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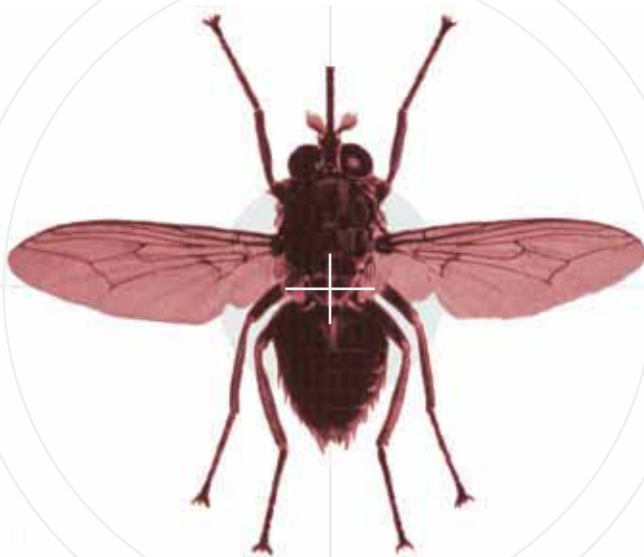
PAAT

Programme
Against
African
Trypanosomosis



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TSETSE AND TRYPANOSOMOSIS INFORMATION



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TSETSE AND TRYPANOSOMOSIS INFORMATION

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TSETSE AND TRYPANOSOMOSIS INFORMATION

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

The half-yearly periodical is prepared for publication, in both English and French editions, by the Food and Agriculture Organization of the United Nations. Each annual volume consists of two parts and an index. Subscription is free for all recipients engaged in trypanosomosis research and control, and requests for enrolment may be sent to: Ms Maria Grazia Solari, AGAH, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy (fax +39 06 5705 3057; e-mail MariaGrazia.Solari@fao.org).

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

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

Distribution dates and copy deadlines

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

The Index will be distributed as soon as possible after the completion of each volume.

ABBREVIATIONS USED IN TTI

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

Organizations

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

Tsetse and Trypanosomosis Information

| | |
|---------|--|
| FITCA | Farming in Tsetse Control Areas of Eastern Africa |
| GTZ | Deutsche Gesellschaft für Technische Zusammenarbeit |
| IAEA | International Atomic Energy Agency |
| IBAR | Interafrican Bureau for Animal Resources |
| ICIPE | International Centre of Insect Physiology and Ecology |
| ICPTV | Integrated Control of Pathogenic Trypanosomes and their Vectors |
| IFAD | International Fund for Agricultural Development |
| ILRI | International Livestock Research Institute |
| INRA | Institut National de Recherche Agronomique |
| IPR | Institut Pierre Richet |
| IRD | Institut de Recherche et de Développement (formerly ORSTOM) |
| ISCTRC | International Scientific Council for Trypanosomiasis Research and Control |
| ISRA | Institut Sénégalais de Recherches Agricoles |
| ITC | International Trypanotolerance Centre |
| KARI | Kenya Agricultural Research Institute |
| KETRI | Kenya Trypanosomiasis Research Institute |
| LCV | Laboratoire Central Vétérinaire |
| LNERV | Laboratoire National de l'Élevage et de Recherches Vétérinaires |
| LSHTM | London School of Hygiene and Tropical Medicine |
| MRC | Medical Research Council |
| MRU | Mano River Union |
| NITR | Nigerian Institute for Trypanosomiasis Research |
| NRI | Natural Resources Institute |
| OCCGE | Organisation de Coopération et de Coordination pour la Lutte contre les Grande Endémies |
| OCEAC | Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale |
| OGAPROV | Office Gabonais pour l'Amélioration de la Production de la Viande |
| OIE | Office International des Epizooties |
| OMVG | Organisation pour la Mise en Valeur du Fleuve Gambie |
| PAAT | Programme against African Trypanosomosis |
| PATTEC | Pan-African Tsetse and Trypanosomiasis Eradication Campaign |
| PRCT | Projet de Recherches Cliniques sur la Trypanosomiase |
| RDI | Rural Development International |
| RUCA | Rijksuniversitair Centrum Antwerpen |
| SADC | Southern African Development Community |
| SIDA | Swedish International Development Authority |
| SODEPRA | Société pour le Développement des Productions Animales |
| TDR | UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases |
| TDRC | Tropical Diseases Research Centre |
| TPRI | Tropical Pesticides Research Institute |
| TTRI | Tsetse and Trypanosomiasis Research Institute |
| UNDP | United Nations Development Programme |
| USAID | United States Agency for International Development |
| USDA | United States Department of Agriculture |
| UTRO | Uganda Trypanosomiasis Research Organisation |
| WHO | World Health Organization |

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SECTION A – NEWS

REPORT FROM THE 30TH INTERNATIONAL SCIENTIFIC COUNCIL FOR TRYPANOSOMIASIS RESEARCH AND CONTROL CONFERENCE (ISCTRC), KAMPALA, UGANDA, 21–25 SEPTEMBER 2009

1. International Organizations

Moderator: Prof. Ahmed Elsawalhy

Rapporteur: Dr. Raffaele Mattioli

The International Organizations presented their activities over the last two years and came up with the following recommendations:

1.1 FAO

The meeting acknowledges the complexity of the tsetse and trypanosomosis problem and notes that various aspects underpinning livestock-agriculture development, including land use and natural resources and socio-economic development are affected by the presence of the disease.

The meeting welcomes the work of FAO in:

- generating standardized information pertaining to animal health, livestock and agricultural production systems, environment and agro-ecology in areas infested by tsetse fly;
- providing assistance to tsetse affected countries in the formulation of policies, strategies, and guidelines for tsetse and trypanosomosis (T&T) interventions.

The meeting recommends:

- to increase efforts for greater and wider adoption of developed policies and guidelines for planning T&T field programmes;
- to pursue endeavours for increased efficacy and impact at field and country levels and for enhancing synergies with national, regional, and international entities and initiatives.

1.2 WHO

The meeting acknowledges the achievements made in the area of sleeping sickness control. It is, however, concerned by the lack of appropriate tools to develop adapted control methodologies, and the weaknesses of health systems to integrate sleeping sickness control and surveillance to sustain current results.

The meeting recommends:

- WHO to continue its support to countries to adapt control strategies taking into consideration the evolution of the disease;
- R&D groups to develop new diagnostic tools and drugs to ensure cost-effective, adapted and sustainable control strategies for sleeping sickness;

- to increase awareness and advocacy for decision-makers and donors to ensure that sleeping sickness is kept on their agenda.

The meeting notes that transmission rates of sleeping sickness are still high in areas of Central African Republic and Democratic Republic of Congo where security constraints hamper control activities.

The meeting recommends:

- NGOs that have previously been showing commitment and success in sleeping sickness control to continue providing support and maintain their efforts and assistance.

The meeting recognizes the leadership and efforts of WHO on mapping sleeping sickness distribution and acknowledges the assistance and support provided by FAO/PAAT; it welcomes the outcomes already obtained on the development of this tool.

The meeting recommends:

- countries to continue providing necessary inputs to WHO to complete the Atlas of Human African Trypanosomosis;
- WHO to provide countries with the necessary equipment and training to own the Atlas and be able to continue its updating for use as a tool for planning control activities and monitoring disease evolution.

1.3 PAAT

The meeting notes with appreciation the progress made by PAAT after the last ISCTRC conference, in the continuous production and wide distribution of the TTI, the PAAT Technical and Scientific Series, and the decision support information on the PAAT website for the T&T family. It welcomes the annual organization of the meetings of the Panel of PAAT Advisory Group Coordinators and of the Programme Committee. ISCTRC also welcomes the decision to review PAAT and its structures.

The meeting urges:

- that this review will result in greater consultation and coordination with project activities in countries implementing large-scale T&T projects in Africa;
- the use of the agreed harmonized guidelines/criteria for the selection of areas intended for T&T intervention to ensure the achievement of sustainable agriculture and rural development (SARD), improved human and animal livelihood, and poverty alleviation.

1.4 DNDi

The meeting notes the unbalanced ratio between the global disease burden and the development of new drugs for tropical diseases, including human African trypanosomosis, and acknowledges the achievements and actions taken by DNDi in addressing the needs for novel treatments of neglected tropical diseases.

The meeting recommends:

- DNDi to further progress its strategy and enlarge its strategic partnership with the private sector and WHO for joint development of new drugs and/or treatment protocols for sleeping sickness.

1.5 FIND

The meeting commends the actions undertaken by FIND in the development of diagnostic tools for poverty related diseases of public health importance such as sleeping sickness and recognizes the need to develop accurate diagnostic tools applicable in endemic (rural) areas. The meeting also notes the MoU between FIND and PATTEC for advocacy.

The meeting recommends:

- FIND to pursue the process of development, evaluation, demonstration, and implementation of diagnostic tests for human African trypanosomosis.

1.6 PATTEC

The ISCTRC notes with pleasure the establishment of the monitoring and evaluation (M&E) Unit in the PATTEC Coordination Office. This Unit has been able to monitor and evaluate the performance of the six countries currently implementing the AfDB-funded projects, thus providing information on their levels of individual project achievements in implementation. The ISCTRC commends this step and hopes that by this process, these projects will be challenged so that success stories will be reported and recorded. The ISCTRC also notes that those countries currently implementing PATTEC projects and the second batch of countries have pledged funds for the next phase.

Based on the experience gained in the implementation by the current beneficiaries of the AfDB loans, the meeting urges:

- these countries to immediately release part of their pledges to be used for the collection of standardized baseline data, crucial to the success of the projects.

2. Research Centres/Institutions

The meeting notes with appreciation the research themes developed by the international and regional research centres/institutions (CIRDES, ICIPE, ILRI) leading to provision of new insights and increased scientific knowledge supporting planners in conceiving T&T intervention projects and translating them into field application for improved efficiency of control techniques. However, the meeting also notes that there is no silver bullet and magic solutions for the creation of T&T free zones. An array of tools has been developed and used with different degrees of success. In order to achieve greater impact and cost-effective disease management, eventually leading to disease elimination,

The meeting recommends:

- the research with impact to focus on the development of cost-effective control measures based also on the principles of integrated pest and disease management and using a phased conditional approach;
- the technology to be developed and used be environmentally acceptable and economically justified;

- proper, detailed baseline surveys be conducted, data collected and analysed as necessary pre-requisites for supporting the formulation of T&T field intervention campaigns;
- to use the international/regional research centres/institutions as entities for coordinating, harmonizing and collating national and regional research data on T&T and related matters, and establishing data banks that are publicly accessible.

3. PATTEC

Moderator: Dr. Issa Sidibe

Rapporteur: Dr. Solomon Hale Mariam

Twenty three papers were presented during a special session that was for the first time devoted entirely to reports on activities within the PATTEC initiative. Presenting the overall progress report on PATTEC, Dr John Kabayo, the AU-PATTEC Coordinator, presented a summary of the activities undertaken within the PATTEC initiative during the past two years since the ISCTRC Conference in Angola. He recounted the progress made, including the successful eradication of tsetse and trypanosomosis in Botswana and Namibia, and the various activities that have been carried out aimed at consolidating the full extent and purposes of the campaign. These activities included progress in the execution of tsetse and trypanosomiasis eradication projects in Burkina Faso, Kenya, Ethiopia, Ghana, Mali and Uganda using support in the form of soft loans from the African Development Bank; initiation of self-funded tsetse eradication activities in Angola and Zambia using the sequential aerosol technique (SAT); the development of several multi-national bankable project proposals (including: Mozambique, South Africa, and Swaziland; Sudan and Ethiopia; Uganda and Sudan; Chad, CAR, Cameroon and Nigeria; Tanzania, Burundi and Rwanda; Burkina Faso, Nigeria and Togo); extensive consultations with several affected countries; resources mobilization; and monitoring, evaluation and training.

He appreciated the financial and technical support provided to the PATTEC initiative by various partners and urged those willing to help to liaise with the PATTEC Coordination Office in planning intended interventions for maximum synergy, harmony, and coordination. He expressed his gratitude for the sense of commitment shown by affected countries; his satisfaction for the cooperation and spirit of the African Union in the planning and execution of PATTEC projects; and shared his feelings of optimism and hope that the objective of the PATTEC initiative will be realized.

Papers were also presented on the progress made by the PATTEC Coordination Office in the area of strengthening advocacy activities; monitoring and evaluation efforts; the development of the PATTEC website; and the dynamic database systems for PATTEC. It was also reported that PATTEC had signed MoUs with FIND to strengthen advocacy activities and with WHO on cooperation in training and capacity-building activities; these were gratefully acknowledged. The PATTEC Coordination Office is in the process of establishing regional PATTEC Coordination Offices to enhance cooperation on PATTEC activities in response to increasing levels of intervention within the PATTEC initiative.

