQR 0.5-11 months). Those first presenting to public health centres had a median systems delay of seven months (IQR 2-14 months, n = 23). On median, patients were diagnosed upon the forth visit to a health facility (IQR 3rd-7th visit). It is concluded that substantial patient as well as health system delays are incurred in HAT cases detected passively. Public health centres are performing poorly in the diagnostic work-up for HAT,

mainly because HAT is a relatively rare disease with few and non-specific early symptoms. Integration of HAT diagnosis and treatment into general health services requires strong technical support and well-organized supervision and referral mechanisms.

15680. **Ilboudo, H., Jamonneau, V., Camara, M., Camara, O., Dama, E., Leno, M., Ouendeno, F., Courtin, F., Sakande, H., Sanon, R., Kabore, J., Coulibaly, B., N'Dri, L., Diarra, A., N'Goran, E. & Bucheton, B., 2011.** Diversity of response to *Trypanosoma brucei gambiense* infections in the Forecariah mangrove focus (Guinea): perspectives for a better control of sleeping sickness. *Microbes & Infection*. **E publication ahead of print May 27.**

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At a time when human African trypanosomiasis (HAT) elimination again seems a reachable goal in many parts of sub-Saharan Africa, it is becoming increasingly important to characterise the factors involved in disease resurgence or maintenance to develop sustainable control strategies. In this study conducted in the Forecariah mangrove focus in Guinea, HAT patients and serological suspects (SERO) were identified through mass screening of the population with the card agglutination test for trypanosomiasis (CATT) and were followed up for up to two years. Analysis of the samples collected during the follow-up of HAT patients and SERO was performed with PCR (TBR1/TBR2) and the trypanolysis serological test (TL) in order to clarify the role played by these individuals in the epidemiology of HAT. PCR positivity was higher in TL+ than in SERO TL- (50 percent vs. 18 percent, respectively). Whereas CATT plasma titres decreased both in treated HAT patients and SERO TL-, SERO TL+ maintained high CATT titres. Four out of 17 SERO TL+ developed HAT during the study. These results strongly suggest that SERO TL+ individuals are asymptomatic carriers. In the context where disease prevalence is sufficiently low, treating SERO TL+ individual may thus be of crucial importance in order to cut transmission.

15681. **Kabore, J., Koffi, M., Bucheton, B., Macleod, A., Duffy, C., Ilboudo, H., Camara, M., De Meeus, T., Belem, A. M. & Jamonneau, V., 2011.** First evidence that parasites infecting apparent aparasitaemic serological suspects in human African trypanosomiasis are *Trypanosoma brucei gambiense* and are similar to those found in patients. *Infection, Genetics & Evolution.* **In press, corrected proof; available online 17 April**.

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Thanks to its sensitivity and its ease of use in the field, the card agglutination test for trypanosomiasis (CATT) is widely used for serological screening of *Trypanosoma brucei gambiense* human African trypanosomiasis (HAT). Positive subjects are then examined by microscopy to confirm the disease. However, the CATT exhibits false-positive results raising the question of whether CATT-positive subjects who are not confirmed by microscopic

detection of trypanosomes (SERO) are truly exposed to *T. b. gambiense* infection. For this purpose, we applied microsatellite genotyping on DNA extracted from blood of both HAT confirmed patients and SERO subjects in Guinea and Côte d'Ivoire since microsatellite genotyping has proved useful for the study of *T. b. gambiense* genetic diversity. Problems of amplification failures raise the question of the sensitivity of microsatellite markers when applied on biological samples especially from SERO subjects for who low blood parasitaemia are suspected. Nevertheless, we have shown that the trypanosomes from SERO individuals that have been genotyped belong to *T. b. gambiense* group 1 and were identical to those found in HAT patients. These results constitute the first evidence that at least some SERO are indeed infected by *T. b. gambiense* group 1 and that they may constitute a human reservoir of parasite in HAT foci. Whether these individuals should undergo treatment remains an open question as long as their role in HAT transmission is unknown. Our results strongly recommend the follow-up of such subjects to improve control strategies.

15682. **Mwanakasale, V. & Songolo, P., 2011.** Disappearance of some human African trypanosomiasis transmission foci in Zambia in the absence of a tsetse fly and trypanosomiasis control programme over a period of forty years. *Transactions Royal Society Tropical Medicine & Hygiene*, **105** (3): 167-172.

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We conducted a situation analysis of human African trypanosomiasis (HAT) in Zambia from January 2000 to April 2007. The aim of this survey was to identify districts in Zambia that were still recording cases of HAT. Three districts namely, Mpika, Chama, and Chipata were found to be still reporting cases of HAT and thus lay in HAT transmission foci in north eastern Zambia. During the period under review, 24 cases of HAT were reported from these three districts. We thereafter reviewed literature on the occurrence of HAT in Zambia from the early 1960s to mid-1990s. This revealed that HAT transmission foci were widespread in Western, North Western, Lusaka, Eastern, Luapula, and Northern Provinces of Zambia during this period. In this article we have tried to give possible reasons as to why the distribution of HAT transmission foci is so different between before and after 2000 when there has been no active national tsetse fly and trypanosomiasis control programme in Zambia.

15683. **Rosset, S., Tzur, S., Behar, D. M., Wasser, W. G. & Skorecki, K., 2011.** The population genetics of chronic kidney disease: insights from the MYH9-APOL1 locus. *Nature Reviews. Nephrology,* **7**: 313-326.

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Many rare kidney disorders exhibit a monogenic, Mendelian pattern of inheritance. Population-based genetic studies have identified many genetic variants associated with an increased risk of developing common kidney diseases. Strongly associated variants have potential clinical uses as predictive markers and may advance our understanding of disease pathogenesis. These principles are elegantly illustrated by a region within chromosome 22q12 that has a strong association with common forms of kidney disease. Researchers had identified DNA sequence variants in this locus that were highly associated with an increased prevalence

of common chronic kidney diseases in people of African ancestry. Initial research concentrated on MYH9 as the most likely candidate gene; however, population-based whole-genome analysis enabled two independent research teams to discover more strongly associated mutations in the neighbouring APOL1 gene. The powerful evolutionary selection pressure of an infectious pathogen in West Africa favoured the spread of APOL1 variants that protect against a lethal form of African sleeping sickness but are highly associated with an increased risk of kidney disease. We describe the data sources, process of discovery, and reasons for initial misidentification of the candidate gene, as well as the lessons that can be learned for future population genetics research.

15684. **Wastling, S. L., Picozzi, K., Wamboga, C., B, V. O. N. W., Amongi-Accup, C., Wardrop, N. A., Stothard, J. R., Kakembo, A. & Welburn, S. C., 2011.** Latent *Trypanosoma brucei gambiense* foci in Uganda: a silent epidemic in children and adults? *Parasitology.* **Published online 8 April.**

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Trypanosoma brucei gambiense sleeping sickness follows a long asymptomatic phase and persists in ancient foci from which epidemic clinical disease arises. A putative focus of *T. b. gambiense* infections has been identified, initially in mothers and young children, on the Lake Albert shoreline of Western Uganda leading to mass screening of 6 207 individuals in September 2008. *T. b. gambiense* infections were identified by the card agglutination test for trypanosomiasis (CATT) and sub-species-specific PCR although parasitological methods failed to confirm any patent trypanosome infections. In April 2009, CATT positives were re-visited; diagnosis of individuals by CATT and PCR was unstable over the two time points and parasites remained undetected, even using mini anion exchange centrifugation technique (mAECT). These observations suggest the possibility of a silent focus of disease, where all infected individuals are in a latent stage, and highlight our limited understanding of the local natural history and disease progression of *T. b. gambiense* in children and adults.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **34**: 15617, 15633]

15685. **Dill, E. A., Renault, C. & Kirkpatrick, B. D., 2011.** *Trypanosoma brucei* infection in a HIV positive Ugandan male. *Clinical & Laboratory Science*, **24** (2): 85-88.

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Human African trypanosomiasis, or African sleeping sickness, is a parasitic infection caused by *Trypanosoma brucei* (*gambiense* or *rhodesiense)*, and one of the declared neglected tropical diseases. Sleeping sickness has high fatality rates and is a continued threat in several African countries. We present characteristic clinical and microbiologic features of a fatal case of African sleeping sickness in an HIV-infected individual.

(c) TREATMENT

[See also **34**: 15616, 15619, 15621, 15623, 15624, 15625, 15630, 15632, 15635, 15640]

15686. **Deborggraeve, S., Lejon, V., Ekangu, R. A., Mumba Ngoyi, D., Pati Pyana, P., Ilunga, M., Mulunda, J. P. & Buscher, P., 2011.** Diagnostic accuracy of PCR in *gambiense* sleeping sickness diagnosis, staging and post-treatment follow-up: a 2-year longitudinal study. *PLoS Neglected Tropical Diseases*, **5** (2): e972.

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The polymerase chain reaction (PCR) has been proposed for diagnosis, staging and post-treatment follow-up of sleeping sickness but no large-scale clinical evaluations of its diagnostic accuracy have taken place yet. An 18S ribosomal RNA gene targeting PCR was performed on blood and cerebrospinal fluid (CSF) of 360 *T. brucei gambiense* sleeping sickness patients and on blood of 129 endemic controls from the Democratic Republic of Congo. Sensitivity and specificity (with 95 percent confidence intervals) of PCR for diagnosis, disease staging and treatment failure over two years follow-up post-treatment were determined. Reference standard tests were trypanosome detection for diagnosis and trypanosome detection and/or increased white blood cell concentration in CSF for staging and detection of treatment failure. PCR on blood showed a sensitivity of 88.4 percent (84.4-92.5 percent) and a specificity of 99.2 percent (97.7-100 percent) for diagnosis, while for disease staging the sensitivity and specificity of PCR on cerebrospinal fluid were 88.4 percent (84.8-91.9 percent) and 82.9 percent (71.2-94.6 percent), respectively. During follow-up after treatment, PCR on blood had low sensitivity to detect treatment failure. In cerebrospinal fluid, PCR positivity vanished slowly and was observed until the end of the two year follow-up in around 20 percent of successfully treated patients. It is concluded that for *T. b. gambiense* sleeping sickness diagnosis and staging, PCR performed better than, or similar to, the current parasite detection techniques but it cannot be used for post-treatment follow-up. Continued PCR positivity in one out of five cured patients points to persistence of living or dead parasites or their DNA after successful treatment and may necessitate the revision of some paradigms about the pathophysiology of sleeping sickness.

15687. **Hainard, A., Tiberti, N., Robin, X., Ngoyi, D. M., Matovu, E., Enyaru, J. C., Muller, M., Turck, N., Ndung'u, J. M., Lejon, V. & Sanchez, J. C., 2011.** Matrix metalloproteinase-9 and intercellular adhesion molecule 1 are powerful staging markers for human African trypanosomiasis. *Tropical Medicine & International Health,* **16** (1): 119-126.

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A critical step before treatment of human African trypanosomiasis (HAT) is the correct staging of the disease. As the late stage is established when trypanosomes cross the blood-brain barrier and invade the central nervous system, we hypothesized that matrix metalloproteinases and cell adhesion molecules could indicate, alone or in combination, the disease progression

from the first to the second stage of HAT. We measured the levels of MMP-2, MMP-9, ICAM-1, VCAM-1 and E-selectin in the cerebrospinal fluid (CSF) of 63 *Trypanosoma brucei gambiense*-infected patients (15 stage 1 and 48 stage 2). Staging was based on counting of white blood cells (WBC) and/or parasite detection in CSF. Concentrations were obtained either by ELISA or multiplex bead suspension assays, and results were compared with three known HAT staging markers (CXCL10, CXCL8 and H-FABP). ICAM-1 and MMP-9 accurately discriminated between stage 1 and stage 2 patients with HAT with 95 percent sensitivity (SE) for 100 percent specificity (SP), which was better than CXCL10 (93 percent SE for 100 percent SP), one of the most promising known markers. Combination of ICAM-1 and MMP-9 with H-FABP provided a panel that resulted in 100 percent of SE and SP for staging HAT. In conclusion, ICAM-1 and MMP-9, alone or in combination, appeared as powerful CSF staging markers of HAT. Final validation of all newly discovered staging markers on a large multi-centric cohort including both forms of the disease as well as patients with others infections should be performed.

15688. **Kuepfer, I., Hhary, E. P., Allan, M., Edielu, A., Burri, C. & Blum, J. A., 2011.** Clinical presentation of *T. b. rhodesiense* sleeping sickness in second stage patients from Tanzania and Uganda. *PLoS Neglected Tropical Diseases*, **5** (3): e968.

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A wide spectrum of disease severity has been described for human African trypanosomiasis (HAT) due to *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*), ranging from chronic disease patterns in southern countries of East Africa to an increase in virulence towards the north. However, only limited data on the clinical presentation of *T. b. rhodesiense* HAT is available. From 2006-2009 we conducted the first clinical trial programme (Impamel III) in *T. b. rhodesiense* endemic areas of Tanzania and Uganda in accordance with international standards (ICH-GCP). The primary and secondary outcome measures were safety and efficacy of an abridged melarsoprol schedule for treatment of second stage disease. Based on diagnostic findings and clinical examinations at baseline we describe the clinical presentation of *T. b. rhodesiense* HAT in second stage patients from two distinct geographical settings in East Africa. One hundred and thirty eight second stage patients from Tanzania and Uganda were enrolled. Blood samples were collected for diagnosis and molecular identification of the infective trypanosomes, and *T. b. rhodesiense* infection was confirmed in all trial subjects. Significant differences in diagnostic parameters and clinical signs and symptoms were observed: the median white blood cell (WBC) count in the cerebrospinal fluid (CSF) was significantly higher in Tanzania (134 cells/mm^3) than in Uganda (20 cells/mm^3); $p<0.0001$). Unspecific signs of infection were more commonly seen in Uganda, whereas neurological signs and symptoms specific for HAT dominated the clinical presentation of the disease in Tanzania. Co-infections with malaria and HIV did not influence the clinical presentation nor treatment outcomes in the Tanzanian study population. In the on-going absence of sensitive diagnostic tools and safe drugs to diagnose and treat second stage *T. b. rhodesiense* HAT an early identification of the disease is essential. A detailed understanding of the clinical presentation of *T. b. rhodesiense* HAT among health personnel and affected communities is vital, and awareness of regional characteristics, as well as implications of co-infections, can support decision making and differential diagnosis.

15689. **Mpandzou, G., Cespuglio, R., Ngampo, S., Bandzouzi, B., Bouteille, B., Vincendeau, P. & Buguet, A., 2011.** Polysomnography as a diagnosis and post-treatment follow-up tool in human African trypanosomiasis: a case study in an infant. *Journal of Neurological Sciences*, **305** (1-2): 112-115.

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Gambian (*Trypanosoma brucei gambiense*) human African trypanosomiasis (HAT) evolves from the haemolymphatic stage 1, treated with pentamidine, to the meningoencephalitic stage 2, often treated with melarsoprol. This arsenate may provoke a deadly reactive encephalopathy. It is therefore crucial to diagnose precisely the stages of HAT, especially when clinical and biological examinations are doubtful. We present here the case of a 30-month old girl (E20 KOLNG) diagnosed with stage 1 HAT during a field survey in June 2007 in Congo. She was followed-up every six months for 18 months in a village dispensary facility at Mpouya. Her health status deteriorated in December 2008, although cerebrospinal fluid (CSF) white blood cell (WBC) count was normal. The child was hospitalized at Brazzaville and a daytime polysomnographic recording (electroencephalogram, electrooculogram, and electromyogram) was performed (Temec Vitaport 3(R) portable recorder) to avoid a new lumbar puncture. The child presented a complete polysomnographic syndrome of HAT with a major disturbance of the distribution of sleep and wake episodes and the occurrence of sleep onset REM periods (SOREMPs). The relapse at stage 2 was confirmed by a new CSF examination that showed an elevated WBC count (23 cells/μL^{-1}) with the presence of B lymphocytes. Melarsoprol treatment was undertaken. A post-treatment recording was immediately performed, showing the resolution of sleep-wake pattern abnormalities. Another polysomnography, taken four months later, confirmed the normalization of sleep-wake patterns indicating healing. We therefore propose that polysomnography, being a non-invasive technique, should be used in children to alleviate the burden caused by HAT staging procedures, especially regarding lumbar punctures in remote African villages.

6. ANIMAL TRYPANOSOMOSIS

(a) SURVEY AND DISTRIBUTION

[See also **34**: 15673]

15690. **Gillingwater, K., Mamabolo, M. V. & Majiwa, P. A., 2010.** Prevalence of mixed *Trypanosoma congolense* infections in livestock and tsetse in KwaZulu-Natal, South Africa. *Journal of the South African Veterinary Association*, **81** (4): 219-223.

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Trypanosoma congolense causes the most economically important animal trypanosomosis in Africa. In South Africa, a rinderpest pandemic of the 1890s removed many host animals, resulting in the near-eradication of most tsetse species. Further suppression was achieved through spraying with dichlorodiphenyltrichloroethane (DDT); however, residual

populations of *Glossina austeni* and *G. brevipalpis* remained in isolated pockets. A total of 506 of these tsetse flies were captured in the Hluhluwe-iMfolozi Park, the St Lucia Wetland Park and Boomerang commercial farm. The polymerase chain reaction (PCR) was used to determine the infection rate and frequency of mixed infections of these flies. Additionally, 473 blood samples were collected from cattle at communal diptanks and a commercial farm in the area and each one examined by the haematocrit centrifugation technique (HCT). Furthermore, buffy coats from these blood samples were spotted onto FTA eluted cards and the DNA extracted from each one tested using three separate PCRs. The HCT revealed the presence of trypanosomes in only 6.6 percent of the blood samples; by contrast, species-specific PCR detected trypanosome DNA in 50 percent of the samples. The species-specific PCR detected trypanosome DNA in 17 percent of the tsetse flies, compared with the nested PCR targeting rDNA which detected trypanosome DNA in only 14 percent of the samples. Over time, the transmission of savannah-type *T. congolense* and Kilifi-type *T. congolense* as mixed infections could have an impact on disease manifestation in different hosts in the area.

15691. **Simukoko, H., Marcotty, T., Vercruysse, J. & Van den Bossche, P., 2011.** Bovine trypanosomiasis risk in an endemic area on the eastern plateau of Zambia. *Research in Veterinary Science*, **90** (1): 51-54.

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The control of bovine trypanosomiasis could be improved by using the available control tools during periods when the incidence of the disease is highest. The present study assessed the monthly risk of bovine trypanosomiasis in 85 sentinel cattle kept on the tsetse-infested eastern plateau of Zambia during a period of 19 consecutive months. To avoid problems associated with persistence of infections because of trypanocidal drug resistance and/or the time lag between sampling and molecular analysis, a survival analysis and the subsequent calculation of risk was used as an indicator of challenge. Results showed that the average monthly risk of infection (92.3 percent due to *Trypanosoma congolense*) was 6 percent. It was significantly higher (7.7 percent) during the beginning of the rainy season (December-February). According to the outcome of the study, bovine trypanosomiasis control in the study area can be improved through increasing control efforts during this period of highest challenge.

15692. **Tadesse, A. & Tsegaye, B., 2010.** Bovine trypanosomosis and its vectors in two districts of Bench Maji zone, South Western Ethiopia. *Tropical Animal Health & Production*, **42** (8): 1757-1762.