From the 23 papers, 18 papers give more details on country on-going activities including in the six first phase countries which are sponsored by AfDB, the national programmes started with their own government funds, and the multinational regional project draft to be submitted for financing. Many countries expressed their commitment to support PATTEC initiatives and to start their own activities. Following the presentations of the 23 papers, a brief discussion ensued.

The following recommendations were made:

- The 30th. ISCTRC Conference commends with satisfaction the progress and achievements so far made by PATTEC and calls upon other tsetse-affected countries to join the PATTEC initiative if they have not already done so.
- While PATTEC welcomes partners willing to provide support in the implementation of PATTEC it is essential that those partners discuss the area in which support is anticipated with the PATTEC Coordination Office for the purpose of effective coordination.
- The conference noted with satisfaction that all projects which were presented under the umbrella of the PATTEC initiative demonstrated the strength and consolidation of the PATTEC programme. The PATTEC session should be a permanent feature of future ISCTRC conferences and should include country reports.
- PATTEC programmes should also put emphasis on non-tsetse transmitted trypanosomosis in the future.
- Noting that the decision of the African Heads of State and Government will only end when trypanosomiasis is eliminated from Africa, and considering that research institutions are engaged in activities to support the eradication process, it is recommended that the AU Commissioner for Rural Economy and Agriculture considers the appropriateness of ISCTRC as the Technical Advisory Council to PATTEC.
- Considering the trans-boundary nature of tsetse distribution and the divergence of T&T management systems between countries, it is recommended that project proposals for the creation of tsetse-free areas be developed and operated independently for tsetse belts that occupy more than one country as sub-regional projects.

4. Country Reports

Moderator: Theophile Josenando

Rapporteur: Louis Banipe

Given the fact that most countries are involved in the PATTEC programme, this session was devoted to some aspects which were not well highlighted during the PATTEC presentations. Reports were received from six countries and from the East African Trypanosomiasis Control Network. All reports highlighted the increasingly important consideration given to trypanosomosis and vector control. The meeting also welcomed the increasing commitment of NGOs in the control activities and drew attention to the fact that a lot is still to be done.

The six countries providing reports were:

- *Angola*, which highlighted the enormous efforts being made by the Government and various partners not only for identifying the disease but also to control it. Angola expressed concerns over the proposed delimitation area designed by PATTEC within the ongoing sub-regional project with the DRC "Cleaning-up of pastoral areas of Kasai and Luanda provinces". In fact, Angola pointed out that the delimitation does not take care of the reality and requested that henceforth, extensive studies should be carried out to design consistent proposals.

- *The Democratic Republic of Congo* underlined once more that three quarters of the recently reported cases of human African trypanosomiasis are from the DRC. This requires further sustained attention. Welcoming the multifaceted support, the DRC wished that the support is maintained and hoped that the control is incorporated into traditional structures in charge of health aiming at improvement of cases' care.
- *Uganda*, presenting all the gift nature offered the country, namely the rich hydrographical net, pointed that this remains the heart of many diseases, in particular trypanosomosis and its vectors. Thanking the partners, it pleaded that the support be sustained to help achieving the effective control of the plague.
- *Tanzania* indicated that wildlife cases remain a concern with an important infestation of livestock at the edge of protected areas. The African Human Trypanosomosis (AHT) centre of Serengeti, formerly nearly extinct, is now revived. Personnel training needs to be pursued and even backed up by various actors.
- *Sudan* made a special presentation on the situation in the South. Faced with a war situation prevailing in that locality, NGOs are increasingly leaving the area and creating a vacuum that is not really filled. This worrisome situation which prevents the production of reliable reports of disease cases is not likely to reassure. In addition, efforts are being made to conduct a general census upon which field activities should be based.
- *Guinea (Conakry)* provided an update on ongoing studies and the difficulties to access targeted sites.
- *The Eastern Africa Network of Trypanosomosis (EANETT)*

During the presentation, it emerged that the Network was founded in 1999, but its activities started in 2000. It is the only research network on human African trypanosomosis (HAT) in the sub-region. The founding institutions involved are: The Swiss Tropical Institute (STI), KETRI (currently KARI-TRC, Kenya), TTRI (Tanzania), TMRI (Sudan), and LIRI (currently NaLIRRI, Uganda). Three other countries joined the Network. These are: Malawi, Zambia, and the Democratic Republic of Congo. The Network organizes scientific conferences once a year and also offers an opportunity for young scientists to intervene, share and be backed up in research activities on trypanosomiasis and tsetse flies. Young scientists in postgraduate education are encouraged to seize the opportunity.

The meeting recommends:

- that country reports should strictly follow the outline developed by the ISCTRC Secretariat;
- that the reports should only focus on the specified period.

5. Human African Trypanosomosis (HAT)

Moderator: Pere Simarro

Sessions 6 & 7: Basic Research on Trypanosomes and Diagnostics

Rapporteurs: Dawson Mbulamberi & Jose Ramon Franco

Sessions 6 and 7 focused on the diagnosis of human African trypanosomiasis, including some basic research on trypanosomes. Ten papers were presented during these sessions.

The first three presentations were related to basic research on the parasite (population genetics of *T. b. gambiense*, an improved *in vitro* medium for the bloodstream form of *T. b. gambiense*, and comparative genomic analysis of procyclic *T. b. rhodesiense* with DNA microarrays). All these presentations addressed the improvement of knowledge on the genetic characteristics of the trypanosome and the search for an improved culture medium. The subsequent presentations tried to demonstrate the impact of HAT on the sensitivity of HIV diagnostic tests and the public health implications of this impact. A study performed with samples coming from Mbuji-Mayi, (DRC) revealed a decrease in the sensitivity of the usual HIV rapid tests.

The presentation on immune trypanolysis revisited this test as described in 1995 and tried to explore the possibility of using it as tool for epidemiological decisions, with examples in some countries in West Africa. This presentation raised a number of questions and comments about the interpretation of seropositive individuals without parasitological confirmation, particularly those who spontaneously turn seronegative.

The presentations 3.06 and 3.07 were about research on new staging markers. The use of some proteins showed promising preliminary results, thus emphasizing the need to combine several markers such as CXCL10, CXCL8 and H-FABP.

Presentations 3.08 and 3.09 dealt with molecular methods of sleeping sickness diagnosis (NASBA and Trypanozoon OligoC-strip). They gave a review of the efforts to standardize and simplify these tools, highlighting the use of oligochromatography as a simpler amplicon method.

The last presentation of the session addressed the use of algorithms combining different field criteria (presence of trypanosomes and white blood cell count in CSF) with the aim of shortening the follow-up period. The result seemed to support the possibility of shortening the follow up-period of second stage *gambiense* HAT patients. However, the application of these results was limited because of the peculiarity of the cohort studied. The other limiting factor for the application of these results was the unusually high failure rate of treatment with melarsoprol. There was a possibility of these results being different if another treatment had been used.

Recommendations:

- further evaluation of the impact of HAT on HIV rapid tests, coordinating with HIV programmes for the assessment of HIV tests in HAT patients;
- to assess the utility of the immune trypanolysis tests in other different epidemiological situations; comparatively evaluate and standardize the different PCR tests available to highlight their real value in the diagnosis process;
- to encourage the efforts made to shorten the follow-up period and recommend the application of the proposed algorithms in different cohorts (including stage 1 cases), considering always the variability according to the drug used for treatment.

Session 8: Epidemiology of HAT

Rapporteurs: Dawson Mbulamberi & Diarra Abdoulaye

Four presentations were made during this session. Two presentations dealt with epidemiology, one with long-term follow up of sleeping sickness patients, and one with the

impact of interventions targeting the control of animal reservoir of *T. b. rhodesiense* within the framework of public/private partnership and community involvement.

The presentations on disease epidemiology attempted to describe the current trends of the disease, the ongoing control activities, and the perspectives in terms of surveillance and control. In general, there seems to be an increase in control activities leading to decreases in the number of reported cases over the past ten years. However, it was observed that there was still low coverage of endemic areas.

Parameters underlying the epidemiology of *rhodesiense* sleeping sickness, as well as the role of domestic reservoir hosts were described.

The effectiveness of PPPs (public-private partnerships) and the very important role of communities in controlling the animal reservoir were described as an important factor for sustainability of activities and their expansion. Sleeping sickness patients who refused to be treated were regularly followed up by using serological and parasitological methods to monitor the evolution of the disease without treatment linked to the individual susceptibility (human trypanotolerance or self cure). Some of them died after developing second stage disease while some cured spontaneously and a few remained positive as asymptomatic carriers with low parasitaemias.

Recommendations:

The meeting acknowledged the complex epidemiological features of the *rhodesiense* form of sleeping sickness and its zoonotic component, and recommended that:

- *T. b. rhodesiense* endemic countries should develop integrated control approaches including human, animal and vector components, fostering PPPs and community involvement.

Session 9: Disease distribution and Treatment of HAT

Rapporteurs: Dawson Mbulamberi & Jose Postigo

Seven presentations were made during this session. Two presentations dealt with epidemiology and five with treatment.

One presentation on epidemiology in West Africa showed how the spatial evolution of HAT over the past 100 years seems to have moved from the North to the South, that it has disappeared from savannah areas, and is currently occurring in forest and mangrove areas. The second presentation on epidemiology showed the current status of the Atlas of HAT which includes three quarters of the cases reported during the period 2000–2008 already mapped at the village level. Representatives from Angola and DRC expressed their full support for this initiative and committed themselves to share their data with WHO in order to finalize the mapping exercise. The final outcome of the Atlas will be made available in the public domain through WHO and FAO/PAAT websites.

Out of the five presentations on the subject of treatment, the first one dealt with the nifurtimox-eflornithine combination therapy clinical study and its implications for the national sleeping sickness programme in Uganda. The second presentation showed the results of a study to use the 10-day melarsoprol schedule for second stage *T. b. rhodesiense* patients. The study concluded that the 10-day schedule does not expose patients to a higher risk of serious adverse events or death and that it has high efficacy. The third presentation dealt with the Phase III trial on the safety and efficacy of pafuramidine maleate (DB289) for the

treatment of first stage *T. b. gambiense* sleeping sickness. The study had to be stopped due to severe adverse events associated with the new medicine. The fourth and fifth presentations dealt with molecular parasitological studies carried out in relapsed *T. b. gambiense* patients. The studies did not yield conclusive results on the molecular mechanisms leading to relapses.

Recommendations:

Recalling the recommendation of the 27th ISCTRC meeting held in Pretoria, South Africa in 2003 to adopt the 10 days' course of melarsoprol for treatment of late stage *T. b. gambiense* sleeping sickness and to request undertaking similar studies for *T. b. rhodesiense* sleeping sickness, on the basis of the results of the clinical trials conducted in Tanzania and Uganda,

the ISCTRC recommends:

- disease-endemic countries to adopt the abridged 10-day melarsoprol schedule as the new regimen for the treatment of the late-stage *T. brucei rhodesiense* sleeping sickness.

Considering that nifurtimox-eflornithine combination treatment for the second stage of *T. brucei gambiense* infections was included in the WHO Essential List of Medicines in May 2009 after a successful clinical trial developed by a collaborative partnership carried out in Congo and the Democratic Republic of the Congo, and that a similar clinical trial in Uganda also shows preliminary positive results of this combination treatment, ***the ISCTRC recommends:***

- countries to include nifurtimox-eflornithine combination as a treatment option in their national protocols to treat sleeping sickness and initiate the process of ordering this combination treatment through WHO.
- WHO to make available to countries all necessary training, information and support to implement this combination treatment.

6. African Animal Trypanosomiasis (AAT)

Moderator: A. Ilemobade

Rapporteur: A. H. A Rahman

A keynote address was given by Prof. Eli Katunguka-Rwakishaya who gave a historical perspective of the disease and the efforts made into its control, stressing the need for concerted multidisciplinary approaches to control the disease. Out of the 15 papers accepted for oral presentation only 12 were presented. The papers addressed five major areas, epidemiology and baseline data collection, trypanocides, trypanotolerance, drug quality, and safety assurance and chemotherapy.

In the area of animal trypanosomosis epidemiology, surveys and base line data collection four papers were presented:

- i. *Baseline survey on bovine trypanosomosis and chemo-resistance in the Sikasso Cercle of Mali as a preamble to a vector control operation.*
- ii. *Bovine antibody response directed against *Glossina saliva*: An epidemiological marker of cattle exposure to tsetse bites*
- iii. *Donkey trypanosomiasis, their vectors, Helminthiasis in Pate Island of Lamu District, Kenya.*

- iv. *Sero-epidemiology of dourine in Bale Highlands of Oromyia Region, Ethiopia.*

In the area of drug resistance the following papers were presented:

- i. *Occurrence of diminazene, homidium, and isometamidium resistant T. congolense strains isolated from cattle in Ghibe Valley and Lake Abaya localities, South-west Ethiopia.*
- ii. *Field detection and evaluation of trypanocidal drug resistance in the Sissala East District of Northern Ghana.*
- iii. *Field detection of chemo-resistance to isometamidium and diminazene in the region of Boucle Du Mouhoun, Burkina Faso.*

In the area of drug quality and safety assurance two papers were presented:

- i. *Determination of diminazene aceturate in animal tissues by enzyme-linked immunosorbent assay (ELISA).*
- ii. *Poor quality and fake trypanocidal drugs, a real threat for a sustainable and profitable livestock production in sub-Saharan Africa.*

In the area of chemotherapy two papers were presented:

- i. *Cymelarsan effectively cures trypanosomes in dourine infections and with no relapses*
- ii. *Identification and experimental validation of potential drug targets in Trypanosoma brucei.*

The baseline data reported in the presentations included trypanosomiasis prevalence and tsetse densities. Also the surveys included the non-tsetse transmitted *T. equiperdum*. The antibody response directed against *Glossina* saliva was suggested to be used as a marker of the exposure of cattle to tsetse flies. Drug resistance against trypanocidals was reported from Ethiopia, Burkina Faso and Ghana, while the paper from Ethiopia recommended the use of Cymelarsan in the treatment of dourine. Methionine synase and homocysteine methyltransferase were reported to be potential drug targets against *T. brucei* in one of the presentations. Two presentations warned about the presence of residues of diminazene and other trypanocides in addition to the fake drugs in the different tissues of animals and about their severe implication on both animal health and food safety.

In the area of trypanotolerance, there was one paper under the title:

- i. *Effect of N'Dama origin marker alleles on trypanotolerance in a backcross cattle population under natural tsetse and trypanosomosis challenge.*

Recommendations:

Recognizing the alarming and long-lasting problem of the presence of substandard veterinary drugs in the free market with specific trypanocides which are among the causes of drug resistance in chemotherapy, ***the ISCTRC recommends that the Food and Drug Agencies in the African countries:***

- inspect, follow up, and take legal action against manufacturers and importers who do their business against Pharmaceutical SOPs and rules of quality and safety assurance;

- The ISCTRC recognizes with appreciation the success WHO/FIND has achieved in developing diagnostics for HAT requesting them to respond similarly to the diagnostic needs in AAT;
- Referring to reports on the widespread resistance to all the available trypanocidals, FAO is requested to assist in developing a standard protocol that is to be applied in the different countries to reduce the presence of drug resistance;
- Management of this problem requires concerted efforts, using parasitological and molecular tools to quantify the level of resistance, its distribution and where possible conduct remedial action

7. *Glossina* Biology

Moderator: Ambrose Gidudu

Rapporteur: Joyce Daffa

Eleven papers presented in this session. It was noted from the lead paper that there is no easy single method that can be applied alone, and that not much work has been done on savannah *Glossina* spp. Due to the feeding behaviour of tsetse flies, insecticide application in cattle can be restricted to legs only. There was a proposition that the target size can be reduced from 1 x 1 m to 0.5 x 0.5 m as the efficacy is almost the same. There are new advances in tsetse genomics and bioinformatics, and on the molecular biology specifically of fungus and bacteria towards improving tsetse control. Symbionts have expressed trypanocidal agents. It was noted that due to technical problems in rearing and colonization of different species, SIT is not available for the current eradication programme period. Other papers covered: detection of salivary gland hypertrophy virus (SGHV); developments on research for tsetse and host attractants; nutritional stress in tsetse for trypanosome susceptibility; improved visual baits; genetic diversity among geographically separated tsetse populations; and management and control of vectors for improved productivity. Furthermore, spatial analytical tools and mathematical models can be used in baseline data collection to develop stratified entomological sampling protocols. Finally there are areas which were previously announced to be free of tsetse but are currently infested by tsetse flies.

Recommendations drawn from these presentation and discussion were:

- it was noted that due to technical problems in rearing and colonization of different species, SIT is not available for the current eradication programme period; therefore countries were urged to undertake area-wide integrated tsetse suppression and eradication techniques until the SIT is available for field use;
- insecticide treated target/screen sizes may be reduced; however, this should be further investigated;
- undertake further investigation on fungi and bacteria for future use in tsetse control programmes;
- conduct surveys to monitor tsetse flies in tsetse free countries to ensure their current status in these areas;
- undertake area-wide integrated tsetse control/eradication until the SIT is available for use.

8. Socio-Economics

Moderator: Prof. Hippolyte Affognon

Rapporteur: Dr Cecchi Giuliani

During this session five presentations were given.

The first was entitled “*Community-based livestock health delivery services: The case of the medium to high tsetse/trypanosomiasis challenge areas of the Ghibe Valley, south west Ethiopia*”. This presentation focused on an ILRI project in the Ghibe Valley of south western Ethiopia where pour-on insecticide treated-cattle were used, and improved income and welfare of farmers was achieved. The method was well accepted by the community which was willing to pay for the treatment. ILRI facilitated a series of consultation workshops with farmers’ representatives and service providing institutions with a view towards institutionalizing sustainable service delivery. Farmers’ animal health service cooperatives were formed, which were successfully implemented for about five years. Such community-based animal health delivery was the first of its kind in Ethiopia and experiences from such institutional innovations could be scaled out/up to areas with similar challenges.

The second presentation (i.e. “*Do social networks influence livestock keepers’ know-how on animal trypanosomiasis and its control?*”) concerned social networks and their contribution to the diffusion of cattle farmers’ knowledge on animal trypanosomiasis and its control in Solenzo in Burkina Faso. A knowledge, attitude and practices (KAP) survey and a social network analysis were conducted in two villages where all cattle farmers in both villages were involved. A knowledge score was developed as a percentage point of total knowledge and a regression analysis conducted. Results suggest that besides other means of information dissemination, farmer-to-farmer information sharing should be promoted in order to improve farmers’ know-how on animal trypanosomiasis and its control.

The third presentation was entitled “*Livelihood strategies in endemic livestock breeds based production systems: Trends, tradeoffs and implications*” and concentrated on trypanotolerant endemic ruminant livestock breeds, whose relative population is decreasing as a result of both increased crossbreeding with Zebu cattle and Sahelian sheep, and degraded habitat due to forest conversion and bushfires. These trends suggest tradeoffs between livelihoods (cross-breeding and cotton cultivation) and ecosystem preservation (endemic ruminant genetic resources and their habitat). The paper used a Participatory Rural Appraisal in selected communities to look into the current trends, tradeoffs, and implications of observed breeding strategies and natural resource management. Results indicated that trends in habitat quality tend to drive changes in breed composition at the site levels but also changes (tradeoffs) in livelihoods (income generating activities). Habitat degradation as suggested by the results is related to an increase or decrease of particular animal breeds. Furthermore, the analysis revealed that livelihood options were largely defined by the assets base (resources), which were mostly common property in the study areas (in the Gambia). Community-based management of natural resources was indicated as “critical” to sustainable endemic ruminant livestock, natural resources and livelihoods.

The fourth presentation was entitled “*Trypanocidals cost as an economic parameter in the socio-economic surveys of animal trypanosomiasis in the Sudan*”. For this study, surveys were conducted in two tsetse-infested areas in the Sudan (one in the Blue Nile State and one in Central Equatoria State) to estimate the cost of trypanosomiasis treatment. Both areas possess huge numbers of livestock, particularly cattle; and are infested with several species of

tsetse flies. Questionnaires and interviews of community leaders and group discussions were used for data collection. Usage of Berenil (diminazene aceturate) was found to be at an average rate of 2.15 doses/animal/year, Homidium (ethidium bromide) at average rate of 1.9 and Antrycide (quinopyramine sulphate) at average rate of 0.45. The cost of trypanosomiasis treatment amounted to 7.9 percent and 6.3 percent of the gross livestock production cost in the two study areas. The results of the study indicate that trypanosomiasis treatment cost may be used as a tool for assessing the economic impact of African animal trypanosomiasis.

The last paper was entitled “*Improving food security through facilitation of community-based management of trypanotolerant cattle in the high disease challenge Ghibe Valley*”. The study was aimed at facilitating community action-learning in the participatory screening and verification of allegedly trypanotolerant cattle breeds from traditionally managed herds in four villages in the Ghibe valley. Animals were identified that had better tolerance to trypanosomiasis, as measured in terms of less or even no infection, maintenance of reasonably high PCV values after infection, and the need for only few trypanocidal treatments in a year. Participating communities also recognized these attributes of their animals, and accepted the results as true. They have expressed strong interest for continuation of activities initiated by this project. All the participating farmers recognized the genetic basis of trypanotolerance, and those farmers who own selected animals started to record pedigrees of these animals to help them in selecting replacement breeding animals. A series of village-level and regional consultation workshops was held. The reporting-back and policy dialogue workshop which were held discussed the outcomes of the study, and recommended that the process of screening continues. A unanimous recommendation was made to try to encourage farmers to use the established farmers groups for enhanced breeding and reproduction of selected animals.

Recommendations:

It was noted that the socio-economic component of the ISCTRC meeting is getting weaker and weaker, that few papers were presented, and that many participants were absent.

- It was recommended that projects and programmes developed for trypanosomiasis and its control put more emphasis on the socio-economic component and encourage participation in ISCTRC meetings.
- The meeting recognized the need for countries to have flexible policies to allow the communities and their partners to organize themselves in groups and play active roles in the sustainable management of tsetse and trypanosomiasis control.
- The meeting recognized the need for appropriate support by projects and the communities and their partners engaged in them through income generation practices using their own resources.
- The meeting encouraged and supported the dissemination of successful experiences to communities and their partners.

9. Land Use and Environment

Moderator: Dr Okoth O. Josue

Rapporteur: Joseph Maitima

Recommendations:

- Realizing the importance of climate change on the dynamics of ecosystems that determine the distribution of tsetse habitats, the meeting recommended further work to show the impacts of climate change on future habitats of tsetse and the implications on PATTEC activities.
- The meeting recommended harmonization of socio-economic impacts assessment across regions so that PATTEC achievements can be compared across regions, across tsetse belts and across countries.
- The meeting recommended more training of communities in PATTEC working sites on the proper use of chemicals to ensure that chemicals used in tsetse control are used safely and that trypanocides are applied to animals using the correct procedures.

10. Posters

Moderator: Dr. Mamadou Lamine Dia

Rapporteur: Dr. James Wabacha

It was noted that the announcement required that posters should be in English or French and should carry the following information: title, author(s), institutional affiliation, address(es) and email, indicate the corresponding author with an asterisk after the name. Also, the space allocated for posters is 100 x 150cm., posters must be easy to read, and they should bear the following sections: an introduction stating the purpose of the study, material and methods, results (text and illustrations), conclusions/recommendations and references.

The following were the key observations:

- No posters were posted on the first day as the poster stands were not ready.
- Out of the 42 posters selected for presentation, only 26 posters had been posted by the third day of the conference.
- The scientific content of all the posters was of high quality.
- The visual quality of most posters was high.
- However, for some posters the following were the shortcomings:
 - some included the abstract/summary;
 - some discussed the results at length rather than providing conclusions only;
 - some had small font sizes and made them not visually attractive;
 - some contained a lot of information and therefore made the posters clumsy;
 - some did not contain the references;
 - some did not carry the acknowledgements.

DISCUSSIONS BETWEEN FAO, IAEA AND AU-PATTEC ON TSETSE FLY MANAGEMENT. 5–8 OCTOBER 2010, ADDIS ABABA, ETHIOPIA

Two staff members from the FAO Animal Production and Health Division, Rome, and the Joint FAO/IAEA Division, Vienna, Raffaele Mattioli and Udo Feldmann, respectively, paid a visit to the Headquarters of the African Union (AU) Commission in Addis Ababa in an effort to further streamline and harmonise the support of the two mandated specialized UN organizations to the AU–Pan African Tsetse and Trypanosomosis Eradication Campaign (AU-PATTEC). During a courtesy visit to Her Excellency Rhoda P. Tumusiime, AU-Commissioner for Rural Economy and Agriculture (see picture), the Commissioner thanked the IAEA for its role in supporting the establishment of PATTEC and acknowledged the assistance of the FAO in its efforts to support the implementation of the PATTEC initiative. With reference to a Memorandum of Understanding in support of PATTEC, signed at the request of the AU Commission with IAEA in November 2009, and respective resolutions both from the FAO Conference and the IAEA General Conference to assist in planning and implementing of projects under the PATTEC initiative, detailed discussions were held on several general areas of collaboration:

- IAEA was specifically requested to provide support on tsetse SIT as part of an area-wide integrated pest management (AW-IPM) effort on tsetse mass rearing, on baseline data collection, and on relevant operational research.
- FAO was requested to support efforts directed at sustainable agriculture and rural development (SARD) as well as aspects relevant to land use and animal health.
- Continued WHO support is needed on intervention against human African trypanosomosis (HAT, i.e. sleeping sickness).
- Cooperation is needed in the development of national legislation and relevant regulatory measures.
- An effort will be made to enhance joint planning, implementing and monitoring of intervention projects.
- A major focus of the cooperation will be joint training and capacity development in three main areas: (i) project management; (ii) laboratory and field techniques; (iii) studies and project implementation, including baseline data collection and feasibility confirmation.