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A cross-sectional study was carried out from November 2008 to February 2009 in Guraferda and Sheko districts of Bench Maji Zone, South Western Ethiopia. The objective of the study was to determine the prevalence of bovine trypanosomosis and the density of its vectors. An overall prevalence of trypanosome infection in the study area was 4.4 percent. *Trypanosoma congolense* (36.36 percent) was the dominant trypanosome species followed by *Trypanosoma vivax* (18.18 percent) and *Trypanosoma brucei* (9.09 percent). Mean packed cell

volume value of parasitaemic animals (21.8 percent) was significantly ($p<0.05$) lower than that of aparasitaemic animals (27.7 percent). Biconical and NGU traps were deployed for 72 h, and the result indicated *Glossina pallidipes* followed by *Glossina fuscipes* as the only tsetse fly species caught in the study area along with other biting flies like *Stomoxys* and *Tabanus.* The apparent density of tsetse flies was 2.83 flies $trap^{-1}$ day^{-1}. NGU trap caught more of *G. pallidipes* while biconical trap caught more *G. fuscipes*, and the difference was significant ($p<0.05$). Although the current study indicated low prevalence of trypanosomosis in the study area, the impacts of trypanosomosis on cattle production and productivity should not be neglected. Therefore, attention should be given to control the disease and also the vector.

15693. **von Wissmann, B., Machila, N., Picozzi, K., Fevre, E. M., de, C. B. B. M., Handel, I. G. & Welburn, S. C., 2011.** Factors associated with acquisition of human infective and animal infective trypanosome infections in domestic livestock in Western Kenya. *PLoS Neglected Tropical Diseases*, **5** (1): e941.

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Trypanosomiasis is regarded as a constraint on livestock production in Western Kenya where the responsibility for tsetse and trypanosomiasis control has increasingly shifted from the state to the individual livestock owner. To assess the sustainability of these localised control efforts, this study investigates biological and management risk factors associated with trypanosome infections detected by polymerase chain reaction (PCR), in a range of domestic livestock at the local scale in Busia, Kenya. Busia District also remains endemic for human sleeping sickness with sporadic cases of sleeping sickness reported. In total, trypanosome infections were detected in 11.9 percent (329) out of the 2 773 livestock sampled in Busia District. Multivariable logistic regression revealed that host species and cattle age affected overall trypanosome infection, with significantly increased odds of infection for cattle older than 18 months, and significantly lower odds of infection in pigs and small ruminants. Different grazing and watering management practices did not affect the odds of trypanosome infection, adjusted by host species. Neither anaemia nor condition score significantly affected the odds of trypanosome infection in cattle. Human infective *Trypanosoma brucei rhodesiense* were detected in 21.5 percent of animals infected with *T. brucei* s.l. (29/135) amounting to 1 percent (29/2773) of all sampled livestock, with significantly higher odds of *T. brucei rhodesiense* infections in *T. brucei* s.l. infected pigs (OR = 4.3, 95 percentCI 1.5-12.0) than in *T. brucei* s.l. infected cattle or small ruminants. Although cattle are the dominant reservoir of trypanosome infection it is unlikely that targeted treatment of only visibly diseased cattle will achieve sustainable interruption of transmission for either animal infective or zoonotic human infective trypanosomiasis, since most infections were detected in cattle that did not exhibit classical clinical signs of trypanosomiasis. Pigs were also found to be reservoirs of infection for *T. b. rhodesiense* and present a risk to local communities.

(b) PATHOLOGY AND IMMUNOLOGY

(c) TRYPANOTOLERANCE

15694. **Behnke, J. M., Chiejina, S. N., Musongong, G. A., Nnadi, P. A., Ngongeh, L. A., Abonyi, F. O. & Fakae, B. B., 2011.** Resistance and resilience of traditionally managed West African Dwarf goats from the savanna zone of northern Nigeria to naturally acquired trypanosome and gastrointestinal nematode infections. *Journal of Helminthology*, **85** (1): 80-91.

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A survey was conducted of gastrointestinal nematode infections and trypanosomosis in Nigerian West African Dwarf (WAD) goats from the savannah region of the country. Animals were screened at two markets, Gboko and Akpagher, from the beginning of April until the end of September, coinciding with the end of the dry season and the first five months of the wet season. Of 1 054 goats that were examined, 80.5 percent carried gastrointestinal (GI) nematodes belonging to the genera *Haemonchus* (61.0 percent), *Oesophagostomum* (21.0 percent) and *Trichostrongylus* (17.9 percent). Faecal egg counts (FEC) increased very slowly but significantly from April to maximum levels in September, and varied marginally between the two market sources. The majority of goats (68.8 and 70.1 percent at the two markets) had low FEC not exceeding 50 eggs/g (epg). FEC did not differ significantly between the sexes or between age classes. Packed cell volume (PCV) also declined significantly with month of the study, but was affected by host sex (a significant month x sex interaction) being generally higher in male animals throughout the period. There was a highly significant negative correlation between log(FEC+1) and PCV, when all other factors had been taken into account. Body condition scores (BCS) also declined with month of the study, but there was a marked difference between the two sexes, with male animals generally showing a greater stability of BCS across the months compared with females. Trypanosome infections were found in only 4 percent of the goats and only during the rainy season. Most infections (92.86 percent) were caused by *Trypanosoma brucei* alone although *T. vivax* and *T. congolense* were occasionally detected. Overall, the majority of goats sampled each month maintained generally good body condition (BCS 3.0-5.0), normal or slightly reduced PCV, even when concurrently infected with trypanosomes and GI nematodes. However, four concurrently infected goats showed signs of overt anaemia during periods of peak infection, during the late rainy season, with marked reductions in PCV (< 15 percent). Two of the infected goats were also in poor body condition with BCS of < 2.0. There was no evidence of additive or synergistic pathogenic effects of the two parasites. These results are discussed in the context of the unexpectedly strong resistance and resilience of the savannah WAD ecotype to its native strains of GI nematode and trypanosome parasites.

15695. **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** (1): 12.

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West African Dwarf (WAD) goats serve an important role in the rural village economy of West Africa, especially among small-holder 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.

15696. **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 of the United States of America*, **108** (22): 9304-9309.

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African bovine trypanosomiasis caused by *Trypanosoma* sp., 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 ploughing 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.

15697. **Orenge, C., Munga, L., Kimwele, C., Kemp, S., Korol, A., Gibson, J., Hanotte, O. & Soller, M., 2011.** Expression of trypanotolerance in N'Dama x Boran crosses under field challenge in relation to N'Dama genome content. *BMC Proceedings*, **5 Suppl 4**: S23.

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Animal trypanosomosis in sub-Saharan Africa is a major obstacle to livestock based agriculture. Control relies on drugs with increasing incidence of multiple-drug resistance. A previous mapping experiment in an F_2 population derived from the indigenous trypanotolerant N'Dama cattle crossed to susceptible (Kenya)-Boran cattle under controlled challenge, uncovered a number of trypanotolerance QTL (T-QTL). The present study was to determine expression of N'Dama trypanotolerance in a backcross to the Boran under conditions of field challenge, and whether chromosomal regions associated with trypanotolerance in the F2 experiment showed similar effects in the BC population. One hundred and ninety two backcross animals to the Boran were produced in six batches from June 2001 to December 2006. At one year of age animals were moved to the field and exposed to natural challenge over about one year in Southwest Kenya (Narok). The animals were individually recorded weekly for body weight, packed cell volume, parasitaemia score, and drug treatments, and were genotyped using 35 microsatellite markers spanning five chromosomes found in the F_2 study to harbour T-QTL. The F_1 were most trypanotolerant, Boran least, and BC intermediate. Females showed distinctly higher trypanotolerance than males. There was a positive correlation in the BC population between trypanotolerance and number of N'Dama origin marker alleles. QTL mapping revealed T-QTL distributed among all five targeted chromosomes, corresponding in part to the results obtained in the F_2 experiment. In conclusion, N'Dama origin trypanotolerance is expressed in a BC population under field conditions in proportion to N'Dama origin marker alleles. Consequently, marker assisted selection in such populations may be a means of increasing trypanotolerance, while retaining the desirable productive qualities of the recurrent parent.

15698. **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; International Livestock Research Institute, P.O. Box 5689, Addis Ababa, Ethiopia. [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 (1 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

7. EXPERIMENTAL TRYPANOSOMOSIS

(a) DIAGNOSTICS

[See also **34**: 15639]

15699. **Ahmed, H. A., Macleod, E. T., Hide, G., Welburn, S. C. & Picozzi, K., 2011.** The best practice for preparation of samples from FTA^R cards for diagnosis of blood borne infections using African trypanosomes as a model system. *Parasites & Vectors*, **4**: 68.

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Diagnosis of blood borne infectious diseases relies primarily on the detection of the causative agent in the blood sample. Molecular techniques offer sensitive and specific tools for this although considerable difficulties exist when using these approaches in the field environment. In large scale epidemiological studies, FTA^R cards are becoming increasingly popular for the rapid collection and archiving of a large number of samples. However, there are some difficulties in the downstream processing of these cards which is essential for the accurate diagnosis of infection. Here we describe recommendations for the best practice approach for sample processing from FTA^R cards for the molecular diagnosis of trypanosomiasis using PCR. A comparison of five techniques was made. Detection from directly applied whole blood was less sensitive (35.6 percent) than whole blood which was subsequently eluted from the cards using Chelex^R 100 (56.4 percent). Better apparent sensitivity was achieved when blood was lysed prior to application on the FTA cards (73.3 percent) although this was not significant. This did not improve with subsequent elution using Chelex^R 100 (73.3 percent) and was not significantly different from direct DNA extraction from blood in the field (68.3 percent). Based on these results, the degree of effort required for each of these techniques and the difficulty of DNA extraction under field conditions, we recommend that blood is transferred onto FTA cards whole followed by elution in Chelex^R 100 as the best approach.

15700. **Holm, S. H., Beech, J. P., Barrett, M. P. & Tegenfeldt, J. O., 2011.** Separation of parasites from human blood using deterministic lateral displacement. *Lab on a Chip*, **11** (7): 1326-1332.

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We present the use of a simple microfluidic technique to separate living parasites from human blood. Parasitic trypanosomatids cause a range of human and animal diseases. African trypanosomes, responsible for human African trypanosomiasis (sleeping sickness), live free in the blood and other tissue fluids. Diagnosis relies on detection and due to their often low numbers against an overwhelming background of predominantly red blood cells it is crucial to separate the parasites from the blood. By modifying the method of deterministic lateral displacement, confining parasites and red blood cells in channels of optimized depth which accentuates morphological differences, we were able to achieve separation thus offering a potential route to diagnostics.

15701. **Njiru, Z. K., 2011.** Rapid and sensitive detection of human African trypanosomiasis by loop-mediated isothermal amplification combined with a lateral-flow dipstick. *Diagnostic Microbiology & Infectious Disease*, **69** (2): 205-209.

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A combined loop-mediated isothermal amplification lateral flow dipstick (LAMP-LFD) format was evaluated in the detection of human infective trypanosome DNA from clinical samples. The LAMP-LFD showed analytical sensitivity equivalent to 0.01 tryps/mL, levels that were identical to using gel electrophoresis and SYBR Green I dye. The LAMP-LFD showed superior specificity to SYBR Green I when supernatant prepared from boiled human biological samples was used as template. These results indicate that the use of nonspecific DNA intercalators may produce false positives when partially processed templates are used. The LAMP-LFD format presented here is simple, rapid, and has future potential use in diagnosis of sleeping sickness.

15702. **Njiru, Z. K., Traub, R., Ouma, J. O., Enyaru, J. C. & Matovu, E., 2011.** Detection of Group 1 *Trypanosoma brucei gambiense* by loop-mediated isothermal amplification. *Journal of Clinical Microbiology*, **49** (4): 1530-1536.

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Trypanosoma brucei gambiense group 1 is the major causative agent of the Gambian human African trypanosomiasis (HAT). Accurate diagnosis of Gambian HAT is still challenged by lack of precise diagnostic methods, low and fluctuating parasitaemia, and generally poor services in the areas of endemicity. In this study, we designed a rapid loop-mediated isothermal amplification (LAMP) test for *T. b. gambiense* based on the 3' end of the *T. b. gambiense*-specific glycoprotein (TgsGP) gene. The test is specific and amplifies

DNA from *T. b. gambiense* isolates and clinical samples at 62 ^{0}C within 40 min using a normal water bath. The analytical sensitivity of the TgsGP LAMP was equivalent to 10 trypanosomes/ml using purified DNA and approximately 1 trypanosome/ml using supernatant prepared from boiled blood, while those of classical PCR tests ranged from 10 to 10^3 trypanosomes/ml. There was 100 percent agreement in the detection of the LAMP product by real-time gel electrophoresis and the DNA-intercalating dye SYBR green I. The LAMP amplicons were unequivocally confirmed through sequencing and analysis of melting curves. The assay was able to amplify parasite DNA from native cerebrospinal fluid (CSF) and double-centrifuged supernatant prepared from boiled buffy coat and bone marrow aspirate. The robustness, superior sensitivity, and ability to inspect results visually through colour change indicate the potential of TgsGP LAMP as a future point-of-care test.

15703. **Ramirez-Iglesias, J. R., Eleizalde, M. C., Gomez-Pineres, E. & Mendoza, M., 2011.** *Trypanosoma evansi*: a comparative study of four diagnostic techniques for trypanosomosis using rabbit as an experimental model. *Experimental Parasitology*, **128** (1): 91-96.

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The goal of this study was to compare two parasitological diagnostic techniques, such as by micro-haematocrit centrifugation technique (MHCT) and direct microscopic examination (DME) with a serological method (iELISA), and a molecular procedure PCR, in rabbits experimentally infected with *Trypanosoma evansi*, in order to determine their sensitivity throughout the course of disease. The parasitological methods were not able to detect the presence of the parasite during the phases of low parasitaemia, the prepatent period and the chronic phase. In contrast, PCR detected *T. evansi* in the prepatent and chronic phases, when the amount of DNA increased from 100 to 300ng. 100 percent detection was observed with iELISA only in the chronic stage of the disease. In the acute phase, all samples were positively diagnosed using either MHCT or PCR, whereas only few samples were diagnosed by DME. Samples obtained from day 15 post infection were also detected by iELISA. The highest rate of diagnossis during the course of infection was achieved by the PCR technique (93.8 percent), followed by iELISA (71.1 percent), MHCT (59 percent) and DME (13.6 percent). Therefore, we recommend the use of PCR in epidemiological studies in order to implement sanitary control plans for the improvement of livestock productivity in the country.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **34**: 15766, 15767, 15772]

15704. **Akhoon, B. A., Slathia, P. S., Sharma, P., Gupta, S. K. & Verma, V., 2011.** *In silico* identification of novel protective VSG antigens expressed by *Trypanosoma brucei* and an effort for designing a highly immunogenic DNA vaccine using IL-12 as adjuvant. *Microbial Pathogenesis*, **51** (1-2): 77-87.

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African trypanosomiasis continues to be a major health problem, with manyadults dying from this disease world-wide. As the sequence diversity of *Trypanosoma brucei* is extreme, with VSGs having 15-25 percent identity with most other VSGs, hence it displays a huge diversity of adaptations and host specificities. Therefore the need for an improved vaccine has become an international priority. The highly conserved and specific epitopes acting as both $CD8^+$ and $CD4^+$ T-cell epitopes (FLINKKPAL and FTALCTLAA) were predicted from large bunch of VSGs of *T. brucei*. Besides, some other potential epitopes with very high affinity for MHC I and II molecules were also determined while taking into consideration the most common HLA in the general population which accounts for major ethnicities. The vaccine candidates were found to be effective even for non-African populations as predicted by population coverage analysis. Hence the migrating travellers acting as a spread means of the infection can probably also be treated successfully after injection of such a multiepitopic vaccine. Exploiting the immunoinformatics approaches, we designed a potential vaccine by using the consensus epitopic sequence of 388 VSG proteins of *T. brucei* and performed *in silico* cloning of multiepitopic antigenic DNA sequence in pBI-CMV1 vector. Moreover, various techniques like codon adaptation, CpG optimization, removal of self-recognized epitopes, use of adjuvant and co-injection with plasmids expressing immune-stimulatory molecules were implemented to enhance the immunogenicity of the proposed *in silico* vaccine.

15705. **Amrouni, D., Meiller, A., Gautier-Sauvigne, S., Piraud, M., Bouteille, B., Vincendeau, P., Buguet, A. & Cespuglio, R., 2011.** Cerebral changes occurring in arginase and dimethylarginine dimethylaminohydrolase (DDAH) in a rat model of sleeping sickness. *PloS One*, **6** (3): e16891.

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Involvement of nitric oxide (NO) in the pathophysiology of human African trypanosomiasis (HAT) was analysed in a HAT animal model (rat infected with *Trypanosoma brucei brucei*). With this model, it was previously reported that trypanosomes were capable of limiting trypanocidal properties carried by NO by decreasing its blood concentration. It was also observed that brain NO concentration, contrary to blood, increases throughout the infection process. The present approach analyses the brain impairments occurring in the regulations exerted by arginase and N(G), N(G)-dimethylarginine dimethylaminohydrolase (DDAH) on NO synthases (NOS). In this respect: (i) cerebral enzymatic activities, mRNA and protein expression of arginase and DDAH were determined; (ii) immunohistochemical distribution and morphometric parameters of cells expressing DDAH-1 and DDAH-2 isoforms were examined within the diencephalon; (iii) amino acid profiles relating to NOS/arginase/DDAH pathways were established. Arginase and DDAH activities together with mRNA (RT-PCR) and protein (western-blot) expressions were determined in diencephalic brain structures of healthy or infected rats at various days post-infection (D5, D10, D16, D22). While arginase activity remained constant, that of DDAH increased at D10 (+65 percent) and D16 (+51 percent) in agreement with Western-blot and amino acids data (liquid chromatography tandem-mass spectrometry). Only DDAH-2 isoform appeared to be up-regulated at the transcriptional level throughout the infection process. Immunohistochemical staining further revealed that DDAH-1 and DDAH-2 are contained

within interneurons and neurons, respectively. In the brain of infected animals, the lack of change observed in arginase activity indicates that polyamine production is not enhanced. Increases in DDAH-2 isoform may contribute to the overproduction of NO. These changes are at variance with those reported in the periphery. As a whole, the above processes may ensure additive protection against trypanosome entry into the brain, i.e., maintenance of NO trypanocidal pressure and limitation of polyamine production, necessary for trypanosome growth.

15706. **As, D. A. S., Belle, L. P., Bitencourt, P. E., Souza, V. C., Costa, M. M., Oliveira, C. B., Jaques, J. A., Leal, D. B., Moretto, M. B., Mazzanti, C. M., Lopes, S. T. & Monteiro, S. G.,** 2011. Activity of the enzyme adenosine deaminase in serum, erythrocytes and lymphocytes of rats infected with *Trypanosoma evansi*. *Parasitology,* **138:** 201-208.

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In *Trypanosoma evansi* infections changes in the haemogram are commonly observed, and the enzyme adenosine deaminase (ADA) plays an important role in the production and differentiation of blood cells. Thus, the aim of this study was to evaluate the activity of ADA in serum, erythrocytes and lymphocytes of rats infected with *T. evansi* compared with non-infected rats. Thirty adult rats were used, divided into three uniform groups. The animals in groups A and B were infected intraperitoneally with 2 x 10^6 trypomastigotes/rat. Rodents from group C (control group), were not infected. Blood collection was performed on days 4 and 20 post-infection (p.i.) in order to obtain acute and chronic infection stages of disease. The blood was used to assess the activity of ADA. In the blood, reduced haematocrit and increased lymphocytes were correlated with ADA activity in erythrocytes and lymphocytes. We observed reduction of ADA activity in serum and erythrocytes in rats infected with *T. evansi* compared to non-infected rats ($p<0.05$). ADA activity in lymphocytes was decreased after four days, when the parasitaemia was high and increased after 20 days, when the number of circulating parasites was low. In conclusion, our results showed that the ADA activity was altered in the serum, lymphocytes and erythrocytes of rats, concomitantly with haematological parameters, in experimental infection by *T. evansi*.

15707. **Boulange, A. F., Khamadi, S. A., Pillay, D., Coetzer, T. H. & Authie, E., 2011.** Production of congopain, the major cysteine protease of *Trypanosoma* (Nannomonas) *congolense,* in *Pichia pastoris* reveals unexpected dimerization at physiological pH. *Protein Expression & Purification*, **75** (1): 95-103.