The meeting also discussed the need to revise the PATTEC Plan of Action, in an effort to ensure that all available tactics for T&T intervention are appropriately considered and made use of as part of an AW-IPM campaign. The meeting agreed to intensify the exchange of relevant information, to organize frequent joint meetings and to support each other in awareness generation and fund raising activities.



FAO, IAEA and AU-PATTEC experts meet with African Union Commissioner for Rural Economy and Agriculture

SECRETARIAT REPORT ON IMPLEMENTATION OF VARIOUS DECISIONS OF COUNCIL FOR STRENGTHENING ISCTRC. JANUARY 2011, NAIROBI, KENYA.

Below is the Executive Summary of this interesting report. The full report can be obtained by making a request to ibar.office@au-ibar.org.

The International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) is a strategic partnership platform to promote international cooperation in the fight against trypanosomiasis, a disease that is one of Africa's greatest constraints to socio-economic development and that severely affects human and livestock health, limits livestock productivity and land use, causes poverty and perpetuates underdevelopment on the continent. It is a statutory council of the African Union with the Secretariat based at AU-IBAR, Nairobi, Kenya.

The ISCTRC was established in 1949 and became an organ of OAU, now AU, in 1963. This report was prepared by the ISCTRC Secretariat and contains recommendations on the implementation of the various decisions of the Council for strengthening ISCTRC, as recommended in the 2006 Consultants Report "Strengthening the International Council for Trypanosomiasis Research and Control (ISCTRC): Meeting the Challenges of the Present and the Future", and in various minutes and reports of the Council.

A major concern about ISCTRC is that it has erroneously been associated with the Biennial Conference and has had minimal focus on its other core functions. Furthermore, key areas of the Conference have not met the stakeholders' expectations. Moreover, the lack of institutionalization of ISCTRC in the structure of AU Commission (AUC) and provision of a budget and staff had compromised its operations. Furthermore, the changing operating environment, such as the increasing role of the Regional Economic Communities (RECs) and the increased demands on ISCTRC services, require that ISCTRC realigns itself accordingly.

The report focuses on the following key areas: (i) membership and functions of the Council, membership and functions of the Executive Committee; (ii) institutionalization of ISCTRC within the current structure of African Union Commission and its relationship with

PATTEC; (iii) Biennial Conference; and (iv) role and function(s) of ISCTRC Secretariat and ISCTRC publications.

The report has made several recommendations that will form the basis for the implementation of the various decisions and recommendations of the Council for strengthening ISCTRC. Some of the key recommendations include the need to enhance communication with Member States, especially reminding them about the mandate of ISCTRC and to formally submit the ISCTRC recommendations to them. The report also recommends that RECs, Veterinary Faculties and associations such as FARA and ASARECA join ISCTRC Executive Committee. It is also observed that capacity building in tsetse and trypanosomiasis has not been emphasized in the past as a key function of ISCTRC and it is recommended that capacity building to support ongoing projects and field activities in Member States be strengthened.

In a key departure from tradition it is recommended that the Chairman of ISCTRC Executive Committee be elected and not tied to any specific country and he/she may not necessarily be the president/chairman of the General Assembly/General Conference. The report further recommends that country reports be analyzed and the outputs of the analysis should include both control and research activities and the agenda of ISCTRC meetings. The output should also be stored in an electronic database to be established.

The 2006 consultancy report and the 30th ISCTRC Conference recommended that ISCTRC be institutionalized within the new structure of AUC and the report recommends that this be considered by AUC. In view of the overlapping mandates of AU-IBAR- ISCTRC on one hand and AU- PATTEC on the other, it is recommended that AUC considers the need to develop a framework that will facilitate synergy and complementarity between the two organizations in order to ensure better implementation of their respective mandates. The report has also made several recommendations on the improvement of the various aspects of the General Conference especially in the following areas: structure of the Conference; hosting of the Conference; Conference programme and its content; the organization of the Conference and follow-up of recommendations of the Conference. Recommendations on enhancement of the Secretariat and enhancing ISCTRC publications have also been made.

For ISCTRC to meet its objectives and to continue attracting approval by stakeholders, there is need for timely and effective implementation of the recommendations. A vibrant ISCTRC will provide the much needed platform for information sharing and exchange and will act as an important source for evidence-based information for decision making at country and institutional level to support human and animal health, research, control, and development.

**MEETING OF PAAT SECRETARIAT TO DISCUSS THE EXTERNAL PAAT
EVALUATION REPORT AND ACTIONS FOR IMPLEMENTING ITS
RECOMMENDATIONS. INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE
(ILRI), 26–27 JANUARY 2011, ADDIS ABABA, ETHIOPIA.**

This meeting was attended by Albert Ilemobade - PAAT Chairman, James Wabacha - African Union–Inter-African Bureau for Animal Resources (AU–IBAR) and ISCTRC Focal Point, Pere Simarro - World Health Organization (WHO), Oumar Diall – FAO Consultant based in the FAO Regional Office, Accra, Giuliano Cecchi – FAO Consultant PAAT Information System, Hassane Mahamat and Solomon Hailemariam - AU–Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), Rajinder Saini - International Centre of Insect Physiology and Ecology (ICIPE) and Tadelle Dessie (ILRI).

Emmanuelle GuerneBleich, Livestock Officer, FAO Sub Regional Office for Eastern Africa, on behalf of the FAO Representative Castro Camarada, provided an introductory note to the meeting and Albert Hemobade described the purpose of the meeting, namely to discuss the report “*The PAAT: Its Performance and Recommendations for its Future Direction and Governance*”, prepared by the external evaluation team (J. Dargie, P. Van den Bossche and O. Diall).

The discussion focused on how to reinvigorate the Programme and to ensure synergy and collaboration with PATTEC. The meeting expresses profound appreciation to the team leader of the review panel, Dr. Jim Dargie, and members of the panel for the excellent work done. It learned with regret the tragic death of Dr. Peter Van den Bossche, a member of the panel soon after the completion of its work and acknowledged his contributions to T&T both in the field and in research. It welcomes the report, and considers it as the basis for defining a PAAT roadmap. Recommendations formulated by the external evaluation were discussed and proposed actions defined.

The PAAT Secretariat meeting provided the following recommendations:

- Steps should be taken to correct the perception that PAAT is synonymous with FAO.
- A Strategic Framework for PAAT has to be developed in a participatory manner (PAAT Secretariat members) as a matter of high priority. In this document, the Vision and Mission of PAAT should be set out, as well its development goals, objectives, membership, etc. Attention should be given to the added-value of PAAT to its stakeholders.
- A results-based (bi-) annual work plan with realistic goals and activities, related budget and timeframes has to be developed, in line with the Strategic Framework. Nevertheless it is recognized that this recommendation will need to take into account the work plans of the individual members of the Secretariat without prejudice to their independence. Monitoring and evaluation of PAAT outputs and outcomes should be considered in the formulation of the work plan.
- The PAAT Research and Development Policy, and the Planning and Implementation modules should be abolished (their roles have already been overtaken by events).
- Structure, membership, and funding of PAAT and its organs should be refined once the Strategic Framework (that realigns PAAT to the new political, institutional, and operational environment) is agreed within the PAAT Secretariat.
- PAAT should focus and explicitly build on the added-values it provides to its stakeholders, both on those already well established (i.e. normative and standardization activities, development and guidance on policy analysis and strategy formulation, knowledge sharing [e.g. TTI], technical and scientific publications [T&S Series etc.]), and on those in need of strengthening (i.e. coordination of capacity-building, planning and implementation of training activities, quality control and assurance, technical assistance to T&T affected countries, production of middle-level training and technical materials, assistance in the development and quality assurance of project proposals and their implementation, support for fund raising and advocacy activities).
- The PAAT Secretariat should send formal invitations to AU-PATTEC, ICIPE, ILRI, and the Centre International de Recherche-Développement sur l'Élevage en Zone Subhumide (CIRDES) to join the PAAT Secretariat.

THE ATLAS OF TSETSE AND AFRICAN ANIMAL TRYPANOSOMOSIS

Giuliano Cecchi¹, Massimo Paone¹, Udo Feldmann², Marc J. B. Vreysen², Raffaele C. Mattioli¹

¹Food and Agriculture Organization of the United Nations (FAO), Animal Production and Health Division, Viale delle Terme di Caracalla, 00153, Rome, Italy.

²Joint Food and Agriculture Organization/International Atomic Energy Agency Programme, Wagramerstraße 5, 1400, Vienna, Austria.

The Atlas of Tsetse and African Animal Trypanosomosis (AAT) is a newly launched initiative of the Food and Agriculture Organization of the United Nations (FAO), jointly implemented with the International Atomic Energy Agency (IAEA) in the framework of the Programme Against African Trypanosomosis (PAAT). The Atlas aims to assemble, analyse, and disseminate up-to-date and comprehensive information on the occurrence and distribution of tsetse and animal trypanosomosis in sub-Saharan Africa.

Accurate knowledge of the geographic distribution of AAT and its biological vector are essential to plan, implement, and monitor a broad range of interventions against tsetse and trypanosomosis (T&T), such as those undertaken under the umbrella of the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC). The key role played by geo-referenced data is clearly illustrated in programmes that are implemented according to the principles of area-wide integrated pest management, whereby the distribution of the pest population in space and time has to be accurately known so that the entire population can be targeted.

The Atlas will include a geo-database of absence/presence and abundance of tsetse flies. Priority will be given to species of medical and veterinary importance. The geographic coordinates of the tsetse catching device (e.g. trap) will represent the basic geospatial unit of recording. At the same time, allowance for coarser geospatial representations will be made, with a view to including information reported at the village or district level. A set of ancillary information items will also be recorded (e.g. type of trap used, use of attractants, capture date, etc.), thus broadening the range of potential applications of the database.

The AAT component will include prevalence data as estimated with a wide spectrum of diagnostic techniques. Herd-level information will form the building block for the AAT geo-database, which will include date of survey/monitoring, diagnostic method, species and breed of animals and husbandry system.

Input data will be identified and collated through a systematic review of formal and grey literature, as well as through direct contacts and collaboration with the extensive network of FAO and IAEA partners in the field, including national animal health authorities and national, regional, and international research institutes.

While the specific objective of the Atlas is to develop maps of tsetse and AAT distribution at a range of geographic scales, the overall aim of the initiative is to strengthen and streamline data collection, reporting, and analysis for improved planning and implementation of interventions at the country and regional levels. To meet this general objective, capacity building and technical assistance to T&T affected countries are already being addressed by FAO and IAEA as a matter of priority. The Atlas will also promote data harmonization and data sharing at the regional and international levels, which, given the transboundary nature of

the T&T problem, have enormous implications on the feasibility and sustainability of interventions.

SPECIAL ISSUE OF PARASITOLOGY ON AFRICAN TRYPANOSOMIASIS

Readers should note that Cambridge University Press, the publishers of the journal *Parasitology* have devoted Volume 137, Issue 14 (December 2010) entirely to issues surrounding African trypanosomosis. Entitled “*African Trypanosomiasis: New Insights for Disease Control*”, it consists of papers written by world-renowned experts on subjects ranging from diagnosis, chemotherapy, pathology, vaccination, and innate and acquired immunity. Abstracts of the papers are contained in this volume of TTI.

THE JOINT FAO/IAEA PROGRAMME OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

Most activities in relation to addressing the tsetse and trypanosomosis (T&T) problem are planned and carried out through the Insect and Pest Control (IPC) sub-Programme of the Joint FAO/IAEA Division and the IAEA Technical Cooperation Programme. Activities include (i) need-driven R&D activities implemented for the most part through Coordinated Research Projects, (ii) support to operational field activities on planning, feasibility assessment and implementing area-wide integrated national and regional tsetse and trypanosomosis control efforts, and (iii) assistance to policy and strategy development to Member States in close collaboration with AU, FAO and WHO, making use of the PAAT forum. Each six months the IPC sub-Programme publishes a Newsletter. The most recent one is available at <http://www-pub.iaea.org/MTCD/publications/PDF/Newsletters/IPC-NL-76.pdf>

Below is a summary of some of the main activities conducted during the period July–December 2010 concerning T&T. Full details are available by consulting the Newsletter itself.

1. Creating a Tsetse-Free Zone in the Southern Rift Valley

In tropical and subtropical rural areas of Africa several tsetse fly species, while sucking blood on humans and livestock, transmit trypanosomes, unicellular blood parasites which eventually affect the central nervous system, causing sleeping sickness among humans and a similar disease among livestock, called nagana. The diseases particularly affect poor rural communities and their livestock and agriculture, which is why the tsetse fly vector is often referred to as the “poverty insect”. Since its inception in 1997, the FAO/IAEA-supported Southern Rift Valley Tsetse Eradication Project (STEP) has managed to train and involve more than 220 000 farmers in methods for suppressing tsetse fly populations and the disease they transmit, African animal trypanosomosis (AAT). The project applies pour-on formulations of insecticides onto livestock and, in addition, positions into the fly habitats insecticide-impregnated blue/black fabric targets, which attract tsetse flies and kill them.

It is anticipated that once developed for large-scale application in Ethiopia, the sterile insect technique will complement the area-wide pest management efforts, aiming at a complete elimination of the tsetse and trypanosomosis (T&T) problem in the Ethiopian Southern Rift Valley.

In July a review of STEP was jointly undertaken by the Ethiopian Government, FAO, IAEA, African Development Bank (AfDB) and other relevant partners. While the above

mentioned field progress was commended, the review team also discussed some critical issues – managerial and technical – to be addressed with a sense of urgency, before the SIT component will be available to STEP:

- The project will need to consider in some areas the area-wide use of the sequential aerosol technique (SAT) for pre-SIT tsetse suppression.
- Particular attention will need to be given to T&T control in the Nech Sar National Park.
- A decision is needed on the mass-rearing and field use of most appropriate (mass rearing adapted) strains of the target tsetse fly species (*Glossina pallidipes* and *G. f. fuscipes*).
- The implementation of a small SIT pilot / demonstration operation in a confined part of the project area needs to be considered.
- The establishment of a maintenance unit for the Kaliti insectary has been advised.
- With increasing size of mass-reared fly colonies substantial amounts of blood diet will need to be decontaminated, which will require respective-size sterilization equipment.
- Some issues relevant to STEP management need to be addressed.

So far about 10 000 km² of land with good opportunities for sustainable agricultural and rural development have been covered by the STEP tsetse suppression activities. The experienced substantial reduction of the T&T problem already permitted an increase of productive livestock in the area. For the first time the rural communities can make use of horses and donkeys in the Southern Rift Valley, where previously they were unable to be used, because of their high susceptibility to tsetse-transmitted trypanosomosis. The project intends to expand the tsetse suppression operations to some 25 000 km² in the next years.

A workshop in Nairobi, July 2010, of the EU-funded and FAO-executed Livestock Policy Initiative under the Intergovernmental Authority on Development (IGAD-LPI) in the Horn of Africa concluded that rural livestock development areas like the Southern Rift Valley will benefit substantially from a complete elimination of the T&T problem: Investments between USD1 500 and USD3 000 per km² are likely to result over a 20-year period in benefits of up to USD12 500 to USD15 000 per km². Excluding urban areas in the Southern Rift Valley, some 700 000 rural households are expected to eventually benefit from the efforts coordinated by STEP.

2. Supporting the Creation of a Tsetse-Free Zone in Southern Mozambique and Northeast South Africa

2.1 FAO/IAEA Regional Training Course

An FAO/IAEA Regional Training Course on “Surveillance of Tsetse Flies in Support of Planning and Implementing Area-wide Integrated Pest Management in Southern Mozambique and Northeast South Africa” was hosted by the Veterinary Faculty of the University of Eduardo Mondlane and the National Directorate of Veterinary Services in Maputo, Mozambique from 7–24 June 2010. The counterpart of TC project RAF5059, Luis Neves was the course director and 15 participants from Malawi, Mozambique, South Africa, United Republic of Tanzania, Zambia, and Zimbabwe attended the course. The course covered topics from basic tsetse biology, anatomy, and ecology of the tsetse fly, species identification, principles of developing a grid-based sampling frame, data bases and data management, GPS, GIS and remote sensing, population genetics and morphometrics, dissection techniques and a field exercise to put these issues in practice. Lectures were

likewise given on principles of area-wide integrated pest management and on the sterile insect technique. Most of the participants found the course extremely useful, well organised, and with adequate facilities. From the feedback received from the participants, it was obvious that the “take home” message of the training course was very clear, i.e. that a lot of the planning and preparations for an entomological base line data collection exercise (i.e. the development of a grid-based sampling frame using GIS and remote sensing and the selection of representative sampling sites in suitable habitat) can be done in the office behind a computer rather than in the field. In this way, surveys will not only be more accurate but also more cost effective.

2.2 Second Technical Regional Meeting

The Agency is supporting a tsetse project in southern Africa that comprises Matutuini province in Mozambique, KwaZulu Natal in South Africa, and Eastern Swaziland. The second regional technical meeting of this joint regional TC project was hosted by the Veterinary Faculty of the University of Eduardo Mondlane, Maputo, Mozambique from 28–30 June 2010 and attended by staff of the Onderstepoort Veterinary Institute (OVI), Pretoria, South Africa, of the Directorate of Veterinary Services of KwaZulu Natal (DVS KZN), South Africa, of the Veterinary Faculty of the University of Eduardo Mondlane, Mozambique and of the Ministry of Agriculture, Mozambique.

Reports were presented on:

- the workshop that was held in December 2009 in Maputo to develop a detailed action plan for the collection of entomological base line data. The area to be surveyed is close to 40 000 km² and is divided into three blocks. The first block (9 000 km²) is bordered in the south by South Africa and in the north by the imaginary horizontal line between Maputo and the border with Swaziland. The action plan follows the principles of grid-based sampling and representative deployment of traps in various vegetation types;
- increased resistance of trypanosomes to the commonly used trypanocidal drugs. Isolates of *T. congolense* were collected from all regions in Mozambique and the isolates originating from the middle and northern parts of Mozambique were all resistant to the drugs. Only in Matutuine District, some isolates were still sensitive or heterogeneous. This clearly indicates that chemotherapy is not sustainable in Mozambique and preferably, needs to be replaced or supplemented by vector control strategies;
- population genetics studies. *Glossina austeni* populations sampled in Swaziland, Mozambique, and South Africa were compared using mitochondrial DNA and wing morphometrics as a marker. Initial results indicated limited gene flow between populations of Swaziland and South Africa. Separation between both the populations became more distinct when the geographical distances between the populations increased;
- the collection of entomological base line data in Matutuine District. Data were collected from 73 trapping sites in 10 grids (each of 10 × 10 km²). A total of 368 flies were sampled of which *G. brevipalpis* was the most prevalent. This study was complemented with a veterinary survey in the Bela Vista and Zitundo areas. A total of

1 789 blood samples were collected in 49 sampling sites. A prevalence of 18.8 percent and 16 percent was found in Bela Vista and Zitundo, respectively.

Future activities will focus on

- collection of flies from more areas for population genetic studies, including the use of microsatellites as markers;
- collection of entomological base line data;
- assessment of gaps in the distribution north of Maputo;
- collection of data on the dynamics of the population of both tsetse species in South Africa and Mozambique;
- field cage studies using colony flies from both species (*G. austeni* and *G. brevipalpis*) to assess various components of the behaviour of sterilised males (e.g. irradiation as pupae or as adults, optimal age for mating, optimal age for irradiation, remating capacity, etc.) under semi-natural environment.

3. Third Research Coordination Meeting on Improving SIT for Tsetse Flies through Research on their Symbionts and Pathogens. 26–30 July 2010, Nairobi, Kenya

This meeting was hosted by the International Centre of Insect Physiology and Ecology (ICIPE) and locally organized by Jean Maniania, the Head of Entomopathology Unit. Twenty participants from fifteen countries attended together with one consultant from the United States and four observers (three from Kenya and one from the USA). The first two days were devoted to presentations of the agreement/contract holders and a consultant, whereas during the remainder of the meeting the participants discussed in two working groups further research on tsetse pathogens and on tsetse symbionts. During the discussions it was concluded that tsetse physiology, including fecundity, seems to depend on the fitness of its symbiotic fauna. Therefore, it was decided that correlations and interactions between the presence of virus, disease symptoms, and the occurrence of bacterial symbionts (*Wigglesworthia*, *Sodalis*, and *Wolbachia*) need to be explored further. The group discussed the various management strategies of the salivary gland hypertrophy virus which have been proposed and are being researched and validated to mitigate/manage the disease in tsetse colonies. These strategies are based on:

- monitoring viral loads for colony quality control;
- blocking virus transmission using specific antibodies, and/or clean feeding practices;
- blocking virus replication by applying specific inhibitors of virus replication.

The group also recommended strategies designed to:

- monitor prevalence and loads of tsetse symbionts and pathogens;
- augment current feeding regimes to improve tsetse fecundity;
- improve the application of the SIT by harnessing tsetse symbionts to develop pathogen resistant fly lines and to introduce natural sterility.

Prior to the RCM, a workshop was held at ICIPE on “Genotyping Analysis of Tsetse Fly Symbionts and Pathogens”, attended by twelve participants and three lecturers from nine

countries. Several oral presentations were given that provided an overview on general principles of genotyping analysis, the genotyping methods used for the insect symbiont *Sodalis*, the Multi Locus Sequence Typing (MLST) method used for *Wolbachia* research and the methods used for virus genotyping. Several practical sessions were organized on DNA extraction for virus and detection of symbionts and demonstrations were given on extraction of the genomic DNA of tsetse flies using DNeasy kit (Qiagen) followed by PCR to detect various genes from *Sodalis*, *Wolbachia* and the salivary gland hypertrophy virus. Demonstrations on PCR product purification and preparation of the DNA samples for sequence were also given. Several online training sessions of the manipulation of sequence data and using the online software to do the genotyping analysis were given.

4. Research at the Insect Pest Control Laboratory, Seibersdorf

Details of this research which is currently directed at examining the mating compatibility between mass-reared and field strains of tsetse and developing strategies to manage the salivary gland hypertrophy virus (SGHV) that reduces the productivity and hence colony development of *Glossina pallidipes*, are available in the Newsletter.

INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE (ILRI)

This volume of TTI contains the abstracts of several papers and theses covering research into the epidemiology and treatment of bovine trypanosomiasis, including the issue of drug resistance to trypanocides, conducted by ILRI staff and partners. The Editor thanks Dr. Tom Randolph of ILRI not only for providing these abstracts in their original language (French) (and therefore did not appear in the normal literature searches conducted), but also for providing translations into the English language. Abstracts appearing in this Volume of TTI arising from ILRI's research with partners, including students, are numbers 15427, 15488, 15489, 15490, 15491, 15492, 15494, 15495, and 15496.

SECTION B - ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

15404. **Akritidis, N.**, 2010. Parasitic, fungal and prion zoonoses: an expanding universe of candidates for human disease. *Clinical Microbiology & Infection*. **e Publication ahead of print, December 24.**

Internal Medicine Department, General Hospital "G. Hatzikosta", Ioannina, Greece.

Zoonotic infections have emerged as a burden for millions of people in recent years owing to re-emerging or novel pathogens often causing outbreaks in the developing world in the presence of inadequate public health infrastructure. Among zoonotic infections, those caused by parasitic pathogens are the ones that affect millions of humans worldwide, who are also at risk of developing chronic disease. The present review discusses the global effect of protozoan pathogens such as *Leishmania* sp., *Trypanosoma* sp., and *Toxoplasma* sp., as well as helminthic pathogens such as *Echinococcus* sp., *Fasciola* sp., and *Trichinella* sp. The zoonotic aspects of agents that are not essentially zoonotic are also discussed. The review

further focuses on the zoonotic dynamics of fungal pathogens and prion diseases as observed in recent years, in an evolving environment in which novel patient target groups have developed for agents that were previously considered to be obscure or of minimal significance.

15405. **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.

Epidemiology and Disease Control Unit, Department of Public Health, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium. [mboelaert@itg.be].

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.

15406. **Crowther, G. J., Shanmugam, D., Carmona, S. J., Doyle, M. A., Hertz-Fowler, C., Berriman, M., Nwaka, S., Ralph, S. A., Roos, D. S., Van Voorhis, W. C. & Agüero, F.,** 2010. Identification of attractive drug targets in neglected-disease pathogens using an *in silico* approach. *PLoS Neglected Tropical Diseases*, **4** (8): e804.

Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington, Seattle, Washington, USA. [crowther@u.washington.edu].