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African animal trypanosomosis (nagana) is arguably the most important parasitic disease affecting livestock in sub-Saharan Africa. Since none of the existing control measures are entirely satisfactory, vaccine development is being actively pursued. However, due to antigenic variation, the quest for a conventional vaccine has proven elusive. As a result, we have sought an alternative “anti-disease vaccine approach”, based on congopain, a cysteine protease of *Trypanosoma congolense*, which was shown to have pathogenic effects *in vivo*.

Congopain was initially expressed as a recombinant protein in bacterial and baculovirus expression systems, but both the folding and yield obtained proved inadequate. Hence alternative expression systems were investigated, amongst which *Pichia pastoris* proved to be the most suitable. We report here the expression of full length, and C-terminal domain-truncated congopain in the methylotrophic yeast *P. pastoris*. Differences in yield were observed between full length and truncated proteins, the full length producing 2-4 mg of protein per litre of culture, while the truncated form produced 20-30 mg/l. The protease was produced as a proenzyme, but underwent spontaneous activation when acidified (pH <5). To investigate whether this activation was due to autolysis, we produced an inactive mutant (active site Cys-->Ala) by site-directed mutagenesis. The mutant form was produced at a much higher rate, up to 100mg/L culture, as a proenzyme. It did not undergo spontaneous cleavage of the propeptide when subjected to acidic pH suggesting an autocatalytic process of activation for congopain. These recombinant proteins displayed a very unusual feature for cathepsin L-like proteinases, i.e. complete dimerization at pH >6, and by reversibly monomerising at acidic pH <5. This attribute is of utmost importance in the context of an anti-disease vaccine, given that the epitopes recognised by the sera of trypanosome-infected trypanotolerant cattle appear dimer-specific.

15708. **Horn, D. & McCulloch, R., 2010.** Molecular mechanisms underlying the control of antigenic variation in African trypanosomes. *Current Opinion on Microbiology*, **13** (6): 700-705.

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African trypanosomes escape the host adaptive immune response by switching their dense protective coat of variant surface glycoprotein (VSG). Each cell expresses only one VSG gene at a time from a telomeric expression site (ES). The 'pre-genomic' era saw the identification of the range of pathways involving VSG recombination in the context of mono-telomeric VSG transcription. A prominent feature of the early post-genomic era is the description of the molecular machineries involved in these processes. We describe the factors and sequences recently linked to mutually exclusive transcription and VSG recombination, and how these act in the control of the key virulence mechanism of antigenic variation.

15709. **Jia, Y., Zhao, X., Zou, J. & Suo, X., 2011.** *Trypanosoma evansi*: identification and characterization of a variant surface glycoprotein lacking cysteine residues in its C-terminal domain. *Experimental Parasitology*, **127** (1): 100-106.

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African trypanosomes are flagellated unicellular parasites which proliferate extracellularly in the mammalian host blood-stream and tissue spaces. They evade the hosts' antibody-mediated lyses by sequentially changing their variant surface glycoprotein (VSG). VSG tightly coats the entire parasite body, serving as a physical barrier. In *Trypanosoma brucei* and the closely related species *Trypanosoma evansi* and *Trypanosoma equiperdum*, each VSG polypeptide can be divided into N- and C-terminal domains, based on cysteine distribution and sequence homology. N-terminal domain, the basis of antigenic variation, is

hypervariable and contains all the exposed epitopes; C-terminal domain is relatively conserved and a full set of four or eight cysteines were generally observed. We cloned two genes from two distinct variants of *T. evansi*, utilizing RT-PCR with VSG-specific primers. One contained a VSG type A N-terminal domain followed by a C-terminal domain lacking cysteine residues. To confirm that this gene is expressed as a functional VSG, the expression and localization of the corresponding gene product were characterized using Western blotting and immunofluorescent staining of living trypanosomes. Expression analysis showed that this protein was highly expressed, variant-specific, and had a ubiquitous cellular surface localization. All these results indicated that it was expressed as a functional VSG. Our finding showed that cysteine residues in VSG C-terminal domain were not essential; the conserved C-terminal domain generally in *T. brucei* like VSGs would possibly evolve for regulating the VSG expression.

15710. **Johanson, C., Stopa, E., McMillan, P., Roth, D., Funk, J. & Krinke, G., 2011.** The distributional nexus of choroid plexus to cerebrospinal fluid, ependyma and brain: toxicologic/pathologic phenomena, periventricular destabilization, and lesion spread. *Toxicologic Pathology*, **39** (1): 186-212.

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Bordering the ventricular cerebrospinal fluid (CSF) are epithelial cells of choroid plexus (CP), ependyma and circumventricular organs (CVOs) that contain homeostatic transporters for mediating secretion/reabsorption. The distributional pathway ("nexus") of CP-CSF-ependyma-brain furnishes peptides, hormones, and micronutrients to periventricular regions. In disease/toxicity, this nexus becomes a conduit for infectious and xenobiotic agents. The sleeping sickness trypanosome (a protozoan) disrupts CP and downstream CSF-brain. Piperamide is anti-trypanosomic but distorts CP epithelial ultrastructure by engendering hydropic vacuoles; this reflects phospholipidosis and altered lysosomal metabolism. CP swelling by vacuolation may occlude CSF flow. Toxic drug tools delineate injuries to choroidal compartments: cyclophosphamide (vasculature), methylcellulose (interstitium), and piperazine (epithelium). Structurally perturbed CP allows solutes to penetrate the ventricles. There, CSF-borne pathogens and xenobiotics may permeate the ependyma to harm neurogenic stem cell niches. Amoscanate, an anti-helminthic, potently injures rodent ependyma. Ependymal/brain regions near CP are vulnerable to CSF-borne toxicants; this proximity factor links regional barrier breakdown to nearby periventricular pathology. Diverse diseases (e.g. African sleeping sickness, multiple sclerosis) take early root in choroidal, circumventricular, or perivascular loci. Toxicokinetics informs on pathogen, anti-parasitic agent, and auto-antibody distribution along the CSF nexus. CVOs are susceptible to plasma-borne toxicants/pathogens. Countering the physico-chemical and pathogenic insults to the homeostasis-mediating ventricle-bordering cells sustains brain health and fluid balance.

15711. **Lanca, A. S., de Sousa, K. P., Atouguia, J., Prazeres, D. M., Monteiro, G. A. & Silva, M. S., 2011.** *Trypanosoma brucei*: immunisation with plasmid DNA encoding invariant surface glycoprotein gene is able to induce partial protection in experimental African trypanosomiasis. *Experimental Parasitology*, **127** (1): 18-24.

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Trypanosoma brucei is the etiological agent responsible for African trypanosomiasis, an infectious pathology which represents a serious problem of public health and economic losses in sub-Saharan Africa. As one of the foremost neglected illnesses, few resources have been available for the development of vaccines or new drugs, in spite of the current therapeutical drugs showing little efficiency and high toxicity. Hence, it is obviously important to widen effective therapeutics and preventive strategies against African trypanosomiasis. In this work, we use the DNA vaccine model to evaluate immunisation effectiveness in mice challenged with *Trypanosoma brucei brucei*. We demonstrate that Balb/C mice immunised intramuscularly with a single dose of a DNA plasmid encoding a bloodstream-stage specific invariant surface glycoprotein (ISG) are partially protected from a lethal dose of *T. b. brucei.* Interestingly, the surviving animals show high levels of IgG_{2a} anti-trypanosoma antibodies, suggesting that the Th1 response profile seems important for the induced mechanisms of immune protection.

15712. **MacGregor, P. & Matthews, K. R., 2010.** New discoveries in the transmission biology of sleeping sickness parasites: applying the basics. *Journal of Molecular Medicine*, **88** (9): 865-871.

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The sleeping sickness parasite *Trypanosoma brucei* must differentiate in response to the changing environments that it encounters during its complex life cycle. One developmental form, the bloodstream stumpy stage, plays an important role in infection dynamics and transmission of the parasite. Recent advances have shed light on the molecular mechanisms by which these stumpy forms differentiate as they are transmitted from the mammalian host to the insect vector of sleeping sickness, tsetse flies. These molecular advances now provide improved experimental tools for the study of stumpy formation and function within the mammalian bloodstream. They also offer new routes to therapy via high-throughput screens for agents that accelerate parasite development. Here, we shall discuss the recent advances that have been made and the prospects for future research now available.

15713. **Macgregor, P., Savill, N. J., Hall, D. & Matthews, K. R., 2011.** Transmission stages dominate trypanosome within-host dynamics during chronic infections. *Cell Host & Microbe*, **9** (4): 310-318.

Centre for Immunity, Infection, and Evolution, Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JT, UK. [keith.matthews@ed.ac.uk].

Sleeping sickness is characterized by waves of the extracellular parasite *Trypanosoma brucei* in host blood, with infections continuing for months or years until inevitable host death. These waves reflect the dynamic conflict between the outgrowth of a succession of parasite

antigenic variants and their control by the host immune system. Although a contributor to these dynamics is the density-dependent differentiation from proliferative "slender forms" to transmissible "stumpy forms," an absence of markers discriminating stumpy forms has prevented accurate parameterization of this component. Here, we exploit the stumpy-specific PAD1 marker, which functionally defines transmission competence, to quantitatively monitor stumpy formation during chronic infections. This allows reconstruction of the temporal events early in infection. Mathematical modelling of these data describes the parameters controlling trypanosome within-host dynamics and provides strong support for a quorum-sensing-like mechanism. Our data reveal the dominance of transmission stages throughout infection, a consequence being austere use of the parasite's antigen repertoire.

15714. **Magez, S. & Caljon, G., 2011.** Mouse models for pathogenic African trypanosomes: unravelling the immunology of host-parasite-vector interactions. *Parasite Immunology,* **33**(8): 423-429.

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African trypanosomiasis is a parasitic disease that affects a variety of mammals, including humans, on the sub-Saharan African continent. In order to understand the diverse parameters that govern the host-parasite-vector interactions, mouse models for the disease have proven to be a corner stone. Despite the fact that most trypanosomes cannot be considered as natural pathogens for rodents, experimental infections in mice have shed a tremendous amount of light on the general biology of these parasites and their interaction with and evasion of the mammalian immune system. Different aspects including inflammation, vaccine failure, antigenic variation, resistance/sensitivity to normal human serum and the influence of trypanocides on parasite transmission have all been addressed using mouse models. In more recent years, the introduction of various “knock-out” mouse strains has allowed analysis of the implication of various cytokines, particularly TNF, IFNgamma and IL-10 in the regulation of parasitemia and induction of pathological conditions during infection.

15715. **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.

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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 isa 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 intervals 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. In conclusion, the mapped QTL regions encompass genes that are vital to innate immune response and can be potential candidate genes for the underlying QTL.

15716. **Ngotho, M., Kagira, J. M., Kariuki, C., Maina, N., Thuita, J. K., Mwangangi, D. M., Farah, I. O. & Hau, J., 2011.** Influence of trypanocidal therapy on the haematology of vervet monkeys experimentally infected with *Trypanosoma brucei rhodesiense*. *Acta Tropica*, **119** (1): 14-18.

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The aim of this study was to characterizxe the sequential haematological changes in vervet monkeys infected with *Trypanosoma brucei rhodesiense* and subsequently treated with sub-curative diminazene aceturate (DA) and curative melarsoprol (MelB) trypanocidal drugs. Fourteen vervet monkeys, on a serial timed-kill pathogenesis study, were infected intravenously with 10^4 trypanosomes of a stabilate *T. b. rhodesiense* KETRI 2537. They were treated with DA at 28 days post infection (dpi) and with MelB following relapse of infection at 140 dpi. Blood samples were obtained from the monkeys weekly, and haematology conducted using a haematological analyser. All the monkeys developed a disease associated with macrocytic hypochromic anaemia characterised by a reduction in erythrocytes (RBC), haemoglobin (HB), haematocrit (HCT), mean cell volume (MCV), platelet count (PLT), and an increase in the red cell distribution width (RDW) and mean platelet volume (MPV). The clinical disease was characteristic of human African trypanosomiasis (HAT) with a pre-patent period of three days. Treatment with DA cleared trypanosomes from both the blood and cerebrospinal fluid (CSF). The parasites relapsed first in the CSF and later in the blood. This treatment normalised the RBC, HCT, HB, PLT, MCV, and MPV achieving the pre-infection values within two weeks while RDW took up to six weeks to attain pre-infection levels after treatment. Most of the parameters were later characterised by fluctuations, and declined at one to two weeks before relapse of trypanosomes in the haemolymphatic circulation. Following MelB treatment at 140 dpi, most values recovered within two weeks and stabilised at pre-infection levels, during the 223 days post treatment monitoring period. It is concluded that DA and MelB treatments cause similar normalising changes in the haematological profiles of

monkeys infected with *T. b. rhodesiense*, indicating the efficacy of the drugs. The infection related changes in haematology parameters, further characterise the vervet monkey as an optimal induced animal model of HAT. Serial monitoring of these parameters can be used as an adjunct in the diagnosis and prognosis of the disease outcome in the vervet monkey model.

15717. **Nishimura, K., Nakaya, H., Nakagawa, H., Matsuo, S., Ohnishi, Y. & Yamasaki, S., 2011.** Effect of *Trypanosoma brucei brucei* on erythropoiesis in infected rats. *Journal of Parasitology*, **97** (1): 88-93.

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Anaemia generated from African trypanosome infection is considered an important symptom in humans and in domestic animals. In order to recover from anaemia, the process of erythropoiesis is essential. Erythropoiesis is affected by erythropoietin (EPO), an erythropoietic hormone, supplying iron and inflammatory and proinflammatory cytokines. However, the role of these factors in erythropoiesis during African trypanosome infection remains unclear. In the present study, we analyse how erythropoiesis is altered in anaemic *Trypanosoma brucei brucei* (interleukin-tat 1.4 strain [ILS])-infected rats. We report that the packed cell volume (PCV) of blood from ILS-infected rats was significantly lower four days after infection, whereas the number of reticulocytes, as an index of erythropoiesis, did not increase. The level of EPO mRNA in ILS-infected rats did not increase from the third day to the sixth day after infection, the same time that the PCV decreased. Kidney cells of uninfected rats cultured with ILS trypanosome strain for 8 h *in vitro* decreased EPO mRNA levels. Treatment of both ILS and cobalt chloride mimicked hypoxia, which restrained the EPO-production-promoting effect of the cobalt. Messenger RNA levels of beta-globin and transferrin receptor, as markers of erythropoiesis in the bone marrow, also decreased in ILS-infected rats. Levels of hepcidin mRNA, which controls the supply of iron to the marrow in liver, were increased in ILS-infected rats; however, the concentration of serum iron did not change. Furthermore, mRNA levels of interleukin-12, interferon-gamma, tumour necrosis factor-alpha, and macrophage migration inhibitory factor in the spleen, factors that have the potential to restrain erythropoiesis in bone marrow, were elevated in the ILS-infected rats. These results suggest that ILS infection in rats affect erythropoiesis, which responds by decreasing EPO production and restraining its function in the bone marrow.

15718. **Nishimura, K., Nakaya, H., Nakagawa, H., Matsuo, S., Ohnishi, Y. & Yamasaki, S., 2011.** Differential effects of *Trypanosoma brucei gambiense* and *Trypanosoma brucei brucei* on rat macrophages. J*ournal of Parasitology*, **97** (1): 48-54.

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Mammalian immune responses to *Trypanosoma brucei* infection are important to control of the disease. In rats infected with *T. brucei gambiense* (Wellcome strain; WS) or *T. brucei brucei* (interleukin-tat 1.4 strain [ILS]), a marked increase in the number of

macrophages in the spleen can be observed. However, the functional repercussions related to this expansion are not known. To help uncover the functional significance of macrophages in the context of trypanosome infection, we determined the mRNA levels of genes associated with an increase in macrophage number or macrophage function in WS- and ILS-infected rats and in cultured cells. Specifically, we assayed mRNA levels for macrophage colony stimulating factor (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), and macrophage migration inhibitory factor (MIF). Upregulation of GM-CSF and MIF mRNA levels was robust in comparison with changes in M-CSF levels in ILS-infected rats. By contrast, upregulation of M-CSF was more robust in WS-infected rats. The phagocytic activity in macrophages harvested from ILS-infected rat spleens, but not WS-infected spleens, was higher than that in macrophages from uninfected rats. These results suggest that macrophages of WS-infected rats change to an immunosuppressive type. However, when WS or ILS is cocultured with spleen macrophages or HS-P cells, a cell line of rat macrophage origin, M-CSF is upregulated relative to GM-CSF and MIF in both cell types. Anaemia occurs in ILS-, but not WS-infected, rats. Treatment of spleen macrophages or HS-P cells cocultured with ILS with cobalt chloride, which mimics the effects of anaemia-induced hypoxia, led to downregulation of M-CSF mRNA levels, upregulation of GM-CSF and MIF, and an increase in phagocytic activity. However, the effect of cobalt chloride on spleen macrophages and HS-P cells cocultured with WS was restricted. These results suggest that anaemia-induced hypoxia in ILS-infected rats stimulates the immune system and activates macrophages.

15719. **Rodgers, J., McCabe, C., Gettinby, G., Bradley, B., Condon, B. & Kennedy, P. G., 2011.** Magnetic resonance imaging to assess blood-brain barrier damage in murine trypanosomiasis. *American Journal of Tropical Medicine & Hygiene*, **84** (2): 344-350.

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` The ability of trypanosomes to invade the brain and induce an inflammatory reaction is well-recognized. This study uses magnetic resonance imaging (MRI) in conjunction with a murine model of central nervous system (CNS) stage trypanosomiasis to investigate this phenomenon at the level of the blood-brain barrier (BBB). Mice were scanned before and after administration of the contrast agent. Signal enhancement maps were generated, and the percentage signal change was calculated. The severity of the neuroinflammation was also assessed. Statistical analysis of the signal change data revealed a significantly ($P = 0.028$) higher signal enhancement in mice at 28 days post-infection (least squares mean = 26.709) compared with uninfected animals (6.298), indicating the presence of BBB impairment. Leukocytes were found in the meninges and perivascular space of some blood vessels in the infected mice. This study shows that the integrity of the BBB is compromised during CNS stage trypanosomiasis and that the impairment does not correlate with inflammatory cell infiltration.

15720. **Schwede, A., Jones, N., Engstler, M. & Carrington, M.,** 2011. The VSG C-terminal domain is inaccessible to antibodies on live trypanosomes. *Molecular & Biochemical Parasitology,* **175** (2): 201-204.

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In the mammalian host, the *Trypanosoma brucei* cell surface is covered with a densely packed protein coat of a single protein, the variant surface glycoprotein (VSG). The VSG is believed to shield invariant surface proteins from host antibodies but there is limited information on how far antibodies can penetrate into the VSG monolayer. Here, the VSG surface coat was probed to determine whether it acts as a barrier to binding of antibodies to the membrane proximal VSG C-terminal domain. The binding of C-terminal domain antibodies to VSG221 or VSG118 was compared with antibodies recognizing the cognate whole VSGs. The C-terminal VSG domain was inaccessible to antibodies on live cells but not on fixed cells. This provides further evidence that the VSG coat acts as a barrier and protects the cell from antibodies that would otherwise bind to some of the other externally disposed proteins.

15721. **Wei, G., Bull, H., Zhou, X. & Tabel, H., 2011.** Intradermal infections of mice by low numbers of African trypanosomes are controlled by innate resistance but enhance susceptibility to reinfection. *Journal of Infectious Diseases*, **203** (3): 418-429.

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Antibodies are required to control blood-stage forms of African trypanosomes in humans and animals. Here, we report that intradermal infections by low numbers of African trypanosomes are controlled by innate resistance but prime the adaptive immune response to increase susceptibility to a subsequent challenge. Mice were found 100 times more resistant to intradermal infections by *Trypanosoma congolense* or *Trypanosoma brucei* than to intraperitoneal infections. B cell-deficient and RAG2(-/-) mice are as resistant as wild-type mice to intradermal infections, whereas inducible nitric oxide synthase (iNOS)(-/-) mice and wild-type mice treated with antibody to tumor necrosis factor (TNF) alpha are more susceptible. We conclude that primary intradermal infections with low numbers of parasites are controlled by innate defence mediated by induced nitric oxide (NO). CD1d(-/-) and major histocompatibility complex (MHC) class II(-/-) mice are more resistant than wild-type mice to primary intradermal infections. Trypanosome-specific spleen cells, as shown by cytokine production, are primed as early as 24 h after intradermal infection. Infecting mice intradermally with low numbers of parasites, or injecting them intradermally with a trypanosomal lysate, makes mice more susceptible to an intradermal challenge. We suggest that intradermal infections with low numbers of trypanosomes or injections with trypanosomal lysates prime the adaptive immune system to suppress protective immunity to an intradermal challenge.

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15722. **Ang, K. K., Ratnam, J., Gut, J., Legac, J., Hansell, E., Mackey, Z. B., Skrzypczynska, K. M., Debnath, A., Engel, J. C., Rosenthal, P. J., McKerrow, J. H., Arkin, M. R. & Renslo, A. R., 2011.** Mining a cathepsin inhibitor library for new antiparasitic drug leads. *PLoS Neglected Tropical Diseases*, **5** (5): e1023.

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The targeting of parasite cysteine proteases with small molecules is emerging as a possible approach to treat tropical parasitic diseases such as sleeping sickness, Chagas disease, and malaria. The homology of parasite cysteine proteases to the human cathepsins suggests that inhibitors originally developed for the latter may be a source of promising lead compounds for the former. We describe here the screening of a unique approximately 2 100-member cathepsin inhibitor library against five parasite cysteine proteases thought to be relevant in tropical parasitic diseases. Compounds active against parasite enzymes were subsequently screened against cultured *Plasmodium falciparum, Trypanosoma brucei brucei* and/or *Trypanosoma cruzi* parasites and evaluated for cytotoxicity to mammalian cells. The end products of this effort include the identification of sub-micromolar cell-active leads as well as the elucidation of structure-activity trends that can guide further optimization efforts.

15723. **Bahar, M., Deng, Y., Zhu, X., He, S., Pandharkar, T., Drew, M. E., Navarro-Vazquez, A., Anklin, C., Gil, R. R., Doskotch, R. W., Werbovetz, K. A. & Kinghorn, A. D., 2011.** Potent antiprotozoal activity of a novel semi-synthetic berberine derivative. *Bioorganic & Medicinal Chemistry Letters*, **21** (9): 2606-2610.

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Treatment of diseases such as African sleeping sickness and leishmaniasis often depends on relatively expensive or toxic drugs, and resistance to current chemotherapeutics is an issue in treating these diseases and malaria. In this study, a new semi-synthetic berberine analogue, 5,6-didehydro-8,8-diethyl-13-oxodihydroberberine chloride, showed nanomolar level potency against *in vitro* models of leishmaniasis, malaria, and trypanosomiasis as well as activity in an *in vivo* visceral leishmaniasis model. Since the synthetic starting material, berberine hemisulphate, is inexpensive, 8,8-dialkyl-substituted analogues of berberine may lead to a new class of affordable antiprotozoal compounds.

15724. **Ding, D., Meng, Q., Gao, G., Zhao, Y., Wang, Q., Nare, B., Jacobs, R., Rock, F., Alley, M. R., Plattner, J. J., Chen, G., Li, D. & Zhou, H., 2011.** Design, synthesis, and structure-activity relationship of *Trypanosoma brucei* leucyl-tRNA synthetase inhibitors as antitrypanosomal agents. *Journal of Medicinal Chemistry*, **54** (5): 1276-1287.

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African trypanosomiasis, caused by the protozoal pathogen *Trypanosoma brucei* (*T. brucei*), is one of the most neglected tropical diseases that are in great need of new drugs. We report the design and synthesis of *T. brucei* leucyl-tRNA synthetase (*Tb*LeuRS) inhibitors and their structure--activity relationship. Benzoxaborole was used as the core structure and C6 was modified to achieve improved affinity based on docking results that showed further binding space at this position. Indeed, compounds with C7 substitutions showed diminished activity due to clash with the eukaryote specific I4ae helix while substitutions at C6 gave enhanced affinity. *Tb*LeuRS inhibitors with IC_{50} as low as 1.6 μM were discovered, and the structure-activity relationship was discussed. The most potent enzyme inhibitors also showed excellent *T. brucei* parasite growth inhibition activity. This is the first time that *Tb*LeuRS inhibitors are reported, and this study suggests that leucyl-tRNA synthetase (LeuRS) could be a

potential target for antiparasitic drug development.

15725. **Eberle, C., Lauber, B. S., Fankhauser, D., Kaiser, M., Brun, R., Krauth-Siegel, R. L. & Diederich, F., 2011.** Improved inhibitors of trypanothione reductase by combination of motifs: synthesis, inhibitory potency, binding mode, and antiprotozoal activities. *ChemMedChem*, **6** (2): 292-301.

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Trypanothione reductase (TR) is an essential enzyme in the trypanothione-based redox metabolism of trypanosomatid parasites. This system is absent in humans and, therefore, offers a promising target for the development of selective new drugs against African sleeping sickness and Chagas disease. Over the past two decades, a variety of nonpeptidic small-molecule ligands of the parasitic enzyme were discovered. A current goal is to decipher the binding mode of these known inhibitors in order to optimize their structures. We analysed the binding mode of recently reported 1-(1-(benzo[b]thiophen-2-yl)cyclohexyl)piperidine (BTCP) analogues using computer modelling methods. This led us to conclude that the analogues occupy a different region of the active site than the diaryl sulphide-based class of inhibitors. A combination of the two motifs significantly increased affinity for the enzyme compared to the respective parent compounds. The newly synthesized conjugates exhibit K_{ic} values for TR as low as 0.51+/-0.1 μM and high selectivity for the parasitic enzyme over the related human glutathione reductase (hGR), as was predicted by our molecular modelling studies. *In vitro* studies showed IC_{50} values in the low micromolar to submicromolar range against *Trypanosoma brucei rhodesiense*, often in combination with low cytotoxicity against mammalian cells. Interestingly, even stronger activities were found against *Plasmodium falciparum.*

15726. **Farahat, A. A., Paliakov, E., Kumar, A., Barghash, A. E., Goda, F. E., Eisa, H. M., Wenzler, T., Brun, R., Liu, Y., Wilson, W. D. & Boykin, D. W., 2011.** Exploration of larger central ring linkers in furamidine analogues: synthesis and evaluation of their DNA binding, antiparasitic and fluorescence properties. *Bioorganic & Medicinal Chemistry*, **19** (7): 2156-2167.

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The effects of replacing the central furan ring of furamidine with indole and benzimidazole on their DNA binding affinity, antiparasitic activity and fluorescence are reported. The bis-cyanophenylindoles required to make the corresponding amidines were prepared by sequential Stille and/or Suzuki coupling reactions. The bis-cyanophenylbenzimidazoles were obtained by coupling 4-cyanobenzaldehydes with the appropriate cyano substituted phenylenediamine. The bis-nitriles were converted to the diamidines by reaction with LiN[Si(CH_3)3] or by Pinner methodology. Specifically, we have prepared new series of 2,6- and 2,5-diaryl indoles and the related benzimidazoles. The new compounds bind in the DNA minor groove in DNA AT base pair sequences and eight of the ten new analogues exhibit DeltaT_m values comparable to or higher than that of furamidine. Six of ten of the new compounds exhibit lower IC_{50} values against *Trypanosoma brucei rhodesiense*

(T. b. r.) and eight of ten exhibit lower IC_{50} values against *Plasmodium falciparum* (P. f.) than furamidine. Four of the ten show greater efficacy than furamidine in the rigorous T. b. r. STIB900 mouse model for African trypanosomiasis. Generally, the fluorescence properties of the new analogues are similar to that of DAPI.

15727. **Garcia, I., Fall, Y., Gomez, G. & Gonzalez-Diaz, H., 2011.** First computational chemistry multi-target model for anti-Alzheimer, anti-parasitic, anti-fungi, and anti-bacterial activity of GSK-3 inhibitors *in vitro, in vivo*, and in different cellular lines. *Molecular Diversity*, **15** (2): 561-567.

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In the work described here, we developed the first multi-target quantitative structure-activity relationship (QSAR) model able to predict the results of 42 different experimental tests for GSK-3 inhibitors with heterogeneous structural patterns. GSK-3beta inhibitors are interesting candidates for developing anti-Alzheimer compounds. GSK-3beta are also of interest as anti-parasitic compounds active against *Plasmodium falciparum, Trypanosoma brucei*, and *Leishmania donovani*- the causative agents for malaria, African trypanosomiasis and leishmaniasis. The MARCH-INSIDE technique was used to quickly calculate total and local polarizability, n-octanol/water partition coefficients, refractivity, van der Waals area and electronegativity values to 4 508 active/non-active compounds as well as the average values of these indexes for active compounds in 42 different biological assays. Both the individual molecular descriptors and the average values for each test were used as input for a linear discriminant analysis (LDA). We discovered a classification function which used in training series correctly classifies 873 out of 1 218 GSK-3 cases of inhibitors (97.4 percent) and 2 140 out of 2 163 cases of non-active compounds (86.1 percent) in the 42 different tests. In addition, the model correctly classifies 285 out of 406 GSK-3 inhibitors (96.3 percent) and 710 out of 721 cases of non-active compounds (85.4 percent) in external validation series. The result is important because, for the first time, we can use a single equation to predict the results of heterogeneous series of organic compounds in 42 different experimental tests instead of developing, validating, and using 42 different QSAR models. Lastly, a double ordinate Cartesian plot of cross-validated residuals (first ordinate), standard residuals (second ordinate), and leverages (abscissa) defined the domain of applicability of the model as a squared area within +/-2 band for residuals and a leverage threshold of h = 0.0044.

15728. **Haanstra, J. R., Kerkhoven, E. J., van Tuijl, A., Blits, M., Wurst, M., van Nuland, R., Albert, M. A., Michels, P. A., Bouwman, J., Clayton, C., Westerhoff, H. V. & Bakker, B. M.,** 2011. A domino effect in drug action: from metabolic assault towards parasite differentiation. *Molecular Microbiology,* **79** (1): 94-108.

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Awareness is growing that drug target validation should involve systems analysis of cellular networks. There is less appreciation, though, that the composition of networks may change in response to drugs. If the response is homeostatic (e.g. through upregulation of the

target protein), this may neutralize the inhibitory effect. In this scenario the effect on cell growth and survival would be less than anticipated based on affinity of the drug for its target. Glycolysis is the sole free-energy source for the deadly parasite *Trypanosoma brucei* and is therefore a possible target pathway for anti-trypanosomal drugs. Plasma membrane glucose transport exerts high control over trypanosome glycolysis and hence the transporter is a promising drug target. Here we show that at high inhibitor concentrations, inhibition of trypanosome glucose transport causes cell death. Most interestingly, sub lethal concentrations initiate a domino effect in which network adaptations enhance inhibition. This happens via (i) metabolic control exerted by the target protein, (ii) decreases in mRNAs encoding the target protein and other proteins in the same pathway, and (iii) partial differentiation of the cells leading to (low) expression of immunogenic insect-stage coat proteins. We discuss how these "anti-homeostatic" responses together may facilitate killing of parasites at an acceptable drug dosage.

15729. **Helena, K. J., N'Da, D. D., Johansson, C. C., Breytenbach, J. C. & Ashton, M., 2010.** Effects of oral administration of synthesized delta-amides of eflornithine in the rat. *Arzneimittelforschung*, **60** (11): 682-688.

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The purpose of this study was to synthesize a series of delta-amide derivatives of the antitrypanosomal drug eflornithine (2,5-diamino-2-(difluoromethyl)pentanoic acid hydrochloride, DMFO, CAS 70052-12-9), to determine their physicochemical properties and to assess whether they convert to eflornithine *in vivo* and if so, whether higher systemic exposure to eflornithine could be achieved by increased intestinal absorption, suggesting an oral treatment to be possible. The derivatives were synthesized by amidation of eflornithine on its delta-amino group using acyl chlorides. The partition coefficients (log D, pH = 7.4) were found to be between -0.78 +/- 1.07 and -0.07 +/- 1.08 while the aqueous solubility (Sw), which was determined in phosphate buffered solution (pH 7.4), ranged from 11.13 +/- 0.32 to 28.74 +/- 0.36 mg/mL. The synthesized compounds were thus mostly more lipophilic than eflornithine itself (log D = -0.98 +/- 0.88, Sw = 34.96 +/- 0.37 mg/mL). The intestinal absorption was assessed by plasma analysis after oral administration of each compound to Sprague-Dawley rats. The biological data revealed that the derivatives were either not absorbed from the gastro-intestinal tract or not metabolized into eflornithine as no parent drug was detected in the plasma.

15730. **Herculano, R. D., Pavinatto, F. J., Caseli, L., D'Silva, C. & Oliveira, O. N., Jr., 2011.** The lipid composition of a cell membrane modulates the interaction of an antiparasitic peptide at the air-water interface. *Biochimica et Biophysica Acta*, **1808** (7): 1907-1912.

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The antiparasitic property of peptides is believed to be associated with their interactions with the protozoan membrane, which calls for research on the identification of membrane sites capable of peptide binding. In this study we investigated the interaction of a lipophilic

glutathioine peptide known to be effective against the African sleeping sickness (ASS - African trypanosomiasis) and cell membrane models represented by Langmuir monolayers. It is shown that even small amounts of the peptide affect the monolayers of some phospholipids and other lipids, which points to a significant interaction. The latter did not depend on the electrical charge of the monolayer-forming molecules but the peptide action was particularly distinctive for cholesterol + sphingomyelin monolayers that roughly resemble rafts on a cell membrane. Using *in situ* polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS), we found that the orientation of the peptide is affected by the phospholipids and dioctadecyldimethylammonium bromide (DODAB), but not in monolayers comprising cholesterol + sphingomyelin. In this mixed monolayer resembling rafts, the peptide still interacts and has some induced order, probably because the peptide molecules are fitted together into a compact monolayer. Therefore, the lipid composition of the monolayer modulates the interaction with the lipophilic glutathioine peptide, and this may have important implications in understanding how the peptide acts on specific sites of the protozoan membrane.

15731. **Ismail, M. A., Bialy, S. A., Brun, R., Wenzler, T., Nanjunda, R., Wilson, W. D. & Boykin, D. W., 2011.** Dicationic phenyl-2,2'-bichalcophenes and analogues as antiprotozoal agents. *Bioorganic & Medicinal Chemistry*, **19** (2): 978-984.

Department of Chemistry, Mansoura University, Egypt.

A series of phenyl-2,2'-bichalcophene diamidines 1a-h were synthesized from the corresponding dinitriles either via a direct reaction with LiN(TMS), followed by deprotection with ethanolic HCl or through the bis-O-acetoxyamidoxime followed by hydrogenation in acetic acid and EtOH over Pd-C. These diamidines show a wide range of DNA affinities as judged from their DeltaT_m values which are remarkably sensitive to replacement of a furan unit with a thiophene one. These differences are explained in terms of the effect of subtle changes in geometry of the diamidines on binding efficacy. Five of the eight compounds were highly active (below 6 nM IC) *in vitro* against *Trypanosoma brucei rhodesiense* and four gave IC values less than 7 nM against *Plasmodium falciparum.* Only one of the compounds was as effective as reference compounds in the *T. b. rhodesiense* mouse model for the acute phase of African trypanosomiasis.

15732. **Jeganathan, S., Sanderson, L., Dogruel, M., Rodgers, J., Croft, S. & Thomas, S. A., 2011.** The distribution of nifurtimox across the healthy and trypanosome-infected murine blood-brain and blood-cerebrospinal fluid barriers. *Journal Pharmacology & Experimental Therapeutics*, **336** (2): 506-515.

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Nifurtimox, an antiparasitic drug, is used to treat American trypanosomiasis (Chagas disease) and has shown promise in treating central nervous system (CNS)-stage human African trypanosomiasis (HAT; sleeping sickness). In combination with other antiparasitic drugs, the efficacy of nifurtimox against HAT improves, although why this happens is unclear. Studying how nifurtimox crosses the blood-brain barrier (BBB) and reaches the CNS may clarify this issue and is the focus of this study. To study the interaction of nifurtimox with the blood-CNS

interfaces, we used the in situ brain/choroid plexus perfusion technique in healthy and trypanosome-infected mice and the isolated incubated choroid plexus. Results revealed that nifurtimox could cross the healthy and infected blood-brain and blood-cerebrospinal fluid (CSF) barriers (K_{in} brain parenchyma was 50.8 +/- 9.0 mµl. min^{-1}. g^{-1}). In fact, the loss of barrier integrity associated with trypanosome infection failed to change the distribution of [^{3}H] nifurtimox to any significant extent, suggesting there is not an effective paracellular barrier for [^{3}H] nifurtimox entry into the CNS. Our studies also indicate that [^{3}H] nifurtimox is not a substrate for P-glycoprotein, an efflux transporter expressed on the luminal membrane of the BBB. However, there was evidence of [^{3}H] nifurtimox interaction with transporters at both the blood-brain and blood-CSF barriers as demonstrated by cross-competition studies with the other antitrypanosomal agents, eflornithine, suramin, melarsoprol, and pentamidine. Consequently, CNS efficacy may be improved with nifurtimox-pentamidine combinations, but over time may be reduced when nifurtimox is combined with eflornithine, suramin, or melarsoprol.

15733. **Kirmizibekmez, H., Atay, I., Kaiser, M., Brun, R., Cartagena, M. M., Carballeira, N. M., Yesilada, E. & Tasdemir, D.,** 2011. Antiprotozoal activity of *Melampyrum arvense* and its metabolites. *Phytotherapy Research,* **25** (1): 142-146.

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An activity guided isolation of the H_2O subextract of the crude extract of *Melampyrum arvense* L. afforded iridoid glucosides: aucubin, melampyroside, mussaenoside, mussaenosidic acid, 8-epi-loganin; flavonoids: apigenin, luteolin, luteolin 7-O-beta-glucopyranoside; a lignan glycoside dehydrodiconiferyl alcohol 9-O-beta-glucopyranoside; and benzoic acid beta-sitosterol and a fatty acid mixture were identified as the active principles of the $CHCl_3$ subextract. The structures of the isolates were elucidated by spectroscopic methods, while the composition of the fatty acid mixture was identified by GC-MS after methylation. Luteolin appeared as the most active compound against *Trypanosoma brucei rhodesiense* and *Leishmania donovani* (IC_{50} values 3.8 and 3.0 µg/mL). Luteolin 7-O-beta-glucopyranoside displayed the best antiplasmodial activity against *Plasmodium falciparum* (IC_{50} value 2.9 µg/mL). This is the first detailed phytochemical study on Turkish *M. arvense* and the first report of the antiprotozoal effect of *Melampyrum* species and its constituents.

15734. **Konig, J., Wyllie, S., Wells, G., Stevens, M. F., Wyatt, P. G. & Fairlamb, A. H., 2011.** Antitumor quinol PMX464 is a cytocidal anti-trypanosomal inhibitor targeting trypanothione metabolism. *Journal of Biological Chemistry*, **286** (10): 8523-8533.