The increased sequencing of pathogen genomes and the subsequent availability of genome-scale functional datasets are expected to guide the experimental work necessary for target-based drug discovery. However, a major bottleneck in this has been the difficulty of capturing and integrating relevant information in an easily accessible format for identifying and prioritizing potential targets. The open-access resource TDRtargets.org facilitates drug target prioritization for major tropical disease pathogens such as the mycobacteria *Mycobacterium leprae* and *Mycobacterium tuberculosis*; the kinetoplastid protozoans *Leishmania major*, *Trypanosoma brucei*, and *Trypanosoma cruzi*; the apicomplexan protozoans *Plasmodium falciparum*, *Plasmodium vivax*, and *Toxoplasma gondii*; and the helminths *Brugia malayi* and *Schistosoma mansoni*. Here we present strategies to prioritize pathogen proteins based on whether their properties meet criteria considered desirable in a drug target. These criteria are based upon both sequence-derived information (e.g. molecular mass) and functional data on expression, essentiality, phenotypes, metabolic pathways, assayability, and druggability. This approach also highlights the fact that data for many relevant criteria are lacking in less-studied pathogens (e.g. helminths), and we demonstrate how this can be partially overcome by mapping data from homologous genes in well-studied organisms. We also show how individual users can easily upload external datasets and integrate them with existing data in TDRtargets.org to generate highly customized ranked

lists of potential targets. Using the datasets and the tools available in TDRtargets.org, we have generated illustrative lists of potential drug targets for seven tropical disease pathogens. While these lists are broadly consistent with the research community's current interest in certain specific proteins, and suggest novel target candidates that may merit further study, the lists can easily be modified in a user-specific manner, either by adjusting the weights for chosen criteria or by changing the criteria that are included.

15407. **Fournier-Wirth, C., Jaffrezic-Renault, N. & Coste, J.,** 2010. Detection of blood-transmissible agents: can screening be miniaturized? *Transfusion*, **50** (9): 2032-2045.

Laboratoire de R&D-Agents Transmissibles par Transfusion (R&D-ATT),
Etablissement Français du Sang Pyrénées-Méditerranée, Montpellier, France.
[chantal.fournier@efs.sante.fr].

Transfusion safety relating to blood-transmissible agents is a major public health concern, particularly when faced with the continuing emergence of new infectious agents. These include new viruses appearing alongside other known re-emerging viruses (West Nile virus, Chikungunya) as well as new strains of bacteria and parasites (*Plasmodium falciparum*, *Trypanosoma cruzi*) and finally pathologic prion protein (variant Creutzfeldt-Jakob disease). Genomic mutations of known viruses (hepatitis B virus, hepatitis C virus, human immunodeficiency virus) can also be the origin of variants susceptible to escaping detection by diagnostic tests. New technologies that would allow the simultaneous detection of several blood-transmissible agents are now needed for the development and improvement of screening strategies. DNA microarrays have been developed for use in immunohaematology laboratories for blood group genotyping. Their application in the detection of infectious agents, however, has been hindered by additional technological hurdles. For instance, the variability among and within genomes of interest complicates target amplification and multiplex analysis. Advances in biosensor technologies based on alternative detection strategies have offered new perspectives on pathogen detection; however, whether they are adaptable to diagnostic applications for testing biologic fluids is under debate. Elsewhere, current nanotechnologies now offer new tools to improve the sample preparation, target capture, and detection steps. Second-generation devices combining micro- and nanotechnologies have brought us one step closer to the potential development of innovative and multiplexed approaches applicable to the screening of blood for transmissible agents.

15408. **Jordan, K. A. & Hunter, C. A.,** 2010. Regulation of CD8⁺ T cell responses to infection with parasitic protozoa. *Experimental Parasitology*, **126** (3): 318-325.

Department of Pathobiology, University of Pennsylvania, 380 South University
Avenue, Philadelphia, PA 19104, USA. [kajordan@mail.med.upenn.edu].

There are over 10 000 species of parasitic protozoa, a subset of which can cause considerable disease in humans. Here we examine in detail the complex immune response generated during infection with a subset of these parasites: *Trypanosoma cruzi*, *Leishmania* sp., *Toxoplasma gondii*, and *Plasmodium* sp. While these particular species perhaps represent the most studied parasites in terms of understanding how T cells function during infection, it is clear that the lessons learned from this body of work are also relevant to the other protozoa known to induce a CD8⁺ T cell response. This review will highlight some of the key studies

that established that CD8⁺ T cells play a major role in protective immunity to protozoa, the factors that promote the generation as well as maintenance of the CD8⁺ T cell response during these infections, and draw attention to some of the gaps in our knowledge. Moreover, the development of new tools, including MHC-Class I tetramer reagents and the use of TCR transgenic mice or genetically modified parasites, have provided a better appreciation of how parasite specific CD8⁺ T cell responses are initiated and new insights into their phenotypic plasticity.

15409. **Leslie, M.**, 2010. Kidney disease is parasite-slaying protein's downside. *Science*, **329** (5989): 263.

Science mag.org.

This editorial describes work identifying genetic variations that can lead to kidney shutdown but may also fend off a microorganism that causes sleeping sickness in thousands of people in Africa. Specifically, scientists from the United States were searching for genetic risk factors for two renal conditions — focal segmental glomerulosclerosis and hypertension-attributed end-stage kidney disease — that are four to five times more common among African Americans than among people of European ancestry. Previous studies had shown a link with chromosome 22 but by expanding the search they were able to implicate the APOL1 gene which codes for the blood protein apolipoprotein L-1 (ApoL1). By statistically analyzing the gene variants in African Americans who had either of the kidney diseases, the team identified two alterations in the APOL1 gene that correlated with illness. The G1 variant, for example, turned up in 52 percent of glomerulosclerosis patients, versus 18 percent of controls. And the G2 variant was about 50 percent more common in patients with either kidney disease than it was in healthy people. The researchers calculated that if both of a person's APOL1 genes have one of the illness-causing mutations (no gene carries both), the risk of developing hypertension-attributed endstage kidney disease shoots up more than seven times. Given this impact, it is surprising how common G1 and G2 are in Africa. Among the Yoruba people of Nigeria, G2's frequency was 8 percent, and G1's was 38 percent. When the researchers applied a statistical technique that can discern the effects of natural selection, they found that G1's prevalence in Africa had surged within the past 10 000 years and hypothesized that the G1 and G2 versions of ApoL1 better protect against *Trypanosoma brucei*. The standard version of ApoL1 kills one subspecies of the parasite, *T. brucei brucei*, but not another subspecies, *T. brucei rhodesiense*, which makes a protein called SRA that neutralizes the blood defender. But G1 and G2 reconfigure ApoL1, restoring its potency. Blood plasma from people who carried G1 or G2 killed the *rhodesiense* version of the parasite, as did lab-made copies of the altered proteins. However, the study isn't conclusive since the Yoruba people come from West Africa, whereas the altered ApoL1 proteins were effective against the subspecies of *T. brucei* that exists in East Africa. The G1 and G2 variants didn't kill *T. brucei gambiense*, which causes sleeping sickness in West Africa - a discrepancy that needs to be resolved. The results also suggest that synthetic versions of the more effective ApoL1 proteins, or even plasma from people who carry G1 or G2, could provide a new treatment for African sleeping sickness.

15410. **Nimmo, C.**, 2010. Time to put out the lights on sleeping sickness? *Travel Medicine & Infectious Diseases*, **8** (4): 263-268.

University College London Medical School, Gower Street, London WC1E6BT, UK. [camus.nimmo@gmail.com].

Sleeping sickness (or human African trypanosomiasis, HAT) is a potentially fatal parasitic disease that affects a large proportion of sub-Saharan Africa. It was epidemic in the early 20th century before being nearly eradicated through a variety of control programmes. Despite this, there were resurgences in the 1980s and 1990s following relaxation of these programmes. Recent advances are reversing this trend once more. However, more research is required to improve diagnosis and treatment, and to better understand the epidemiology of HAT if complete eradication is to be achieved in the future.

15411. **Simarro, P. P., Cecchi, G., Paone, M., Franco, J. R., Diarra, A., Ruiz, J. A., Fevre, E. M., Courtin, F., Mattioli, R. C. & Jannin, J. G.,** 2010. The Atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. *International Journal of Health Geographics*, **9**: 57.

World Health Organization, Control of Neglected Tropical Diseases, Innovative and Intensified Disease Management, 1211 Geneva 27, Switzerland. [SimarroP@who.int].

Following World Health Assembly resolutions 50.36 in 1997 and 56.7 in 2003, the World Health Organization (WHO) committed itself to supporting human African trypanosomiasis (HAT)-endemic countries in their efforts to remove the disease as a public health problem. Mapping the distribution of HAT in time and space has a pivotal role to play if this objective is to be met. For this reason WHO launched the HAT Atlas initiative, jointly implemented with the Food and Agriculture Organization of the United Nations, in the framework of the Programme Against African Trypanosomosis. The distribution of HAT is presented for 23 out of 25 sub-Saharan countries having reported on the status of sleeping sickness in the period 2000-2009. For the two remaining countries, i.e. Angola and the Democratic Republic of the Congo, data processing is ongoing. Reports by National Sleeping Sickness Control Programmes (NSSCPs), Non-Governmental Organizations (NGOs) and Research Institutes were collated and the relevant epidemiological data were entered in a database, thus incorporating (i) the results of active screening of over 2.2 million people, and (ii) cases detected in health care facilities engaged in passive surveillance. A total of over 42 000 cases of HAT and 6 000 different localities were included in the database. Various sources of geographic coordinates were used to locate the villages of epidemiological interest. The resulting average mapping accuracy is estimated at 900 m. Full involvement of NSSCPs, NGOs and Research Institutes in building the Atlas of HAT contributes to the efficiency of the mapping process and it assures both the quality of the collated information and the accuracy of the outputs. Although efforts are still needed to reduce the number of undetected and unreported cases, the comprehensive, village-level mapping of HAT control activities over a ten-year period ensures a detailed and reliable representation of the known geographic distribution of the disease. Not only does the Atlas serve research and advocacy, but, more importantly, it provides crucial evidence and a valuable tool for making informed decisions to plan and monitor the control of sleeping sickness.

15412. **Sternberg, J. M., Black, S. J. & Magez, S.,** 2010. African trypanosomiasis: new insights for disease control. *Parasitology*, **137** (14): 1975.

IBES, University of Aberdeen, Zoology Building, Aberdeen AB24 2TZ, UK.
[j.sternberg@abdn.ac.uk].

This is the preface to the special edition of *Parasitology* referred to earlier. It basically sets the background for the book, describing in general terms the scope of its various articles and pointing out that despite the undoubted progress made over recent decades, the challenges to be overcome at both basic and applied biological levels are substantial before the diseases caused by African trypanosomes can be effectively diagnosed, controlled and treated.

15413. **Willyard, C.**, 2010. Putting sleeping sickness to bed. *Nature Medicine*, **17** (1): 14-17.

Address not available

This article describes work attempting to combat sleeping sickness through genetic interventions, pointing out that the two parasites involved—*T. b. gambiense* and *T. b. rhodesiense* employ different strategies for evading the immune system, so research on one doesn't necessarily translate to the other. Also, sleeping sickness is treatable, but new drugs are desperately needed. For decades, the main medication used for late-stage sleeping sickness was melarsoprol, a compound derived from arsenic that wipes out the parasite but also kills 3–10 percent of people on the drug. Last year, the World Health Organization introduced a combination of two compounds—eflornithine and nifurtimox—that is safer than melarsoprol, but the treatment doesn't work against *T. b. rhodesiense*. And because eflornithine must be given intravenously for a week, the combination is still difficult to administer in remote parts of Africa. Against this background, studies are underway to examine the possibility of preventing the disease by developing cows with the gene for ApoL1 through genetic modification, thereby reducing the number of infected hosts and curb the spread of the disease in both cattle and people. Resistant cows would also bring benefits in terms of food and incomes. The question remains, however, -even if proven technically feasible (including e.g. not introducing adverse health and productivity side effects as a result of transgenesis) would transgenic cows gain biosafety approval from regulatory authorities and be socially acceptable? Also, wouldn't drug treatment be much simpler and cheaper than going along the GM route for tackling the disease?

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

15414. **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** (12): 3065-3074.

Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria; Department of Biology, Faculty of Arts and Sciences, Giresun

University, 28049 Giresun, Turkey; Laboratory of Biochemistry, Wageningen University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands; and Laboratoire de Pathologie Comparée, Université Montpellier 2, Place Eugene Bataillon, 34095 Montpellier, France. [just.vlak@wur.nl].

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 insects and application 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 the GpSGHV infection with specific antibodies against virion proteins. Two major GpSGHV virion proteins of about 130 and 50 kDa, 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 against viral infections in colonies of *G. pallidipes* is a realistic option.