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Better drugs are urgently needed for the treatment of African sleeping sickness. We tested a series of promising anticancer agents belonging to the 4-substituted 4-hydroxycyclohexa-2,5-dienones class ("quinols") and identified several with potent trypanocidal activity (EC_{50} < 100 nM). In mammalian cells, quinols are proposed to inhibit the thioredoxin/thioredoxin reductase system, which is absent from trypanosomes. Studies with the prototypical 4-benzothiazole-substituted quinol, PMX464, established that PMX464 is

rapidly cytocidal, similar to the arsenical drug, melarsen oxide. Cell lysis by PMX464 was accelerated by addition of sublethal concentrations of glucose oxidase implicating oxidant defences in the mechanism of action. Whole cells treated with PMX464 showed a loss of trypanothione (TSH_2), a unique dithiol in trypanosomes, and tryparedoxin peroxidase (TryP), a 2-Cys peroxiredoxin similar to mammalian thioredoxin peroxidase. Enzyme assays revealed that TSH_2, TryP, and a glutathione peroxidase-like tryparedoxin-dependent peroxidase were inhibited in time- and concentration-dependent manners. The inhibitory activities of various quinol analogues against these targets showed a good correlation with growth inhibition of *Trypanosoma brucei.* The monothiols glutathione and L-cysteine bound in a 2:1 ratio with PMX464 with K_d values of 6 and 27 mμM, respectively, whereas TSH_2 bound more tightly in a 1:1 ratio with a K_d value of 430 nM. Overexpression of trypanothione synthetase in *T. brucei* decreased sensitivity to PMX464 indicating that the key metabolite TSH_2 is a target for quinols. Thus, the quinol pharmacophore represents a novel lead structure for the development of a new drug against African sleeping sickness.

15735. **Lepesheva, G. I. & Waterman, M. R., 2011.** Sterol 14alpha-demethylase (CYP51) as a therapeutic target for human trypanosomiasis and leishmaniasis. *Current Topics in Medicinal Chemistry*. **E publication ahead of print, May 26.**

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Pathogenic protozoa threaten lives of several hundred million people throughout the world and are responsible for large numbers of deaths globally. The parasites are transmitted to humans by insect vectors, more than a hundred of infected mammalian species forming reservoir. With human migrations, HIV-coinfections, and blood bank contamination the diseases are now spreading beyond the endemic tropical countries, being found in all parts of the world including the USA, Canada and Europe. In spite of the widely appreciated magnitude of this health problem, current treatment for sleeping sickness (*Trypanosoma brucei*), Chagas disease (*Trypanosoma cruzi*) and leishmaniasis (*Leishmania* spp.) remains unsatisfactory. The drugs are decades old, their efficacy and safety profiles are unacceptable. This review describes sterol 14 alpha-demethylase, an essential enzyme in sterol biosynthesis in eukaryotes and clinical target for antifungal azoles, as a promising target for antiprotozoan chemotherapy. While several antifungal azoles have been proven active against Trypanosomatidae and are under consideration as antiprotozoan agents, crystal structures of sterol 14 alpha-demethylases from three protozoan pathogens, *Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania infantum* provide the basis for the development of new, highly potent and pathogen-specific drugs with rationally optimized pharmacological properties.

15736. **Marino, K., Guther, M. L., Wernimont, A. K., Qiu, W., Hui, R. & Ferguson, M. A., 2011.** Characterization, localization, essentiality and high-resolution crystal structure of glucosamine 6-phosphate N-acetyltransferase from *Trypanosoma brucei*. *Eukaryotic Cell,* **10**(7): 985-997.

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A gene predicted to encode *Trypanosoma brucei* glucosamine 6-phosphate N-acetyltransferase (EC 2.3.1.4, *Tb*GNA1) was cloned and expressed in *Escherichia coli*. The recombinant protein was enzymatically active and its high-resolution crystal structure obtained at 1.86 A. Endogenous *Tb*GNA1 protein was localized to the peroxisome-like microbody, the glycosome. A bloodstream form *T. brucei* GNA1 conditional null mutant was constructed and shown to be unable to sustain growth *in vitro* under non-permissive conditions, demonstrating that there are no metabolic or nutritional routes to UDP-GlcNAc other than via GlcNAc-6-phosphate. Analysis of the protein glycosylation phenotype of the *Tb*GNA1 mutant under non-permissive conditions revealed that poly-N-acetyllactosamine structures were greatly reduced in the parasite and that the glycosylation profile of the principal parasite surface coat component, the variant surface glycoprotein (VSG) was modified. The significance of results and the potential of *Tb*GNA1 as a novel drug target for African sleeping sickness are discussed.

15737. **Mercer, L., Bowling, T., Perales, J., Freeman, J., Nguyen, T., Bacchi, C., Yarlett, N., Don, R., Jacobs, R. & Nare, B., 2011.** 2,4-Diaminopyrimidines as potent inhibitors of Trypanosoma brucei and identification of molecular targets by a chemical proteomics approach. *PLoS Neglected Tropical Diseases*, **5** (2): e956.

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There is an urgent need to develop new, safe and effective treatments for human African trypanosomiasis (HAT) because current drugs have extremely poor safety profiles and are difficult to administer. Here we report the discovery of 2,4-diaminopyrimidines, exemplified by 4-[4-amino-5-(2-methoxy-benzoyl)-pyrimidin-2-ylamino]-piperidine-1-carboxylic acid phenylamide (SCYX-5070), as potent inhibitors of *Trypanosoma brucei* and the related trypanosomatid protozoans *Leishmania* spp. In this work we show that loss of *T. brucei* viability following SCYX-5070 exposure was dependent on compound concentration and incubation time. Pulse incubation of *T. brucei* with SCYX-5070 demonstrates that a short period of exposure (10-12 hrs) is required to produce irreversible effects on survival or commit the parasites to death. SCYX-5070 cured an acute trypanosomiasis infection in mice without exhibiting signs of compound related acute or chronic toxicity. To identify the molecular target(s) responsible for the mechanism of action of 2,4-diaminopyrimidines against trypanosomatid protozoa, a representative analogue was immobilized on a solid matrix (sepharose) and used to isolate target proteins from parasite extracts. Mitogen-activated protein kinases (MAPKs) and cdc2-related kinases (CRKs) were identified as the major proteins specifically bound to the immobilized compound, suggesting their participation in the pharmacological effects of 2,4-diaminopyrimidines against trypanosomatid protozoan parasites. Results show that 2,4-diaminopyrimidines have a good *in vitro* and *in vivo* pharmacological profile against trypanosomatid protozoans and that MAPKs and CRKs are potential molecular targets of these compounds. The 2,4-diminipyrimidines may serve as suitable leads for the development of novel treatments for HAT.

15738. **Nieto, L., Mascaraque, A., Miller, F., Glacial, F., Rios Martinez, C., Kaiser, M., Brun, R. & Dardonville, C., 2011.** Synthesis and antiprotozoal activity of N-alkoxy analogues of the trypanocidal lead compound

4,4'-bis(imidazolinylamino)diphenylamine with improved human blood-brain barrier permeability. *Journal of Medicinal Chemistry*, **54** (2): 485-494.

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To improve the blood-brain barrier permeability of the trypanocidal lead compound 4,4'-bis(imidazolinylamino)diphenylamine, five N-alkoxy analogues were synthesized from bis(4-isothiocyanatophenyl)amine and N-alkoxy-N-(2-aminoethyl)-2-nitrobenzenesulfonamides following successive chemical reactions in just one reactor ("one-pot procedure"). This involved: (a) formation of a thiourea intermediate, (b) removal of the amine protecting groups, and (c) intramolecular cyclization. The blood-brain barrier permeability of the compounds determined *in vitro* by transport assays through the hCMEC/D3 human cell line, a well-known and characterized human cellular blood-brain barrier model, showed that the N-hydroxy analogue 16 had enhanced blood-brain barrier permeability compared with the unsubstituted lead compound. Moreover, this compound displayed low micromolar IC_{50} against *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum* and moderate activity by intraperitoneal administration in the STIB900 murine model of acute sleeping sickness.

15739. **Oduor, R. O., Ojo, K. K., Williams, G. P., Bertelli, F., Mills, J., Maes, L., Pryde, D. C., Parkinson, T., Van Voorhis, W. C. & Holler, T. P., 2011.** *Trypanosoma brucei* glycogen synthase kinase-3, a target for anti-trypanosomal drug development: a public-private partnership to identify novel leads. *PLoS Neglected Tropical Diseases*, **5** (4): e1017.

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Trypanosoma brucei, the causative agent of human African trypanosomiasis (HAT), expresses two proteins with homology to human glycogen synthase kinase 3 beta (HsGSK-3) designated *Tbru*GSK-3 short and *Tbru*GSK-3 long. *Tbru*GSK-3 short has previously been validated as a potential drug target and since this enzyme has also been pursued as a human drug target, a large number of inhibitors are available for screening against the parasite enzyme. A collaborative industrial/academic partnership facilitated by the World Health Organisation Tropical Diseases Research Division (WHO TDR) was initiated to stimulate research aimed at identifying new drugs for treating HAT. A subset of over 16 000 inhibitors of HsGSK-3 beta from the Pfizer compound collection was screened against the shorter of two orthologues of *Tbru*GSK-3. The resulting active compounds were tested for selectivity versus HsGSK-3beta and a panel of human kinases, as well as *in vitro* anti-trypanosomal activity. Structural analysis of the human and trypanosomal enzymes was also performed. We identified potent and selective compounds representing potential attractive starting points for a drug discovery programme. Structural analysis of the human and trypanosomal enzymes also revealed hypotheses for further improving selectivity of the compounds.

15740. **Oguri, H., Hiruma, T., Yamagishi, Y., Oikawa, H., Ishiyama, A., Otoguro, K., Yamada, H. & Omura, S., 2011.** Generation of anti-trypanosomal agents through concise synthesis and structural diversification of sesquiterpene analogues. *Journal of the American Chemical Society*, **133** (18): 7096-7105.

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To access high-quality small-molecule libraries to screen lead candidates for neglected diseases exemplified by human African trypanosomiasis, we sought to develop a synthetic process that would produce collections of cyclic scaffolds relevant to an assortment of natural products exhibiting desirable biological activities. By extracting the common structural features among several sesquiterpenes, including artemisinin, anthecularin, and transtaganolides, we designed six types of scaffolds with systematic structural variations consisting of three types of stereochemical relationships on the sp_3 ring-junctions and two distinct arrays of tricyclic frameworks. A modular and stereodivergent assembly of dienynes exploiting a versatile manifold produced a series of cyclization precursors. Divergent cyclizations of the dienynes employing tandem ring-closing metathesis reactions overrode variant reactivities of the cyclization precursors, leading to the six canonical sets of the tricyclic scaffolds incorporating a diene group. Screenings of trypanosomal activities of the canonical sets, as well as regio- and stereoisomers of the tricyclic dienes, allowed generation of several anti-trypanosomal agents defining the three-dimensional shape of the pharmacophore. The candidate tricyclic dienes were selected by primary screenings and further subjected to installation of a peroxide bridge, which generated artemisinin analogues that exhibited potent *in vitro* anti-trypanosomal activities comparable or even superior to those of artemisinin and the approved drugs, suramin and eflornithine.

15741. **Pereira, C. A., Bouvier, L. A., Camara Mde, L. & Miranda, M. R., 2011.** Singular features of trypanosomatids' phosphotransferases involved in cell energy management. *Enzyme Research*, **2011**: 576483.

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Trypanosomatids are responsible for economically important veterinary affections and severe human diseases. In Africa, *Trypanosoma brucei* causes sleeping sickness or African trypanosomiasis, while in South America, *Trypanosoma cruzi* is the etiological agent of Chagas disease. These parasites have complex life cycles which involve a wide variety of environments with very different compositions, physicochemical properties, and availability of metabolites. As the environment changes there is a need to maintain the nucleoside homeostasis, requiring a quick and regulated response. Most of the enzymes required for energy management are phosphotransferases. These enzymes present a nitrogenous group or a phosphate as acceptors, and the most clear examples are arginine kinase, nucleoside diphosphate kinase, and adenylate kinase. *Trypanosoma* and *Leishmania* have the largest number of phosphotransferase isoforms ever found in a single cell; some of them are absent in mammals, suggesting that these enzymes are required in many cellular compartments associated to different biological processes. The presence of such a number of phosphotransferases supports the hypothesis of the existence of an intracellular enzymatic phosphotransfer network that communicates the spatially separated intracellular ATP consumption and production processes. All these unique features make phosphotransferases a

promising start point for rational drug design for the treatment of human trypanosomiasis.

15742. **Reid, C. S., Patrick, D. A., He, S., Fotie, J., Premalatha, K., Tidwell, R. R., Wang, M. Z., Liu, Q., Gershkovich, P., Wasan, K. M., Wenzler, T., Brun, R. & Werbovetz, K. A., 2011.** Synthesis and antitrypanosomal evaluation of derivatives of N-benzyl-1,2-dihydroquinolin-6-ols: effect of core substitutions and salt formation. *Bioorganic & Medicinal Chemistry*, **19** (1): 513-523.

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Analogues of the trypanocidal lead compound 1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate were prepared to extend the structure-activity relationship in this series of molecules, improve the *in vivo* antitrypanosomal activity of the lead, and determine whether ester prodrugs are needed to overcome the instability of the dihydroquinolin-6-ols. Two of the most active compounds identified in this study were 1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride and 1-(2-methoxy)benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride. These stable solids possessed low nanomolar IC_{50} values against *Trypanosoma brucei rhodesiense* STIB900 *in vitro* and provided cures in an early treatment acute mouse model of African trypanosomiasis when given ip at 50mg/kg/day for four consecutive days.

15743. **Roy Chowdhury, A., Bakshi, R., Wang, J., Yildirir, G., Liu, B., Pappas-Brown, V., Tolun, G., Griffith, J. D., Shapiro, T. A., Jensen, R. E. & Englund, P. T., 2010.** The killing of African trypanosomes by ethidium bromide. *PLoS Pathogens*, **6** (12): e1001226.

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Introduced in the 1950s, ethidium bromide (EB) is still used as an anti-trypanosomal drug for African cattle although its mechanism of killing has been unclear and controversial. EB has long been known to cause loss of the mitochondrial genome, named kinetoplast DNA (kDNA), a giant network of interlocked minicircles and maxicircles. However, the existence of viable parasites lacking kDNA (dyskinetoplastic) led many to think that kDNA loss could not be the mechanism of killing. When recent studies indicated that kDNA is indeed essential in bloodstream trypanosomes and that dyskinetoplastic cells survive only if they have a compensating mutation in the nuclear genome, we investigated the effect of EB on kDNA and its replication. We here report some remarkable effects of EB. Using EM and other techniques, we found that binding of EB to network minicircles is low, probably because of their association with proteins that prevent helix unwinding. In contrast, covalently-closed minicircles that had been released from the network for replication bind EB extensively, causing them, after isolation, to become highly supertwisted and to develop regions of left-handed Z-DNA (without EB, these circles are fully relaxed). *In vivo*, EB causes helix distortion of free minicircles, preventing replication initiation and resulting in kDNA loss and cell death. Unexpectedly, EB also kills dyskinetoplastic trypanosomes, lacking kDNA, by inhibiting nuclear replication. Since the effect on kDNA occurs at a >10-fold lower EB

concentration than that on nuclear DNA, we conclude that minicircle replication initiation is likely EB's most vulnerable target, but the effect on nuclear replication may also contribute to cell killing.

15744. **Schmidt, T. J., Kaiser, M. & Brun, R., 2011.** Complete structural assignment of serratol, a cembrane-type diterpene from *Boswellia serrata*, and evaluation of its antiprotozoal activity. *Planta Medica*, **77** (8): 849-850.

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From the dichloromethane extract obtained from the gum resin of *Boswellia serrata* Roxb. (Burseraceae), a well-known medicinal plant resin ("Indian Olibanum"), the cembrane-type diterpene serratol was isolated in high yield. Its structure, previously reported without clear specification of double-bond geometry and without specification of stereochemistry, was reanalysed by means of spectroscopic measurements and unambiguously assigned as S(-)-cembra-3 E,7 E,11 E-triene-1-ol. Full assignment of all NMR data is reported for the first time. The compound was found to be identical with a cembrenol previously isolated from *B. carteri.* Serratol was tested for *in vitro* activity against four protozoan human pathogens, namely, *Trypanosoma brucei rhodesiense* (East African human trypanosomiasis, sleeping sickness), *T. cruzi* (Chagas disease), *Leishmania donovani* (Kala-Azar), and *Plasmodium falciparum* (tropical malaria). It was found active against *Trypanosoma brucei* and *Plasmodium falciparum.* These activities were 10- 15-fold higher than its cytotoxicity against rat skeletal myoblasts. While some reports exist on potential anti-inflammatory activity of *Boswelli*a diterpenes, this is the first report on antiprotozoal activity of such a compound.

15745. **Shibata, S., Gillespie, J. R., Kelley, A. M., Napuli, A. J., Zhang, Z., Kovzun, K. V., Pefley, R. M., Lam, J., Zucker, F. H., Van Voorhis, W. C., Merritt, E. A., Hol, W. G., Verlinde, C. L., Fan, E. & Buckner, F. S., 2011.** Selective inhibitors of methionyl-tRNA synthetase have potent activity against *Trypanosoma brucei* infection in mice. *Antimicrobial Agents and Chemotherapy*, **55** (5): 1982-1989.

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Human African trypanosomiasis continues to be an important public health threat in extensive regions of sub-Saharan Africa. Treatment options for infected patients are unsatisfactory due to toxicity, difficult administration regimes, and poor efficacy of available drugs. The aminoacyl-tRNA synthetases were selected as attractive drug targets due to their essential roles in protein synthesis and cell survival. Comparative sequence analysis disclosed differences between the trypanosome and mammalian methionyl-tRNA synthetases (MetRSs) that suggested opportunities for selective inhibition using drug-like molecules. Experiments using RNA interference on the single MetRS of *Trypanosoma brucei* demonstrated that this gene product was essential for normal cell growth. Small molecules (diaryl diamines) similar to those shown to have potent activity on prokaryotic MetRS enzymes were synthesized and observed to have inhibitory activity on the *T. brucei* MetRS (50 percent inhibitory concentration, <50 nM) and on bloodstream forms of *T. brucei* cultures (50 percent effective concentration, as low as 4 nM). Twenty-one compounds had a close correlation between

enzyme binding/inhibition and *T. brucei* growth inhibition, indicating that they were likely to be acting on the intended target. The compounds had minimal effects on mammalian cell growth at 20 µM, demonstrating a wide therapeutic index. The most potent compound was tested in the murine model of trypanosomiasis and demonstrated profound parasite suppression and delayed mortality. A homology model of the *T. brucei* MetRS based on other MetRS structures was used to model binding of the lead diaryl diamine compounds. Future studies will focus on improving the pharmacological properties of the MetRS inhibitors.

15746. **Spencer, J., Rathnam, R. P., Harvey, A. L., Clements, C. J., Clark, R. L., Barrett, M. P., Wong, P. E., Male, L., Coles, S. J. & Mackay, S. P., 2011.** Synthesis and biological evaluation of 1,4-benzodiazepin-2-ones with antitrypanosomal activity. *Bioorganic & Medicinal Chemistry*, **19** (5): 1802-1815.

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A library of 1,4-benzodiazepines has been synthesized and evaluated against *Trypanosoma brucei*, a causative parasite of human African trypanosomiasis. Benzodiazepines possessing a P2- transporter motif were found to have MIC values as low as 0.78 mµM.

15747. **Spinks, D., Ong, H. B., Mpamhanga, C. P., Shanks, E. J., Robinson, D. A., Collie, I. T., Read, K. D., Frearson, J. A., Wyatt, P. G., Brenk, R., Fairlamb, A. H. & Gilbert, I. H.,** 2011. Design, synthesis and biological evaluation of novel inhibitors of *Trypanosoma brucei* pteridine reductase 1. *ChemMedChem,* **6***(2): 209*.

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Genetic studies indicate that the enzyme pteridine reductase 1 (PTR1) is essential for the survival of the protozoan parasite *Trypanosoma brucei*. Herein, we describe the development and optimisation of a novel series of PTR1 inhibitors, based on benzo[d]imidazol-2-amine derivatives. Data are reported on 33 compounds. This series was initially discovered by a virtual screening campaign (*J. Med. Chem.*, 2009, **52**, 4454). The inhibitors adopted an alternative binding mode to those of the natural ligands, biopterin and dihydrobiopterin, and classical inhibitors, such as methotrexate. Using both rational medicinal chemistry and structure-based approaches, we were able to derive compounds with potent activity against *T. brucei* PTR1 which had high selectivity over both human and *T. brucei* dihydrofolate reductase. Unfortunately, these compounds displayed weak activity against the parasites. Kinetic studies and analysis indicate that the main reason for the lack of cell potency is due to the compounds having insufficient potency against the enzyme, which can be seen from the low K_m to K_i ratio (K_m=25 nM and K_i=2.3 nM, respectively).

15748. **Teka, I. A., Kazibwe, A. J., El-Sabbagh, N., Al-Salabi, M. I., Ward, C. P., Eze, A. A., Munday, J. C., Maeser, P., Matovu, E., Barrett, M. P. & De Koning, H. P., 2011.** The diamidine diminazene aceturate is a substrate for the high affinity pentamidine transporter: implications for the development of high resistance levels in trypanosomes. *Molecular Pharmacology,* **80**(1) 110-116.

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African trypanosomiasis is a disease of humans and livestock in many areas south of the Sahara. Resistance to the few existing drugs is a major impediment to control of these diseases and we here investigate how resistance to the main veterinary drug diminazene aceturate correlates with changes in drug transport in resistant strains. The strain tbat1 (-/-), lacking the *Tb*AT1/P2 aminopurine transporter previously implicated in diminazene transport, was adapted to higher levels of diminazene resistance. The resulting cell line was designated ABR and was highly cross-resistant to other diamidines, and moderately resistant to cymelarsan. Procyclic trypanosomes were shown to be a convenient model to study diamidine uptake in *T. b. brucei* given (1) the lack of *Tb*AT1/P2 and (2) a ten-fold higher activity of the high affinity pentamidine transporter HAPT1. Diminazene could be transported by HAPT1 in procyclic trypanosomes; this drug transport activity was lacking in the ABR line, as previously reported for the pentamidine-adapted line B48. The K_m for diminazene transport in bloodstream tbat1 (-/-) trypanosomes was consistent with uptake by HAPT1. Diminazene transport in ABR and B48 cells was reduced compared to tbat1 (-/-) but their resistance phenotype was different: B48 displayed higher levels of resistance to pentamidine and the melaminophenyl arsenicals, whereas ABR displayed higher resistance to diminazene. These results establish loss of HAPT1 function as a contributing factor to diminazene resistance but equally demonstrate for the first time that adaptations other than those determining initial rates of drug uptake contribute to diamidine and arsenical resistance in African trypanosomes.

15749. **Trunz, B. B., Jedrysiak, R., Tweats, D., Brun, R., Kaiser, M., Suwinski, J. & Torreele, E., 2011.** 1-aryl-4-nitro-1H-imidazoles, a new promising series for the treatment of human African trypanosomiasis. *European Journal of Medicinal Chemistry*, **46** (5): 1524-1535.

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Nitroimidazoles are a well-known class of antibacterial and antiprotozoal drugs but in spite of the widespread clinical and veterinary use of these drugs, this family has been stigmatized in part due to associated genotoxicity problems. Here we report the synthesis, the anti-trypanosomal activity and a structure-activity relationship (SAR) study of a series of about fifty 1-aryl-4-nitro-1H-imidazoles, with an emphasis on selected *in vivo* active molecules. Compounds 4-nitro-1-{4-(trifluoromethoxy)phenyl}-1H-imidazole and 1-(3,4-dichlorophenyl)-4-nitro-1H-imidazole are curative in mouse models of both acute and chronic African trypanosomiasis when given orally at doses of 25-50 mg/kg for four days for the acute infection, and 50-100 mg/kg for five days in the chronic model. While both compounds are bacterial mutagens, activity is lost in strains lacking bacterial specific nitro-reductases. Mammalian nitro-reductases do not reduce nitroaromatic compounds with low redox potentials with same avidity as their bacterial counterparts and these compounds were shown to be devoid of genotoxicity in mammalian cells. Both compounds are promising leads for the treatment of human African trypanosomiasis (HAT or sleeping sickness), including the fatal stage 2 of the disease, for which new treatments are urgently needed.

15750. **Walton, J. G., Jones, D. C., Kiuru, P., Durie, A. J., Westwood, N. J. & Fairlamb, A. H.,** 2011. Synthesis and evaluation of indatraline-based inhibitors for trypanothione reductase. *ChemMedChem,* **6**(2): 209.

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The search for novel compounds of relevance to the treatment of diseases caused by trypanosomatid protozoan parasites continues. Screening of a large library of known bioactive compounds has led to several drug-like starting points for further optimisation. In this study, novel analogues of the monoamine uptake inhibitor indatraline were prepared and assessed both as inhibitors of trypanothione reductase (TryR) and against the parasite *Trypanosoma brucei*. Although it proved difficult to significantly increase the potency of the original compound as an inhibitor of TryR, some insight into the preferred substituent on the amine group and in the two aromatic rings of the parent indatraline was deduced. In addition, detailed mode of action studies indicated that two of the inhibitors exhibit a mixed mode of inhibition.

15751. **Wang, Q. P., Lai, D. H., Li, Z., Li, F. J. & Lun, Z. R., 2011.** Semicarbazide-sensitive amine oxidase kills African trypanosomes *in vitro*. *Acta Tropica*, **117** (2): 161-164.

Center for Parasitic Organisms, State Key Laboratory of Biocontrol, School of Life Sciences, and Key Laboratory of Tropical Diseases Control of Ministry of Education, Zhongshan Medical College, Sun Yat-Sen (Zhongshan) University, Guangzhou 510275, PR China. [lsslzr@mail.sysu.edu.cn].

The African trypanosome *Trypanosoma brucei* is the cause of sleeping sickness in humans and nagana in animals. Here we report that semicarbazide-sensitive amine oxidases (SSAOs), enzymes that are abundant in *T. brucei* mammalian hosts, eliminate trypanosomes by oxidation of their substrate *in vitro*. SSAO and its endogenous substrate methylamine are not toxic to *T. brucei*, but parasites were killed in the presence of both of them. SSAO inhibitors antagonized the SSAO-methylamine induced toxicity on *T. brucei*. The trypanocidal activity was mainly associated with formaldehyde generated in the SSAO mediated oxidation of methylamine. This finding suggests that SSAO may play some roles in non-specific defence of trypanosome infection in mammals.

15752. **Ward, C. P., Wong, P. E., Burchmore, R. J., de Koning, H. P. & Barrett, M. P., 2011.** Trypanocidal furamidine analogues: influence of pyridine nitrogens on trypanocidal activity, transport kinetics, and resistance patterns. *Antimicrobial Agents & Chemotherapy*, **55** (5): 2352-2361.

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Current therapies for human African trypanosomiasis (HAT) are unsatisfactory and under threat from emerging drug resistance linked to the loss of transporters, e.g. the P2 aminopurine transporter (*Tb*AT1). Here we compare the uptake and trypanocidal properties of furamidine (DB75), recently evaluated in clinical trials against stage 1 (haemolymphatic)

HAT, and two aza analogues, DB820 and CPD0801 (DB829), which are candidate compounds for treatment of stage 2 (neurological) disease. Values of 50 percent inhibitory concentrations (IC_{50}s) determined *in vitro* against both wild-type and transporter mutant parasites were submicromolar, with DB75 trypanotoxicity shown to be better than and DB820 trypanotoxicity similar to that of the widely used veterinary trypanocide diminazene, while CPD0801 was less active. Activity correlated with uptake and with the minimum drug exposure time necessary to kill trypanosomes: DB75 accumulated at double and 10-fold the rates of DB820 and CPD0801, respectively. All three compounds inhibited P2-mediated adenosine transport with similar K_i values, indicating affinity values for this permease in the low to submicromolar range. Uptake of DB75, DB820, and CPD0801 was significantly reduced in tbat1(-/-) parasites and was sensitive to inhibition by adenine, showing that all three compounds are substrates for the P2 transporter. Uptake *in vitro* was significantly less than that seen with parasites freshly isolated from infected rats, correlating with a downregulation of P2 activity *in vitro*. We conclude that DB75, DB820, and CPD0801 are actively accumulated by *Trypanosoma brucei brucei*, with P2 as the main transport route. The aza analogues of DB75 accumulate more slowly than furamidine itself and reveal less trypanocidal activity in standard *in vitro* drug sensitivity assays.

15753. **Wilkinson, S. R., Bot, C., Kelly, J. M. & Hall, B. S., 2011.** Trypanocidal activity of nitroaromatic prodrugs: current treatments and future perspectives. *Current Topics in Medicinal Chemistry*. **E publication ahead of print, May 26.**

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Chagas disease and African sleeping sickness are trypanosomal infections that represent important public health problems in Latin America and Africa, respectively. The restriction of these diseases to the poorer parts of the world has meant that they have been largely neglected and limited progress has been made in their treatment. The nitroheterocyclic prodrugs nifurtimox and benznidazole, in use against Chagas disease for >40 years, remain the only agents available for this infection. In the case of African sleeping sickness, nifurtimox has recently been added to the arsenal of medicines, with the nitroheterocyclic fexinidazole currently under evaluation. For a long time, the cytotoxic mechanism of these drugs was poorly understood: nifurtimox was thought to act via production of superoxide anions and nitro radicals, while the mode of benznidazole action was more obscure. The trypanocidal activity of nitroheterocyclic drugs is now known to depend on a parasite type I nitroreductase (NTR). This enzyme is absent from mammalian cells, a difference that forms the basis for the drug selectivity. The role of this enzyme in drug activation has been genetically and biochemically validated. It catalyses the 2-electron reduction of nitroheterocyclic compounds within the parasite, producing toxic metabolites without significant generation of superoxide. Recognition that this enzyme is responsible for activation of nitroheterocyclic prodrugs has allowed screening for compounds that preferentially target the parasite. This approach has led to the identification of two new classes of anti-trypanosomal agents, nitrobenzylphosphoramide mustards and aziridinyl nitrobenzamides, and promises to yield new, safer, more effective drugs.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

15754. **Van Reet, N., Pyana, P. P., Deborggraeve, S., Buscher, P. & Claes, F., 2011.** *Trypanosoma brucei gambiense*: HMI-9 medium containing methylcellulose and human serum supports the continuous axenic *in vitro* propagation of the bloodstream form. *Experimental Parasitology*, **128** (3): 285-290.

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Trypanosoma brucei (T. b.) *gambiense* causes the chronic form of human African trypanosomiasis or sleeping sickness. One of the major problems with studying *T. b. gambiense* is the difficulty to isolate it from its original host and the difficult adaptation to *in vivo* and *in vitro* mass propagation. The objective of this study was to evaluate if an established method for axenic culture of pleomorphic bloodstream form *T. b. brucei* strains, based on methylcellulose containing HMI-9 medium, also facilitated the continuous *in vitro* propagation of other bloodstream form Trypanozoon strains, in particular of *T. b. gambiense*. Bloodstream form trypanosomes from one *T. b. brucei*, two *T. b. rhodesiense*, one *T. evansi* and seven *T. b. gambiense* strains were isolated from mouse blood and each was concurrently cultivated in liquid and methylcellulose-containing HMI-9 based medium, either with or without additional human serum supplementation, for over 10 consecutive sub passages. Although HMI-9 based medium supplemented with 1.1 percent (w/v) methylcellulose supported the continuous cultivation of all non-*gambiense* strains better than liquid media could, the *in vitro* cultivation of all *gambiense* strains was only achieved in HMI-9 based medium containing 1.1 percent (w/v) methylcellulose, 15 percent (v/v) foetal calf serum and 5 percent (v/v) heat-inactivated human serum.

(b) TAXONOMY; CHARACTERIZATION OF ISOLATES

15755. **Balmer, O., Beadell, J. S., Gibson, W. & Caccone, A., 2011.** Phylogeography and taxonomy of *Trypanosoma brucei*. *PLoS Neglected Tropical Diseases*, **5** (2): e961.

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Characterizing the evolutionary relationships and population structure of parasites can provide important insights into the epidemiology of human disease. We examined 142 isolates of *Trypanosoma brucei* from all over sub-Saharan Africa using three distinct classes of genetic markers (kinetoplast CO1 sequence, nuclear SRA gene sequence, eight nuclear microsatellites) to clarify the evolutionary history of *Trypanosoma brucei rhodesiense* (*Tbr*) and *T. b. gambiense (Tbg*), the causative agents of human African trypanosomosis (sleeping sickness) in sub-Saharan Africa, and to examine the relationship between *Tbr* and the non-human infective parasite *T. b. brucei* (*Tbb*) in eastern and southern Africa. A Bayesian phylogeny and haplotype network based on CO1 sequences confirmed the taxonomic distinctness of *Tbg* group 1. Limited diversity combined with a wide geographical distribution suggested that this parasite

has recently and rapidly colonized hosts across its current range. The more virulent *Tbg* group 2 exhibited diverse origins and was more closely allied with *Tbb* based on COI sequence and microsatellite genotypes. Four of five COI haplotypes obtained from *Tbr* were shared with isolates of *Tbb*, suggesting a close relationship between these taxa. Bayesian clustering of microsatellite genotypes confirmed this relationship and indicated that *Tbr* and *Tbb* isolates were often more closely related to each other than they were to other members of the same subspecies. Among isolates of *Tbr* for which data were available, we detected just two variants of the SRA gene responsible for human infectivity. These variants exhibited distinct geographical ranges, except in Tanzania, where both types co-occurred. Here, isolates possessing distinct SRA types were associated with identical COI haplotypes, but divergent microsatellite signatures. Our data provide strong evidence that *Tbr* is only a phenotypic variant of *Tbb*; while relevant from a medical perspective, *Tbr* is not a reproductively isolated taxon. The wide distribution of the SRA gene across diverse trypanosome genetic backgrounds suggests that a large amount of genetic diversity is potentially available with which human-infective trypanosomes may respond to selective forces such as those exerted by drugs.

15756. **Kabore, J., Macleod, A., Jamonneau, V., Ilboudo, H., Duffy, C., Camara, M., Camara, O., Belem, A. M., Bucheton, B. & De Meeus, T., 2011.** Population genetic structure of Guinea *Trypanosoma brucei gambiense* isolates according to host factors. *Infection, Genetics & Evolution,* **11(5)**:1129-35.

Centre International de Recherche-Développement sur l'Elevage en zones Subhumides (CIRDES), Unité de recherches sur les bases biologiques de la lutte intégrée, 01 BP 454 Bobo-Dioulasso 01, Burkina Faso. [thierry.demeeus@ird.fr].

Human African trypanosomiasis (HAT) or sleeping sickness is a major public health problem in sub-Saharan Africa and is due to the kinetoplastid parasite *Trypanosoma brucei gambiense* in West and Central Africa. The exact role of multiple infections, the basis of clinical diversity observed in patients and the determinism that leads trypanosomes into different body fluids of the host remain opened questions to date. In this paper we investigate, in three Guinean foci, whether strains found in blood, lymph or cerebrospinal fluid (CSF) or in patients at different phase of HAT (phase 1, early phase 2 and late phase 2) are representative of the focus they belong to. Amplifications of parasites directly from body fluids led to substantial amounts of allelic drop outs, especially so for blood and CSF samples, which required data recoding of all homozygous sites into missing data. While controlling for geography, date of sampling and patient's phase of the disease, we found no effect of body fluids in the genetic structure of *T. b. gambiense* despite the presence of mixed infections. On the contrary, we found that the strains found in patients in different phase of the disease differed genetically, with early phase patients being more likely to be infected with more recent strains than patients at a more advanced phase of the disease. Thus, the combination of date of sampling and patient's status represents a parameter to be controlled for in population genetic structure analyses. Additional studies will also be required to explore further the phenomenon of mixed infections and its consequences.

15757. **Li, J. V., Saric, J., Wang, Y., Utzinger, J., Holmes, E. & Balmer, O., 2011.** Metabonomic investigation of single and multiple strain *Trypanosoma brucei brucei* infections. *The American Journal of Tropical Medicine and Hygiene*, **84** (1): 91-98.

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Although co-infections are common and can have important epidemiologic and evolutionary consequences, studies exploring biochemical effects of multiple-strain infections remain scarce. We studied metabolic responses of NMRI mice to *Trypanosoma brucei brucei* single (STIB777AE-Green1 or STIB246BA-Red1) and co-infections using a H nuclear magnetic resonance (NMR) spectroscopy-based metabolic profiling strategy. All *T. b. brucei* infections caused an alteration in urinary biochemical composition by day 4 post infection, characterized by increased concentrations of 2-oxoisocaproate, D-3-hydroxybutyrate, lactate, 4-hydroxyphenylacetate, phenylpyruvate, and 4-hydroxyphenylpyruvate, and decreased levels of hippurate. Although there were no marked differences in metabolic signatures observed in the mouse infected with a single or dual strain of *T. b. brucei*, there was a slower metabolic response in mice infected with *T. b. brucei* green strain compared with mice infected with either the red strain or both strains concurrently. Pyruvate, phenylpyruvate, and hippurate were correlated with parasitaemia, which might be useful in monitoring responses to therapeutic interventions.

15758. **Pyana, P. P., Ngay Lukusa, I., Mumba Ngoyi, D., Van Reet, N., Kaiser, M., Karhemere Bin Shamamba, S. & Buscher, P., 2011.** Isolation of *Trypanosoma brucei gambiense* from cured and relapsed sleeping sickness patients and adaptation to laboratory mice. *PLoS Neglected Tropical Diseases*, **5** (4): e1025.

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Sleeping sickness due to *Trypanosoma brucei* (*T. b.*) *gambiense* is still a major public health problem in some central African countries. Historically, relapse rates around 5 percent have been observed for treatment with melarsoprol, widely used to treat second stage patients. Later, relapse rates of up to 50 percent have been recorded in some isolated foci in Angola, Sudan, Uganda and Democratic Republic of the Congo (DRC). Previous investigations are not conclusive on whether decreased sensitivity to melarsoprol is responsible for these high relapse rates. Therefore we aimed to establish a parasite collection isolated from cured as well as from relapsed patients for downstream comparative drug sensitivity profiling. A major constraint for this type of investigation is that *T. b. gambiense* is particularly difficult to isolate and adapt to classical laboratory rodents. From 360 patients treated in Dipumba hospital, Mbuji-Mayi, D.R. Congo, blood and cerebrospinal fluid (CSF) was collected before treatment. From patients relapsing during the 24 months follow-up, the same specimens were collected. Specimens with confirmed parasite presence were frozen in liquid nitrogen in a mixture of Triladyl, egg yolk and phosphate buffered glucose solution. Isolation was achieved by inoculation of the cryopreserved specimens in *Grammomys surdaster*, *Mastomys natalensis* and SCID mice. Thus, 85 strains were isolated from blood and CSF of 55 patients. Isolation success was highest in *Grammomys surdaster*. Forty strains were adapted to mice. From 12 patients, matched strains were isolated before treatment and after relapse. All strains belong to *T. b. gambiense* type I. We established a unique collection of *T. b. gambiense* from cured and relapsed patients, isolated in the same disease focus and within a limited period. This collection is available for genotypic and phenotypic characterisation to investigate the mechanism behind abnormally high treatment failure rates in Mbuji-Mayi, D.R. Congo.