15415. **Kibugu, J. K., Kiragu, J. M., Mumba, A. M., Mwangangi, D. M. & Muchiri, M. W.**, 2010. Improved protocol for aseptic collection and handling procedures of bovine blood diet in areas with special contamination challenges for use in tsetse rearing. *Laboratory Animals*, **44** (3): 281-282. **e Publication, May 10.**

Kenya Agricultural Research Institute-Trypanosomiasis Research Centre (KARI-TRC), P.O. Box 362, Kikuyu, Kenya. [jkkibugu@yahoo.com]

Abstract not available

15416. **Kibugu, J. K., Muchiri, M. W., Ngae, G. N., Mumba, A. M. & Kiragu, J. M.**, 2010. Effects of Ethidium® (homidium bromide) on female reproductive performance of laboratory-reared tsetse flies, *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). *Acta Tropica*, **116** (2): 157-160.

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

Ethidium® (homidium bromide) is a trypanocide likely to be encountered as a violative residue in blood collected from abattoirs for feeding laboratory tsetse flies. We investigated its effect on female reproduction of *Glossina morsitans morsitans*. One mg homidium/kg body weight was intra-muscularly administered to four steers and blood aseptically collected from them between 15 and 30 min post-treatment, analysed for homidium levels and

processed for tsetse feeding. Two hundred teneral female flies were fed on a homidium-treated diet while a control group of similar number was given an untreated diet and the reproductive performance of the two groups statistically compared. Ethidium®, at 266.1 ng homidium/ml blood diet, halved the A-class portion of F₁ pupae, highly reduced the decline of F₁ progeny quality associated with aging parents, but had no significant effect on the pupae viability, fecundity and abortion rate of the flies. We therefore concluded that Ethidium® has beneficial effects on laboratory tsetse attributable to clearance of unfavourable microbes mediated by the drug, and could be used as a tsetse diet additive.

15417. **Kibugu, J. K., Mwangi, J. N., Kiragu, J. M., Muchiri, M. W., Ndung'u, K. & Mdachi, R. E.**, 2010. Improved survival of laboratory-reared tsetse flies *Glossina morsitans morsitans* (Diptera: Glossinidae) through use of homidium bromide-treated blood diet. *International Journal of Tropical Insect Science*, **30** (3): 163–166

Kenya Agricultural Research Institute (KARI), Trypanosomiasis
Research Centre, PO Box 362-00902 Kikuyu, Kenya; and KARI, Social
Economics and Biometrics Division, PO Box 57811-00200, Nairobi, Kenya.
[jkkibugu@yahoo.com].

Homidium bromide is a broad-spectrum anti-microbial trypanocide likely to be encountered as a violative residue in blood collected from abattoirs destined for feeding laboratory-reared tsetse colonies. We investigated its effects on longevity of laboratory reared *Glossina morsitans morsitans* Westwood. Four steers were intra-muscularly administered with 1mg homidium bromide/kg of body weight and blood was aseptically collected from them between 15 and 30 min post-administration. This blood was defibrinated, analysed for homidium levels, screened for bacterial contamination, frozen and warmed to 37 °C before feeding to tsetse flies. Teneral male (100) and female (220) *G. m. morsitans* flies were fed on homidium-treated diet, and control flies (99 males and 187 females) on untreated blood diet and their survival monitored for 163 days. Homidium, at 266.15 ng/ml blood diet, significantly ($p < 0.05$) improved fly survival. We concluded that homidium bromide has a beneficial effect on tsetse, probably attributable to its antimicrobial activity against unfavourable microbes mediated by the drug, and could be used as a tsetse diet additive.

15418. **Sasanya, J. J., Abd-Alla, A. M., Parker, A. G. & Cannavan, A.**, 2010. Analysis of the antiviral drugs acyclovir and valacyclovir-hydrochloride in tsetse flies (*Glossina pallidipes*) using LC-MSMS. *Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences*, **878** (26): 2384-2390.

Food and Environmental Protection Laboratory, FAO/IAEA Agriculture and
Biotechnology Laboratories, Joint FAO/IAEA Division of Nuclear Techniques
in Food and Agriculture, International Atomic Energy Agency, Wagramer
Strasse 5, PO Box 100, A-1400 Vienna, Austria.

A new simple, sensitive and precise liquid chromatography-tandem mass spectrometry method has been developed and validated for the determination of valacyclovir-HCl and acyclovir in tsetse flies (*Glossina pallidipes*). Tsetse flies were extracted by ultrasonication with acidified methanol/acetonitrile, centrifuged and cleaned up by solid phase dispersion

using MgSO₄ and MSPD C₁₈ material. Samples were analysed using a Waters Alliance 2695 series HPLC with a C₁₈ Gemini analytical column (150 mm x 4.6 mm x 5 µm) and a guard cartridge column connected to a Waters Quattro-Micro triple-quadrupole mass spectrometer. The isocratic mobile phase consisted of methanol:acetonitrile:water (60:30:10, v/v/v) plus formic acid (0.1 percent) at a flow rate of 0.25 ml/min. The precursor>product ion transition for valacyclovir (m/z 325.1>152) and acyclovir (m/z 226.1>151.9) were monitored in positive electrospray multiple reaction monitoring mode. The method was validated at fortification levels of 0.5, 1, and 2 µg/g. The range of calibration for both drugs was 0.45-4.5 µg/g. The overall accuracy of the method was 92 percent for valacyclovir and 95 percent for acyclovir with corresponding within-laboratory reproducibilities of 4.4 and 3.4 percent, respectively. Mean recoveries were above 80 percent for both drugs and repeatability ranged from 0.7 to 6.1 percent. For both drugs the limits of detection and quantification were 0.0625 and 0.2 µg/g, respectively. The method was applied in experiments on the mass rearing of tsetse flies for sterile insect technique (SIT) applications, in which the flies were fed with blood meals containing acyclovir or valacyclovir-HCl prior to analysis to assess effects on *Glossina pallidipes* salivary gland hypertrophy syndrome.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

15419. **Abd-Alla, A. M., Salem, T. Z., Parker, A. G., Wang, Y., Jehle, J. A., Vreysen, M. J. & Boucias, D.,** 2010. Universal primers for rapid detection of hytrosaviruses. *Journal of Virological Methods*, **171** (1): 280-283.

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

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 °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 than 47 °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.

15420. **Basson, C. H. & Terblanche, J. S.,** 2010. Metabolic responses of *Glossina pallidipes*

(Diptera: Glossinidae) puparia exposed to oxygen and temperature variation: implications for population dynamics and subterranean life. *Journal of Insect Physiology*, **56** (12): 1789-1797.

Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa. [jst@sun.ac.za].

Understanding the factors affecting insect gas exchange in subterranean environments is critical to understanding energy budgets and predicting mortality under field conditions. Here, we examine the metabolic rate (MR) responses of tsetse puparia, which remain underground for ca. one month in this life stage, to varying oxygen and temperature. First, the effects of temperature and oxygen on puparial MR were investigated by ramping temperature from 15 °C to 35 °C under 10, 21 or 40 percent O₂. Overall, temperature was the dominant effect on puparial MR although O₂ had small but significant impacts. Second, critical O₂ concentration (P_{CRIT}) for MR of puparia was examined across a range of oxygen concentrations (0-40 percent). P_{CRIT} was 6 percent O₂ which is similar to P_{CRIT} in other basal arthropods but relatively high for inactive or subterranean insects. Third, we asked if puparia exposed to anoxia might experience oxygen debt, potentially indicative of anaerobic metabolism or cellular repair. Metabolic responses to anoxia were limited or insignificant, but MR was marginally elevated (approximately 15 percent) in anoxia-exposed (four h) puparia by 12h post-anoxia. Finally, we examined the ability of puparia to withstand water submersion, thus simulating flooding conditions frequently experienced in tropical soil habitats. Puparia were unable to survive submersion for >24h suggesting limited flooding tolerance. These novel results suggest that soil conditions experienced by puparia should not be limiting for MR, except possibly under high temperature-low O₂ conditions. Due to a large safety margin between P_{CRIT} and soil oxygen levels and limited effects of oxygen on metabolism during temperature ramping experiments, we suggest that *Glossina pallidipes* puparia are not particularly susceptible to oxygen availability in their natural environment. However, soil flooding associated with tropical rainfall likely imposes strong selection on tsetse populations and may have had important effects for tsetse energy budgets and evolution.

15421. **Lietze, V. U., Abd-Alla, A. M., Vreysen, M. J., Geden, C. J. & Boucias, D. G.,** 2010. Salivary gland hypertrophy viruses: a novel group of insect pathogenic viruses. *Annual Review of Entomology*, **56**: 63-80.

Entomology and Nematology Department, University of Florida, Gainesville, Florida 32611, USA; Insect Pest Control Laboratory, Joint FAO/IAEA Program of Nuclear Techniques in Food and Agriculture, A-1400 Vienna, Austria; and Medical, Agricultural and Veterinary Entomology, USDA, ARS, Gainesville, Florida, USA [vlietze@ufl.edu].

Salivary gland hypertrophy viruses (SGHVs) are a unique, unclassified group of entomopathogenic, double-stranded DNA viruses that have been reported from three genera of Diptera. These viruses replicate in the nuclei of salivary gland cells in adult flies, inducing gland enlargement with little obvious external disease symptoms. Viral infection inhibits reproduction by suppressing vitellogenesis, causing testicular aberrations, and/or disrupting

mating behaviour. Historical and present research findings support a recent proposal of a new virus family, the Hytrosaviridae. This review describes the discovery and prevalence of different SGHVs, summarizes their biochemical characterization and taxonomy, compares morphological and histopathological properties, and details transmission routes and the influence of infection on host biology and reproduction. In addition, the potential use of SGHVs as sterilizing agents for house fly control and the deleterious impact of SGHVs on colonized tsetse flies reared for the sterile insect technique are discussed.

15422. **Pellegrini, A., Bigliardi, E., Bechi, N., Paulesu, L., Lehane, M. J. & Avanzati, A. M., 2010.** Fine structure of the female reproductive system in a viviparous insect, *Glossina morsitans morsitans* (Diptera, Glossinidae). *Tissue Cell.* **e Publication ahead of print, November 20.**

Department of Physiology, Division of Reproductive Physiology and Endocrinology, University of Siena, Via A. Moro 2, 53100 Siena, Italy.

The female reproductive system of the tsetse fly *Glossina morsitans morsitans* was analysed by scanning electron microscopy (SEM). The study focused 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 to be formed by numerous tongue-like folds projecting towards the uterine lumen and lined by a thin cuticle. SEM analysis highlighted for the first time a distinctive new feature that is not visible by traditional histological methods i.e. 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.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

15423. **Belda, E., Moya, A., Bentley, S. & Silva, F. J., 2010.** Mobile genetic element proliferation and gene inactivation impact over the genome structure and metabolic capabilities of *Sodalis glossinidius*, the secondary endosymbiont of tsetse flies. *BMC Genomics*, **11**: 449.

Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de Valencia, Spain.

Genome reduction is a common evolutionary process in symbiotic and pathogenic bacteria. This process has been extensively characterized in bacterial endosymbionts of insects, where primary mutualistic bacteria represent the most extreme cases of genome reduction as a consequence of a massive process of gene inactivation and loss during their evolution from free-living ancestors. *Sodalis glossinidius*, the secondary endosymbiont of tsetse flies, contains one of the few complete genomes of bacteria at the very beginning of the symbiotic association, allowing one to evaluate the relative impact of mobile genetic element proliferation and gene inactivation over the structure and functional capabilities of this bacterial endosymbiont during the transition to a host dependent lifestyle. A detailed characterization of mobile genetic elements and pseudogenes reveals a massive presence of different types of prophage elements together with five different families of IS elements that have proliferated across the genome of *Sodalis glossinidius* at different levels. In addition, a

detailed survey of intergenic regions allowed the characterization of 1 501 pseudogenes, a much higher number than the 972 pseudogenes described in the original annotation. Pseudogene structure reveals a minor impact of mobile genetic element proliferation in the process of gene inactivation, with most of pseudogenes originated by multiple frameshift mutations and premature stop codons. The comparison of metabolic profiles of *Sodalis glossinidius* and tsetse fly primary endosymbiont *Wigglesworthia glossinidia* based on their whole gene and pseudogene repertoires revealed a novel case of pathway inactivation, the arginine biosynthesis, in *Sodalis glossinidius* together with a possible case of metabolic complementation with *Wigglesworthia glossinidia* for thiamine biosynthesis. In conclusion, the complete re-analysis of the genome sequence of *Sodalis glossinidius* reveals novel insights in the evolutionary transition from a free-living ancestor to a host-dependent lifestyle, with a massive proliferation of mobile genetic elements mainly of phage origin although with minor impact in the process of gene inactivation that is taking place in this bacterial genome. The metabolic analysis of the whole endosymbiotic consortia of tsetse flies has revealed a possible phenomenon of metabolic complementation between primary and secondary endosymbionts that can contribute to explain the co-existence of both bacterial endosymbionts in the context of the tsetse host.