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

[See also **34**: 15622]

15759. **Arias, D. G., Cabeza, M. S., Erben, E. D., Carranza, P. G., Lujan, H. D., Tellez Inon, M. T., Iglesias, A. A. & Guerrero, S. A.,** 2011. Functional characterization of methionine sulphoxide reductase A from *Trypanosoma* spp. *Free Radical Biology & Medicine,* **50** (1): 37-46.

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Methionine is an amino acid susceptible to being oxidized to methionine sulphoxide (MetSO). The reduction of MetSO to methionine is catalysed by methionine sulphoxide reductase (MSR), an enzyme present in almost all organisms. In trypanosomatids, the study of antioxidant systems has been mainly focused on the involvement of trypanothione, a specific redox component in these organisms. However, no information is available concerning their mechanisms for repairing oxidized proteins, which would be relevant for the survival of these pathogens in the various stages of their life cycle. We report the molecular cloning of three genes encoding a putative A-type MSR in trypanosomatids. The genes were expressed in *Escherichia coli*, and the corresponding recombinant proteins were purified and functionally characterized. The enzymes were specific for L-Met(S)SO reduction, using *Trypanosoma cruzi* tryparedoxin I as the reducing substrate. Each enzyme migrated in electrophoresis with a particular profile reflecting the differences they exhibit in superficial charge. The *in vivo* presence of the enzymes was evidenced by immunological detection in replicative stages of *T. cruzi* and *Trypanosoma brucei*. The results support the occurrence of a metabolic pathway in *Trypanosoma* spp. involved in the critical function of repairing oxidized macromolecules.

15760. **Bruhn, D. F., Sammartino, M. P. & Klingbeil, M. M., 2011.** Three mitochondrial DNA polymerases are essential for kinetoplast DNA replication and survival of bloodstream form *Trypanosoma brucei*. *Eukaryotic Cell*, **10** (6): 734-743.

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Trypanosoma brucei, the causative agent of human African trypanosomiasis, has a complex life cycle that includes multiple life cycle stages and metabolic changes as the parasite switches between insect vector and mammalian host. The parasite's single mitochondrion contains a unique catenated mitochondrial DNA network called kinetoplast DNA (kDNA) that is composed of minicircles and maxicircles. Long-standing uncertainty about the requirement of kDNA in bloodstream form (BF) *T. brucei* has recently eroded, with reports of posttranscriptional editing and subsequent translation of kDNA-encoded transcripts as essential processes for BF parasites. These studies suggest that kDNA and its faithful replication are indispensable for this life cycle stage. Here we demonstrate that three kDNA replication proteins (mitochondrial DNA polymerases IB, IC, and ID) are required for BF parasite viability. Silencing of each polymerase was lethal, resulting in kDNA loss, persistence of prereplication DNA monomers, and collapse of the mitochondrial membrane potential.

These data demonstrate that kDNA replication is indeed crucial for BF *T. brucei*. The contributions of mitochondrial DNA polymerases IB, IC, and ID to BF parasite viability suggest that these and other kDNA replication proteins warrant further investigation as a new class of targets for the development of antitrypanosomal drugs.

15761. **Bucerius, F., Kador, M., Boshart, M. & Janzen, C. J., 2011.** Reliable quantification of cell cycle-dependent mRNA abundance using fluorescence-activated cell sorting in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology*, **175** (2): 205-208.

University of Munich (LMU), Department Biology I, Genetics, Martinsried, Germany. [cjanzen@lmu.de].

Very little is known about cell cycle-dependent regulation of mRNA in *Trypanosoma brucei*, the causative agent of African sleeping sickness. Methods to synchronize cell cycle progression are inefficient or subject the parasites to non-physiological conditions and stress. We developed a fluorescence-activated cell sorting-based method to analyse steady-state mRNA levels in individual cell cycle phases. Normalization of the data was the most challenging problem because internal standards for cell cycle-regulated genes are not available for trypanosomes. Hence, we introduced an external standard (so-called "spike") to compensate for technically derived variations in processing cells and RNA samples. Validation of this method with a limited number of genes unravelled a transient up-regulation during S and G2/M phases for all mRNAs analysed.

15762. **Cosentino-Gomes, D. & Meyer-Fernandes, J. R., 2011.** Ecto-phosphatases in protozoan parasites: possible roles in nutrition, growth and ROS sensing. *Journal of Bioenergetics & Biomembranes*, **43** (1): 89-92.

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The cellular plasma membrane contains enzymes whose active sites face the external medium rather than the cytoplasm. The activities of these enzymes, referred to as ecto-enzymes, can be measured using living cells. Ecto-phosphatases are ecto-enzymes that presumably hydrolyse extracellular phosphorylated substrates, releasing free inorganic phosphate. Although several alternative functions have been suggested for these enzymes, such as participation in proliferation, differentiation, adhesion, virulence, and infection, little is known about the physiological roles of these enzymes in protozoa parasites. In this review, we discuss the principal features of ecto-phosphatases in protozoan parasites that are causative agents of important diseases such as Chagas disease, leishmaniasis, amoebiasis, giardiasis, trichomoniasis and sleeping sickness.

15763. **Dodson, H. C., Lyda, T. A., Chambers, J. W., Morris, M. T., Christensen, K. A. & Morris, J. C.,** 2011. Quercetin, a fluorescent bioflavonoid, inhibits *Trypanosoma brucei* hexokinase 1. *Experimental Parasitology*, **127**(2): 423-428.

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Hexokinases from the African trypanosome, *Trypanosoma brucei*, are attractive targets for the development of anti-parasitic drugs, in part because the parasite utilizes glycolysis exclusively for ATP production during the mammalian infection. Here, we have demonstrated that the bioflavanoid quercetin (QCN), a known trypanocide, is a mixed inhibitor of *Trypanosoma brucei* hexokinase 1 (*Tb*HK1) (IC_{50}=4.1+/-0.8µM). Spectroscopic analysis of QCN binding to TbHK1, taking advantage of the intrinsically fluorescent single tryptophan (Trp177) in *Tb*HK1, revealed that QCN quenches emission of Trp177, which is located near the hinge region of the enzyme. ATP similarly quenched Trp177 emission, while glucose had no impact on fluorescence. Supporting the possibility that QCN toxicity is a consequence of inhibition of the essential hexokinase, in live parasites QCN fluorescence localizes to glycosomes, the subcellular home of *Tb*HK1. Additionally, RNAi-mediated silencing of *Tb*HK1 expression expedited QCN induced death, while over-expressing *Tb*HK1 protected trypanosomes from the compound. In summary, these observations support the suggestion that QCN toxicity is in part attributable to inhibition of the essential *Tb*HK1.

15764. **Emmer, B. T., Nakayasu, E. S., Souther, C., Choi, H., Sobreira, T. J., Epting, C. L., Nesvizhskii, A. I., Almeida, I. C. & Engman, D. M., 2011.** Global analysis of protein palmitoylation in African trypanosomes. *Eukaryotic Cell*, **10** (3): 455-463.

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Many eukaryotic proteins are posttranslationally modified by the esterification of cysteine thiols to long-chain fatty acids. This modification, protein palmitoylation, is catalysed by a large family of palmitoyl acyltransferases that share an Asp-His-His-Cys Cys-rich domain but differ in their subcellular localizations and substrate specificities. In Trypanosoma brucei, the flagellated protozoan parasite that causes African sleeping sickness, protein palmitoylation has been observed for a few proteins, but the extent and consequences of this modification are largely unknown. We undertook the present study to investigate *T. brucei* protein palmitoylation at both the enzyme and substrate levels. Treatment of parasites with an inhibitor of total protein palmitoylation caused potent growth inhibition, yet there was no effect on growth by the separate, selective inhibition of each of the 12 individual *T. brucei* palmitoyl acyltransferases. This suggested either that *T. brucei* evolved functional redundancy for the palmitoylation of essential palmitoyl proteins or that palmitoylation of some proteins is catalysed by a noncanonical transferase. To identify the palmitoylated proteins in *T. brucei*, we performed acyl biotin exchange chemistry on parasite lysates, followed by streptavidin chromatography, two-dimensional liquid chromatography-tandem mass spectrometry protein identification, and QSpec statistical analysis. A total of 124 palmitoylated proteins were identified, with an estimated false discovery rate of 1.0 percent. This palmitoyl proteome includes all of the known palmitoyl proteins in procyclic-stage *T. brucei* as well as several proteins whose homologues are palmitoylated in other organisms. Their sequences demonstrate the variety of substrate motifs that support palmitoylation, and their identities illustrate the range of cellular processes affected by palmitoylation in these important pathogens.

15765. **Gluenz, E., Povelones, M. L., Englund, P. T. & Gull, K.,** 2011. The kinetoplast duplication cycle in *Trypanosoma brucei* is orchestrated by cytoskeleton-mediated cell

morphogenesis. *Molecular & Cellular Biology,* **31**(5): 1012-1021.

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The mitochondrial DNA of *Trypanosoma brucei* is organised in a complex structure called the kinetoplast. In this study we define the complete kinetoplast duplication cycle in *T. brucei* based on three-dimensional reconstructions from serial-section electron micrographs. This structural model was enhanced by analyses of the replication process of DNA maxi- and minicircles. Novel insights were obtained about the earliest and latest stages of kinetoplast duplication. We show that kinetoplast S-phase occurs concurrent with the re-positioning of the new basal body from the anterior to the posterior side of the old flagellum. This emphasizes the role of basal body segregation in kinetoplast division and suggests a possible mechanism for driving the rotational movement of the kinetoplast during minicircle replication. Fluorescence *in situ* hybridisation with minicircle and maxicircle-specific probes showed that maxicircle DNA is stretched out between segregated minicircle networks, indicating that maxicircle segregation is a late event in the kinetoplast duplication cycle. This new view of the complexities of kinetoplast duplication emphasises the dependencies between dynamic remodelling of the cytoskeleton and inheritance of the mitochondrial genome.

15766. **Goldshmidt, H. & Michaeli, S., 2011.** Induction of ER stress response leading to programmed cell death in *Trypanosoma brucei*. *Methods in Enzymology*, **489**: 189-205.

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Trypanosomes are parasitic protozoans that include several medically and a variety of economically important parasites such as *Trypanosoma brucei*, the causative agent of sleeping sickness. This parasite cycles between the insect host (procyclic form) and mammalian host (bloodstream form). These parasites lack transcription regulation, including factors that govern the unfolded protein response (UPR) in other eukaryotes. Gene expression is controlled posttranscriptionally by unique mechanisms such as trans-splicing and RNA editing and by mRNA stability. In trans-splicing, a common exon, the spliced leader (SL) is donated to all mRNAs from a small RNA, the SL RNA. The SL RNA is transcribed from a defined promoter assisted by the tSNAP complex. Despite the lack of transcriptional regulation, induction of ER stress elicits changes in the transcriptome similar to those induced by conventional UPR found in other eukaryotes. The mechanism of upregulation under UPR is dependent on differential stabilization of mRNAs. The transcriptome changes result in ER expansion and elevation in the ER chaperone, BiP. Prolonged ER stress induces the spliced leader RNA silencing (SLS) pathway. SLS is the trypanosome-specific stress response mechanism that elicits the shut-off of SL RNA transcription by perturbing the binding of the transcription factor tSNAP42 to its cognate promoter, eliminating trans-splicing of all mRNAs. SLS was discovered in the RNAi silenced cells depleted for functions that mediate translocation of proteins to the ER such as the signal recognition particle receptor SRalpha, SEC63- a factor that participates in protein translocation across the ER membrane, or SEC61- the translocation channel. Induction of SLS, either by prolonged ER stress or silencing of the genes associated with the ER membrane that function in ER protein translocation led to programmed cell death (PCD), evident by the

exposure of phosphatidyl serine, DNA laddering, increase in ROS production, increase in cytoplasmic Ca^{2+}, and decrease in mitochondrial membrane potential. Here, we describe the protocols to induce ER stress and to observe the resulting morphological changes by transmission electron microscopy (TEM), changes in cytoplasmic Ca^{2+}, and DNA fragmentation which are the hallmarks of programmed cell death.

15767. **Goto, Y., Duthie, M. S., Kawazu, S., Inoue, N. & Carter, D., 2011.** Biased cellular locations of tandem repeat antigens in African trypanosomes. *Biochemical & Biophysical Research Communications*, **405** (3): 434-438.

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Trypanosoma brucei subspecies cause African trypanosomiasis in humans and animals. These parasites possess genes encoding proteins with large tandem repeat (TR) domains as do the other trypanosomatid parasites. We have previously demonstrated that TR protein of *Leishmania infantum* and *Trypanosoma cruzi* are often targets of B-cell responses. However, African trypanosomes are susceptible to antibody-mediated immunity, and it may be detrimental for the parasites to have such B-cell antigens on the cell surface. Here we show that TR proteins of *T. brucei* subspecies are also antigenic and that recombinant TR proteins of these parasites detect antibodies in sera from mice infected with the parasites by ELISA. Analysis of amino acid sequences revealed that, different from TR proteins of *Leishmania* species or *T. cruzi*, the levels of predicted signal peptides, trans-membrane domains and GPI anchor signals in *T. brucei* TR proteins are significantly lower than those of the whole proteome. Many of the *T. brucei* TR proteins are specific to the species or conserved only in the closely related species, as is the same case for *Leishmania major* and *T. cruzi*. These results suggest that, despite their sharing some common characteristics, such abundance in large TR domains and immunological dominance, TR genes have evolved independently among the trypanosomatid parasites.

15768. **Kuettel, S., Greenwald, J., Kostrewa, D., Ahmed, S., Scapozza, L. & Perozzo, R., 2011.** Crystal structures of *T. b. rhodesiense* adenosine kinase complexed with inhibitor and activator: implications for catalysis and hyperactivation. *PLoS Neglected Tropical Diseases*, **5** (5): e1164.

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The essential purine salvage pathway of *Trypanosoma brucei* bears interesting catalytic enzymes for chemotherapeutic intervention of human African trypanosomiasis. Unlike mammalian cells, trypanosomes lack *de novo* purine synthesis and completely rely on salvage from their hosts. One of the key enzymes is adenosine kinase which catalyses the phosphorylation of ingested adenosine to form adenosine monophosphate (AMP) utilizing adenosine triphosphate (ATP) as the preferred phosphoryl donor. Here, we present the first structures of *Trypanosoma brucei rhodesiense* adenosine kinase (*Tbr*AK): the structure of *Tbr*AK in complex with the bisubstrate inhibitor P^1,P^5-di(adenosine-5')-pentaphosphate (AP5A) at 1.55 A, and *Tbr*AK complexed with the recently discovered activator

4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl]morpholine (compound 1) at 2.8 A resolution. The structural details and their comparison give new insights into substrate and activator binding to *Tbr*AK at the molecular level. Further structure-activity relationship analyses of a series of derivatives of compound 1 support the observed binding mode of the activator and provide a possible mechanism of action with respect to their activating effect towards *Tbr*AK.

15769. **Leroux, A. E., Maugeri, D. A., Opperdoes, F. R., Cazzulo, J. J. & Nowicki, C., 2011.** Comparative studies on the biochemical properties of the malic enzymes from *Trypanosoma cruzi* and *Trypanosoma brucei*. *FEMS Microbiology Letters*, **314** (1): 25-33.

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Comparative studies showed that, like *Trypanosoma cruzi, Trypanosoma brucei* exhibits functional cytosolic and mitochondrial malic enzymes (MEs), which are specifically linked to NADP. Kinetic studies provided evidence that *T. cruzi* and *T. brucei* MEs display similarly high affinities towards $NADP^+$ and are also almost equally efficient in catalysing the production of NADPH. Nevertheless, in contrast to the cytosolic ME from *T. cruzi*, which is highly activated by l-aspartate (over 10-fold), the *T. brucei* homologue is slightly more active (50 percent) in the presence of this amino acid. In *T. brucei*, both isozymes appear to be clearly more abundant in the insect stage, although they can be immunodetected in the bloodstream forms. By contrast, in *T. cruzi* the expression of the mitochondrial ME seems to be clearly upregulated in amastigotes, whereas the cytosolic isoform appears to be more abundant in the insect stages of the parasite. It might be hypothesized that in those environments where glucose is very low or absent, these pathogens depend on NADP-linked dehydrogenases such as the MEs for NADPH production, as in those conditions the pentose phosphate pathway cannot serve as a source of essential reducing power.

15770. **Ling, A. S., Trotter, J. R. & Hendriks, E. F., 2011.** A zinc finger protein, *Tb*ZC3H20, stabilises two developmentally regulated mRNAs in trypanosomes. *Journal of Biological Chemistry*. **Published April 5.**

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CCCH zinc finger proteins (ZC3Hs) are a novel class of RNA binding protein involved in post-transcriptional mechanisms controlling gene expression. We show *Tb*ZC3H20 from *Trypanosoma brucei*, the causative agent of sleeping sickness and other diseases, stabilises two developmentally regulated transcripts encoding a mitochondrial carrier protein (MCP12) and trans-sialidase (TS-like E). *Tb*ZC3H20 is shown to be an RNA binding protein, which is enriched in insect procyclic form *T. brucei*, and is the first ZC3H discovered controlling gene expression through modulating mRNA abundance in trypanosomes. Previous studies have demonstrated that RNA recognition motif-containing and PUF family RNA binding proteins can control gene expression by stabilising specific target mRNA levels. This work is the first to describe a ZC3H stabilising, rather than destabilising, target mRNAs as a regulatory mechanism and the first report of a ZC3H regulating a gene encoding a mitochondrial protein.

This suggests a broader role for ZC3Hs in post-transcriptional regulation of gene expression than previously thought.

15771. **Luginbuehl, E., Kunz, S., Wentzinger, L., Freimoser, F. & Seebeck, T., 2011.** The exopolyphosphatase *Tbr*PPX1 of *Trypanosoma brucei*. *BMC Microbiology*, **11**: 4.

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Exopolyphosphatases and pyrophosphatases play important but still incompletely understood roles in energy metabolism, and also in other aspects of cell biology such as osmoregulation or signal transduction. Earlier work has suggested that a human exopolyphosphatase, Prune, might exhibit cyclic nucleotide phosphodiesterase activity. The Kinetoplastida, a large order of unicellular eukaryotes that contains many important pathogens such as *Trypanosoma brucei* (human sleeping sickness), *Trypanosoma cruzi* (Chagas disease) or *Leishmania* ssp (several clinically distinct leishmaniases) all contain several exo- and pyrophosphatases. The current study provides a systematic classification of these enzymes, which now allows to situate the information that is already available on some of these enzymes. It then analyses the exopolyphosphatase *Tbr*PPX1 of *T. brucei* in detail, using RNA interference and genetic knockouts in an attempt to define its function, and immunofluorescence microscopy to study its subcellular localization.*Tbr*PPX1 is an exopolyphosphatase that does hydrolyse pentasodium triphosphate, but not organic triphosphates such as ATP, pyrophosphate or long-chain polyphosphates. Finally, the study investigates the potential cyclic nucleotide phosphodiesterase activity of *Tbr*PPX1. All kinetoplastid genomes that are currently available contain genes for an exopolyphosphatase and two classes of pyrophosphatases, one associated with the acidocalcisomes and one cytoplasmic. *Tbr*PPX1 represents the *T. brucei* exopolyphosphatase. It is located throughout the cytoplasm, and its genetic ablation does not produce a dramatic phenotype. Importantly, *Tbr*PPX1 does not exhibit any cyclic nucleotide specific phosphodiesterase activity, which definitively eliminates it as an additional player in cAMP signalling of the kinetoplastida.

15772. **Natesan, S. K., Black, A., Matthews, K. R., Mottram, J. C. & Field, M. C., 2011.** *Trypanosoma brucei brucei*: endocytic recycling is important for mouse infectivity. *Experimental Parasitology*, **127** (4): 777-783.

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Endocytosis in the African trypanosome, *Trypanosoma brucei*, is intimately involved in maintaining homeostasis of the cell surface proteome, morphology of the flagellar pocket and has recently been demonstrated as a bona fide drug target. RNAi-mediated knockdown of many factors required for endocytic transport, including several small GTPases, the major coat protein clathrin and a clathrin-associated receptor, epsinR, results in rapid cell death *in vitro.* Rapid loss of viability *in vitro* precludes meaningful investigation by RNAi of the roles of trypanosome endocytosis *in vivo*. Here we have sought to address this issue using strategies designed to produce milder effects on the endocytic system than complete functional ablation. We created a trypanosome clathrin heavy chain hemizygote and several lines expressing mutant forms of Rab5 and Rab11, described previously. All are viable in *in vitro* culture, with

negligible impact to proliferative rates or cell cycle. Clathrin hemizygotes express clathrin heavy chain at approximately 50 percent of wild type levels, but despite this demonstrate no defect to growth in mice, while none of the Rab5 mutants affected proliferation *in vivo*, despite clear evidence for effects on endocytosis. By contrast we find that expressing a dominantly active Rab11 mutant led to compromised growth in mice. These data indicate that trypanosomes likely tolerate the effects of partly decreased clathrin expression and alterations in early endocytosis, but are more sensitive to alterations in the recycling arm of the pathway.

15773. **Ohashi-Suzuki, M., Yabu, Y., Ohshima, S., Nakamura, K., Kido, Y., Sakamoto, K., Kita, K., Ohta, N. & Suzuki, T., 2011.** Differential kinetic activities of glycerol kinase among African trypanosome species: phylogenetic and therapeutic implications. *Journal of Veterinary Medical Science*, **73** (5): 615-621.

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African trypanosome species are causative agents for sleeping sickness in humans and nagana disease in cattle. *Trypanosoma brucei* can generate ATP via a reverse reaction with glycerol kinase (GK) when alternative oxidase (AOX) is inhibited; thus, GK is considered to be a crucial target for chemotherapy combined with AOX. However, the energy metabolism systems of African trypanosome species other than *T. brucei* are poorly understood. Thus, GK genes were surveyed from genome databases and cloned by PCR from *T. vivax* and *T. congolense*. Then, recombinant GK proteins (rGK) of *T. vivax, T. congolense* and *T. brucei* were expressed and purified. Kinetic analysis of these rGK proteins revealed that the K_m values of *T. congolense* rGK for ADP and G-3-P substrates were lower than those of *T. vivax* and *T. brucei.* The expression level of GK molecules was highest in *T. congolense* cells and lowest in *T. vivax* cells. Based on these results, effective combination dosages of ascofuranone, a specific inhibitor of AOX, and glycerol, an inhibitor of the GK reverse reaction, were determined by using *in vitro*-cultured trypanosome cells.

15774. **Ojo, K. K., Arakaki, T. L., Napuli, A. J., Inampudi, K. K., Keyloun, K. R., Zhang, L., Hol, W. G., Verlinde, C. L., Merritt, E. A. & Van Voorhis, W. C.,** 2011. Structure determination of glycogen synthase kinase-3 from *Leishmania major* and comparative inhibitor structure-activity relationships with *Trypanosoma brucei* GSK-3. *Molecular & Biochemical Parasitology,* **176**(2): 98-108.

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Glycogen synthase kinase-3 (GSK-3) is a drug target under intense investigation in pharmaceutical companies and constitutes an attractive piggyback target for eukaryotic pathogens. Two different GSKs are found in trypanosomatids, one about 150 residues shorter than the other. GSK-3 short (GeneDB: *Tb*927.10.13780) has previously been validated genetically as a drug target in *Trypanosoma brucei* by RNAi induced growth retardation; and chemically by correlation between enzyme and *in vitro* growth inhibition. Here, we report investigation of the equivalent GSK-3 short enzymes of *L. major* (*Lmj*F18.0270) and *L. infantum* (*Lin*J18_V3.0270, identical in amino acid sequences to LdonGSK-3 short) and a

crystal structure of *Lmaj*GSK-3 short at 2A resolution. The inhibitor structure-activity relationships (SARs) of *L. major* and *L. infantum* are virtually identical, suggesting that inhibitors could be useful for both cutaneous and visceral leishmaniasis. *Leishmania* spp. GSK-3 short has different inhibitor SARs than *Tbru*GSK-3 short, which can be explained mostly by two variant residues in the ATP-binding pocket. Indeed, mutating these residues in the ATP-binding site of *Lmaj*GSK-3 short to the *Tbru*GSK-3 short equivalents results in a mutant *Lmaj*GSK-3 short enzyme with SAR more similar to that of *Tbru*GSK-3 short. The differences between human GSK-3beta (HsGSK-3beta) and *Lmaj*GSK-3 short SAR suggest that compounds which selectively inhibit *Lmaj*GSK-3 short may be found.

15775. **Peacock, L., Ferris, V., Sharma, R., Sunter, J., Bailey, M., Carrington, M. & Gibson, W., 2011.** Identification of the meiotic life cycle stage of *Trypanosoma brucei* in the tsetse fly. *Proceedings of the National Academy of Sciences of the United States of America*, **108** (9): 3671-3676.

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Elucidating the mechanism of genetic exchange is fundamental for understanding how genes for such traits as virulence, disease phenotype, and drug resistance are transferred between pathogen strains. Genetic exchange occurs in the parasitic protists *Trypanosoma brucei, T. cruzi*, and *Leishmania major*, but the precise cellular mechanisms are unknown, because the process has not been observed directly. Here we exploit the identification of homologues of meiotic genes in the *T. brucei* genome and demonstrate that three functionally distinct, meiosis-specific proteins are expressed in the nucleus of a single specific cell type, defining a previously undescribed developmental stage occurring within the tsetse fly salivary gland. Expression occurs in clonal and mixed infections, indicating that the meiotic programme is an intrinsic but hitherto cryptic part of the developmental cycle of trypanosomes. In experimental crosses, expression of meiosis-specific proteins usually occurred before cell fusion. This is evidence of conventional meiotic division in an excavate protist, and the functional conservation of the meiotic machinery in these divergent organisms underlines the ubiquity and basal evolution of meiosis in eukaryotes.

15776. **Ralston, K. S., Kisalu, N. K. & Hill, K. L., 2011.** Structure-function analysis of dynein light chain 1 identifies viable motility mutants in bloodstream-form *Trypanosoma brucei*. *Eukaryotic Cell*. **Published online ahead of print 4 March.**

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The flagellum of *Trypanosoma brucei* is an essential and multifunctional organelle that is receiving increasing attention as a potential drug target and as a system for studying flagellum biology. RNAi knockdown is widely used to test the requirement for a protein in flagellar motility and has suggested that normal flagellar motility is essential for viability in bloodstream-form trypanosomes. However, RNAi knockdown alone provides limited functional information because the consequence is often loss of a multiprotein complex. We therefore developed an inducible system that allows for functional analysis of point mutations

in flagellar proteins in *T. brucei*. Using this system, we identified point mutations in the outer dynein light chain 1 (LC1) that allow stable assembly of outer dynein motors, but do not support propulsive motility. In procyclic-form trypanosomes, the phenotype of LC1 point mutants differs from the motility and structural defects of LC1 knockdowns, which lack the outer arm dynein motor. Thus, our results distinguish LC1-specific functions from broader functions of outer arm dynein. In bloodstream-form trypanosomes, LC1 knockdown blocks cell division and is lethal. In contrast, LC1 point mutants cause severe motility defects without affecting viability, indicating that the lethal phenotype of LC1 RNAi knockdown is not due to defective motility. Our results demonstrate for the first time that normal motility is not essential in bloodstream-form *T. brucei* and that the presumed connection between motility and viability is more complex than might be interpreted from knockdown studies alone. These findings open new avenues for dissecting mechanisms of flagellar protein function and provide an important step in efforts to exploit the potential of the flagellum as a therapeutic target in African sleeping sickness.

15777. **Rotureau, B., Subota, I. & Bastin, P., 2011.** Molecular bases of cytoskeleton plasticity during the *Trypanosoma brucei* parasite cycle. *Cellular Microbiology*, **13** (5): 705-716.

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African trypanosomes are flagellated protozoan parasites responsible for sleeping sickness and transmitted by tsetse flies. The accomplishment of their parasite cycle requires adaptation to highly diverse environments. These transitions take place in a strictly defined order and are accompanied by spectacular morphological modifications in cell size, shape and positioning of organelles. To understand the molecular bases of these processes, parasites isolated from different tissues of the tsetse fly were analysed by immunofluorescence with markers for specific cytoskeleton components and by a new immunofluorescence-based assay for evaluation of the cell volume. The data revealed striking differences between proliferative stages found in the midgut or in the salivary glands and the differentiating stage occurring in the proventriculus. Cell proliferation was characterized by a significant increase in cell volume, by a pronounced cell elongation marked by microtubule extension at the posterior end, and by the production of a new flagellum similar to the existing one. In contrast, the differentiating stage found in the proventriculus does not display any increase in cell volume neither in cell length, but is marked by a profound remodelling of the posterior part of the cytoskeleton and by changes in molecular composition and/or organization of the flagellum attachment zone.

15778. **Sandhu, R. & Li, B., 2011.** Examination of the telomere G-overhang structure in *Trypanosoma brucei*. *Journal of Visualized Experiments,* 47: January 26.

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The telomere G-overhang structure has been identified in many eukaryotes including yeast, vertebrates, and *Trypanosoma brucei*. It serves as the substrate for telomerase for *de novo* telomere DNA synthesis and is therefore important for telomere maintenance. *T. brucei* is a protozoan parasite that causes sleeping sickness in humans and nagana in cattle. Once a

mammalian host becomes infected, *T. brucei* cell regularly switches its surface antigen to evade the host's immune attack. We have recently demonstrated that the *T. brucei* telomere structure plays an essential role in regulation of surface antigen gene expression, which is critical for *T. brucei* pathogenesis. However, *T. brucei* telomere structure has not been extensively studied due to the limitation of methods for analysis of this specialized structure. We have now successfully adopted the native in-gel hybridization and ligation-mediated primer extension methods for examination of the telomere G-overhang structure and an adaptor ligation method for determination of the telomere terminal nucleotide in *T. brucei* cells. Here, we will describe the protocols in detail and compare their different advantages and limitations.

15779. **Schumann Burkard, G., Jutzi, P. & Roditi, I.,** 2011. Genome-wide RNAi screens in bloodstream form trypanosomes identify drug transporters. *Molecular & Biochemical Parasitology,* **175** (1): 91-94.

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An inducible RNA interference (RNAi) library, consisting of a pool of independent stable transformants with 9-fold genome coverage, was constructed in bloodstream form *Trypanosoma brucei* using an improved transfection protocol. RNAi induction and selection of resistant parasites were performed in the presence of melarsoprol or eflornithine. The former led to the isolation of the adenosine transporter *Tb*AT1, which is known to be involved in melarsoprol uptake, while the latter identified an amino acid transporter, AAT6. Knockdown of AAT6 reduced mRNA levels to 30-35 percent in independent clones and increased resistance to eflornithine >five-fold. Genome-wide screens with this library allow an unbiased approach to gene discovery, are extremely rapid and do not exclude essential genes.

15780. **Serricchio, M. & Butikofer, P.,** 2011. *Trypanosoma brucei*: a model microorganism to study eukaryotic phospholipid biosynthesis. *Febs Journal*. **Published online, February 3.**

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Although the protozoan parasite *Trypanosoma brucei* can acquire lipids from its environment, recent reports have shown that it is also capable of *de novo* synthesis of all major phospholipids. In this paper, we provide an overview of the biosynthetic pathways involved in phospholipid formation in *T. brucei* and highlight differences to corresponding pathways in other eukaryotes, with the aim to promote trypanosomes as an attractive model organism to study lipid biosynthesis. We review that *de novo* synthesis of phosphatidylethanolamine involving CDP-activated intermediates is essential in *T. brucei* and that a reduction in its cellular content affects mitochondrial morphology and ultrastructure. In addition, we highlight that reduced levels of phosphatidylcholine inhibit nuclear division, suggesting a role for PC formation in the control of cell division. Furthermore, we discuss possible routes leading to phosphatidylserine and cardiolipin formation in *T. brucei* and review the biosynthesis of phosphatidylinositol, which seems to take place in two separate compartments. Finally, we emphasize that *T. brucei* represents the only eukaryote so far that synthesizes all three

sphingophospholipid classes, sphingomyelin, inositol phosphorylceramide and ethanolamine phosphorylceramide, and that their production is developmentally regulated.

15781. **Springer, A. L., Bruhn, D. F., Kinzel, K. W., Rosenthal, N. F., Zukas, R. & Klingbeil, M. M.,** 2011. Silencing of a putative inner arm dynein heavy chain results in flagellar immotility in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **175** (1): 68-75.

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The *Trypanosoma brucei* flagellum controls motility and is crucial for cell polarity and division. Unique features of trypanosome motility suggest that flagellar beat regulation in this organism is unusual and worthy of study. The flagellar axoneme, required for motility, has a structure that is highly conserved among eukaryotes. Of the several dyneins in the axonemal inner arm complex, dynein f is thought to control flagellar waveform shape. A *T. brucei* gene predicted to encode the dynein f alpha heavy chain, *Tb*DNAH10, was silenced using RNA interference in procyclic *T. brucei* cells. This resulted in immotile flagella, showing no movement except for occasional slight twitches at the tips. Cell growth slowed dramatically and cells were found in large clusters. Microscopic analysis of silenced cultures showed many cells with detached flagella, sometimes entangled between multiple cells. DAPI staining showed an increased frequency of mis-positioned kinetoplasts and multinucleate cells, suggesting that these cells experience disruption at an early cell cycle stage, probably secondary to the motility defect. TEM images showed apparently normal axonemes and no discernable defects in inner arm structure. This study demonstrates the use of RNAi as an effective method to study very large genes such as dynein heavy chains (HCs), and the immotility phenotype of these dynein knockdowns suggests that an intact inner arm is necessary for flagellar beating in *T. brucei*. Since analogous mutants in *Chlamydomonas reinhardtii* retain motility, this phenotype likely reflects differences in requirements for motility and/or dynein assembly between the two organisms and these comparative studies will help elucidate the mechanisms of flagellar beat regulation.

15782. **Tripodi, K. E., Menendez Bravo, S. M. & Cricco, J. A., 2011.** Role of haeme and haeme-proteins in trypanosomatid essential metabolic pathways. *Enzyme Research*, **2011**: 873230.

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Around the world, trypanosomatids are known for being etiological agents of several highly disabling and often fatal diseases like Chagas disease (*Trypanosoma cruzi*), leishmaniasis (Leishmania spp.), and African trypanosomiasis (*Trypanosoma brucei*). Throughout their life cycle, they must cope with diverse environmental conditions, and the mechanisms involved in these processes are crucial for their survival. In this review, we describe the role of haeme in several essential metabolic pathways of these protozoans. Notwithstanding trypanosomatids lack of the complete haeme biosynthetic pathway, we focus

our discussion on the metabolic role played by important haeme-proteins, like cytochromes. Although several genes for different types of cytochromes involved in mitochondrial respiration, polyunsaturated fatty acid metabolism, and sterol biosynthesis are annotated at the Tritryp Genome Project, the encoded proteins have not yet been deeply studied. We pointed our attention into relevant aspects of these protein functions that are amenable to be considered for rational design of trypanocidal agents.

15783. **Vanichtanankul, J., Taweechai, S., Yuvaniyama, J., Vilaivan, T., Chitnumsub, P., Kamchonwongpaisan, S. & Yuthavong, Y., 2011.** Trypanosomal dihydrofolate reductase reveals natural antifolate resistance. *ACS Chemical Biology.* **E publication ahead of print June 16.**

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Dihydrofolate reductase (DHFR) is a potential drug target for *Trypanosoma brucei*, a human parasite, which is the causative agent for African sleeping sickness. No drug is available against this target, since none of the classical antifolates such as pyrimethamine (PYR), cycloguanil or trimethoprim are effective as selective inhibitors of *T. brucei* DHFR (*Tb*DHFR). In order to design effective drugs that target *Tb*DHFR, co-crystal structures with bound antifolates were studied. On comparison with malarial *Plasmodium falciparum* DHFR (*Pf*DHFR), the co-crystal structures of wild-type *Tb*DHFR reveal greater structural similarities to a mutant *Pf*DHFR causing antifolate resistance than the wild-type enzyme. *Tb*DHFR imposes steric hindrance for rigid inhibitors like PYR around Thr86, which is equivalent to Ser108Asn of the malarial enzymes. In addition, a missing residue on *Tb*DHFR active-site loop together with the presence of Ile51 widens its active site even further than the structural effect of Asn51Ile, which is observed in *Pf*DHFR structures. The structural similarities are paralleled by the similarly poor affinities of the trypanosomal enzyme for rigid inhibitors. Mutations of *Tb*DHFR at Thr86 resulted in ten-fold enhancement or seven-fold reduction in the rigid inhibitors affinities for Thr86Ser or Thr86Asn, respectively. The co-crystal structure of *Tb*DHFR with a flexible antifolate WR99210 suggests that its greater affinity result from its ability to avoid such Thr86 clash and occupy the widened binding space similarly to what observed in the *Pf*DHFR structures. Natural resistance to antifolates of *Tb*DHFR can therefore be explained, and potential antifolate chemotherapy of trypanosomiasis should be possible taking this into account.

15784. **Verner, Z., Cermakova, P., Skodova, I., Kriegova, E., Horvath, A. & Lukes, J., 2011.** Complex I (NADH:ubiquinone oxidoreductase) is active in but non-essential for procyclic *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **175** (2): 196-200.

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The requirement of complex I (NADH:ubiquionone oxidoreductase) for respiration in *Trypanosoma brucei* is controversial. Recent identification of homologues of its subunits in mitochondrial proteome resolved the question of its presence or absence. However, with one

exception, no data have been available concerning the function(s) of complex I or its subunits. Here we present a functional RNAi study of three (NUBM, NUKM, NUEM) putative subunits of this complex. Although no changes were detected in growth, mitochondrial membrane potential or reactive oxygen species production in cell lines depleted for target transcript, the NUBM and NUKM RNAi knock-downs showed decreased specific NADH:ubiquinone oxidoreductase activity. Moreover, glycerol gradients of all cell lines revealed the presence of two distinct peaks of NADH dehydrogenase activity, with shifted sensitivity to inhibitors of complex I upon RNAi induction. Thus complex I is not only present in the procyclic stage of *T. brucei* 29-13 strain, but it does participate in electron transport chain.

15785. **Vigueira, P. A. & Paul, K. S., 2011.** Requirement for acetyl-CoA carboxylase in *Trypanosoma brucei* is dependent upon the growth environment. *Molecular Microbiology*, **80** (1): 117-132.

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Trypanosoma brucei, the causative agent of human African trypanosomiasis, possesses two fatty acid synthesis pathways: a major *de novo* synthesis pathway in the ER and a mitochondrial pathway. The two-carbon donor for both pathways is malonyl-CoA, which is synthesized from acetyl-CoA by acetyl-CoA carboxylase (ACC). Here, we show that *T. brucei* ACC shares the same enzyme architecture and moderate approximately 30 percent identity with yeast and human ACCs. ACC is cytoplasmic and appears to be distributed throughout the cell in numerous puncta distinct from glycosomes and other organelles. ACC is active in both bloodstream and procyclic forms. Reduction of ACC activity by RNA interference (RNAi) resulted in a stage-specific phenotype. In procyclic forms, ACC RNAi resulted in 50-75 percent reduction in fatty acid elongation and a 64 percent reduction in growth in low-lipid media. In bloodstream forms, ACC RNAi resulted in a minor 15 percent decrease in fatty acid elongation and no growth defect in culture, even in low-lipid media. However, ACC RNAi did attenuate virulence in a mouse model of infection. Thus the requirement for ACC in *T. brucei* is dependent upon the growth environment in two different life cycle stages.