15424. **Moore, N. & Messina, J.**, 2010. A landscape and climate data logistic model of tsetse distribution in Kenya. *PLoS One*, **5** (7): e11809.

Department of Environmental and Resource Sciences, Zhejiang University, Hangzhou, China. [moorena@msu.edu].

Trypanosoma spp, biologically transmitted by the tsetse fly in Africa, are a major cause of illness resulting in both high morbidity and mortality among humans, cattle, wild ungulates, and other species. However, tsetse fly distributions change rapidly due to environmental changes, and fine-scale distribution maps are few. Due to data scarcity, most presence/absence estimates in Kenya prior to 2000 are a combination of local reports, entomological knowledge, and topographic information. The availability of tsetse fly abundance data is limited, or at least they have not been collected into aggregate, publicly available national datasets. Despite this limitation, other avenues exist for estimating tsetse distributions including remotely sensed data, climate information, and statistical tools. Here we present a logistic regression model of tsetse abundance. The goal of this model is to estimate the distribution of tsetse flies in Kenya in the year 2000, and to provide a method by which to anticipate their future distribution. Multiple predictor variables were tested for significance and for predictive power; ultimately, a parsimonious subset of variables was identified and used to construct the regression model with the 1973 tsetse map. These data were validated against year 2000 Food and Agriculture Organization (FAO) estimates. Mapcurves goodness-of-fit scores were used to evaluate the modeled fly distribution against FAO estimates and against 1973 presence/absence data, each driven by appropriate climate data. It is concluded that logistic regression can be effectively used to produce a model that projects fly abundance under elevated greenhouse gas scenarios. This model identifies potential areas for tsetse abandonment and expansion.

15425. **Runyen-Janecky, L. J., Brown, A. N., Ott, B., Tujuba, H. G. & Rio, R. V.**, 2010. Regulation of high-affinity iron acquisition homologues in the tsetse fly symbiont *Sodalis glossinidius*. *Journal of Bacteriology*, **192** (14): 3780-3787.

Department of Biology, Gottwald Science Center Room, University of Richmond, Richmond, VA 23173, USA. [rnyen@richmond.edu].

Sodalis glossinidius is a facultative intracellular bacterium that is a secondary symbiont of the tsetse fly (Diptera: Glossinidae). Since studies with other facultative intracellular bacteria have shown that high-affinity iron acquisition genes are upregulated *in vivo*, we investigated the regulation of several *Sodalis* genes that encode putative iron acquisition systems. These genes, SG1538 (hemT) and SG1516 (sitA), are homologous to genes encoding periplasmic haeme and iron/manganese transporters, respectively. HemT promoter- and sitA promoter-gfp fusions were constructed, and in both *Escherichia coli* and *Sodalis* backgrounds, expression levels of these fusions were higher when the bacteria were grown in iron-limiting media than when the bacteria were grown in iron-replete media. The *Sodalis* promoters were tested for iron regulation in an *E. coli* strain that lacks the *fur* gene, which encodes the iron-responsive transcriptional repressor Fur. Expression of the promoter-gfp fusions in the *E. coli fur* mutant was constitutively high in both iron-replete and iron-deplete media, and addition of either *Shigella flexneri fur* or *Sodalis fur* to a plasmid restored normal regulation. A *Sodalis fur* mutant was constructed by intron mutagenesis, and semiquantitative reverse transcription-PCR (RT-PCR) showed that iron repression of sitA expression was also abolished in this strain. *In vivo* expression analysis showed that hemT and sitA are expressed when *Sodalis* is within tsetse fly hosts, suggesting a biological role for these genes when *Sodalis* is within the tsetse fly.

15426. Snyder, A. K., Deberry, J. W., Runyen-Janecky, L. & Rio, R. V., 2010. Nutrient provisioning facilitates homeostasis between tsetse fly (Diptera: Glossinidae) symbionts. *Proceedings of the Royal Society B: Biological Sciences*, **277** (1692): 2389-2397.

Department of Biology, West Virginia University, 53 Campus Drive 5106 LSB, Morgantown, WV 26506, USA.

Host-associated microbial interactions may involve genome complementation, driving enhanced communal efficiency and stability. The tsetse fly (Diptera: Glossinidae), the obligate vector of African trypanosomes (*Trypanosoma brucei* subsp.), harbours two enteric Gammaproteobacteria symbionts: *Wigglesworthia glossinidia* and *Sodalis glossinidius*. Host coevolution has streamlined the *Wigglesworthia* genome to complement the exclusively sanguivorous tsetse lifestyle. Comparative genomics reveal that the *Sodalis* genome contains the majority of *Wigglesworthia* genes. This significant genomic overlap calls into question why tsetse maintains the coresidence of both symbionts and, furthermore, how symbiont homeostasis is maintained. One of the few distinctions between the *Wigglesworthia* and *Sodalis* genomes lies in thiamine biosynthesis. While *Wigglesworthia* can synthesize thiamine, *Sodalis* lacks this capability but retains a thiamine ABC transporter (tbpA_{thiPQ}) believed to salvage thiamine. This genetic complementation may represent the early convergence of metabolic pathways that may act to retain *Wigglesworthia* and evade species antagonism. We show that thiamine monophosphate, the specific thiamine derivative putatively synthesized by *Wigglesworthia*, impacts *Sodalis* thiamine transporter expression, proliferation and intracellular localization. A greater understanding of tsetse symbiont interactions may generate alternative control strategies for this significant medical and

agricultural pest, while also providing insight into the evolution of microbial associations within hosts.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

15427. **Bett, B., Randolph, T. F., Irungu, P., Nyamwaro, S. O., Kitala, P., Gathuma, J., Grace, D., Vale, G., Hargrove, J. & McDermott, J.,** 2010. Field trial of a synthetic tsetse-repellent technology developed for the control of bovine trypanosomosis in Kenya. *Preventive Veterinary Medicine*, **97** (3-4): 220-227.

International Livestock Research Institute, P.O. Box 30709-00100, Nairobi, Kenya; Trypanosomiasis Research Centre, Kenya Agricultural Research Institute, P.O. Box 362-00902, Kikuyu, Kenya; Department of Agricultural Economics, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya; Department of Public Health Pharmacology and Toxicology, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya; and South African Centre for Epidemiological Modelling and Analysis (SACEMA), Private Bag X1, Matieland, Stellenbosch University 7602, South Africa. [b.bett@cgiar.org].

We conducted a field trial among Maasai cattle-keepers in Nkuruman and Nkineji areas of Kenya to evaluate the effectiveness of a synthetic tsetse-repellent technology developed for the control of trypanosomosis in cattle. The technology was a repellent (2-methoxy 4-methylphenol) emitted from dispensers attached to collars worn by cattle. Treatment was allocated at the herd level to ensure adequate protection of all the animals in a herd, with measurements of effectiveness conducted at the individual animal level. The trial began in April 2005 and ran for 16 months including a baseline phase of four months. We recruited 12 herds in each area using a restricted random-sampling technique and distributed them equally into intervention (repellent) and control groups. Sample size was determined using a formal power calculation. Effectiveness or minimal worthwhile difference was defined as a 50 percent reduction in the incidence of trypanosome infection in the treated versus control group (effectiveness below which the technology was considered by experts as not viable compared to existing control techniques). All the animals in the recruited herds were screened monthly (buffy-coat technique) for trypanosome infections. The analysis followed the principle of intention-to-treat by which subjects are analysed according to their initial treatment assignment, regardless of the mechanical performance of the device. Crude and adjusted effects of the technology were 23 percent ($p < 0.001$) and 18 percent ($p = 0.08$) reduction in the infection incidence in the treatment compared to the control groups, respectively. The impact of the technology estimated in this study did not achieve the threshold of 50 percent reduction in the trypanosome infection incidence set a priori to indicate effectiveness ($p < 0.001$). We therefore concluded that the prototype repellent technology package was not sufficiently effective in reducing trypanosome infection incidence under natural tsetse challenge to merit commercial development.

15428. **Bouyer, J., Seck, M. T., Sall, B., Ndiaye, E. Y., Guerrini, L. & Vreysen, M. J.,** 2010. Stratified entomological sampling in preparation for an area-wide integrated pest management program: the example of *Glossina palpalis gambiensis* (Diptera: Glossinidae) in the Niayes of Senegal. *Journal of Medical Entomology*, **47** (4): 543-552.

Unité Mixte de Recherche Centre de Coopération Internationale en Recherche Agronomique pour le Développement-Institut National de la Recherche Agronomique Controle des Maladies Animales, Campus International de Baillarguet, F34398, Montpellier, France; Institut Sénégalais de Recherches Agricoles-Laboratoire National d'Elevage et de Recherches Vétérinaires, Service de Parasitologie, BP 2057, Dakar-Hann, Sénégal; Direction des Services Vétérinaires, 37, avenue Pasteur, BP 67, Dakar, Sénégal; Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Unité Propre de Recherche Animale et gestion intégrée des risques, Campus International de Baillarguet, F34398, Montpellier, France; and Entomology Unit, Food and Agriculture Organization/International Atomic Energy Agency Agriculture and Biotechnology Laboratory, Joint Food and Agriculture Organization/International Atomic Energy Agency Programme, Vienna, Austria. [bouyer@cirad.fr].

The riverine tsetse species *Glossina palpalis gambiensis* Vanderplank 1949 (Diptera: Glossinidae) inhabits riparian forests along river systems in West Africa. The government of Senegal has embarked on a project to eliminate this tsetse species, and African animal trypanosomes, from the Niayes area using an area-wide integrated pest management approach. A stratified entomological sampling strategy was therefore developed using spatial analytical tools and mathematical modelling. A preliminary phytosociological census identified eight types of suitable habitat, which could be discriminated from LandSat 7 ETM+ satellite images and denominated wet areas. At the end of March 2009, 683 unbaited Vavoua traps had been deployed, and the observed infested area in the Niayes was 525 km². In the remaining area, a mathematical model was used to assess the risk that flies were present despite a sequence of zero catches. The analysis showed that this risk was above 0.05 in 19 percent of this area that will be considered as infested during the control operations. The remote sensing analysis that identified the wet areas allowed a restriction of the area to be surveyed to 4 percent of the total surface area (7 150 km²), whereas the mathematical model provided an efficient method to improve the accuracy and the robustness of the sampling protocol. The final size of the control area will be decided based on the entomological collection data. This entomological sampling procedure might be used for other vector or pest control scenarios.

15429. **Childs, S. J.**, 2010. The finite element implementation of a K.P.P. equation for the simulation of tsetse control measures in the vicinity of a game reserve. *Mathematical Biosciences*, **227** (1): 29-43.

ARC - Onderstepoort Veterinary Institute, Pretoria 0110, South Africa. [simonjohnchilds@gmail.com].

An equation, strongly reminiscent of Fisher's equation, is used to model the response of tsetse populations to proposed control measures in the vicinity of a game reserve. The model assumes movement is by diffusion and that growth is logistic. This logistic growth is dependent on an historical population, in contrast to Fisher's equation which bases it on the present population. The model therefore takes into account the fact that new additions to the adult fly population are, in actual fact, the descendants of a population which existed one

pupal duration ago, furthermore, that this puparial duration is temperature dependent. Artificially imposed mortality is modelled as a proportion at a constant rate. Fisher's equation is also solved as a formality. The temporary imposition of a 2 percent day⁻¹ mortality everywhere outside the reserve for a period of 2 years will have no lasting effect on the influence of the reserve on either the *Glossina austeni* or the *G. brevipalpis* populations, although it certainly will eradicate tsetse from poor habitats, outside the reserve. A 5 km-wide barrier with a minimum mortality of 4 percent day⁻¹, throughout, will succeed in isolating a worst-case *G. austeni* population and its associated trypanosomiasis from the surrounding areas. A more optimistic estimate of its mobility suggests a mortality of 2 percent day⁻¹ will suffice. For a given target-related mortality, more mobile species are found to be more vulnerable to eradication than more sedentary species, while the opposite is true for containment.

15430. **Childs, S. J.**, 2010. Theoretical levels of control as a function of mean temperature and spray efficacy in the aerial spraying of tsetse fly. *Acta Tropica*. **In press, corrected proof.**

ARC - Onderstepoort Veterinary Institute, Private Bag X5, Pretoria 0110, South Africa. [simonjohnchild@gmail.com].

The hypothetical impact of aerial spraying on tsetse fly populations is investigated. Spray cycles are scheduled at intervals two days before 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 °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 °C, 19.278 °C and 23.645 °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.

15431. **Drager, N.**, 2010. Tsetse fly control and trypanosomiasis in Africa, quo vadis? *Bulletin de la Société de pathologie exotique*, 2010.

Private Bag 1671, ZA-6140, Grahamstown, South Africa, [norbertdrager@gmail.com].

National and international efforts to eradicate tsetse fly-borne human and animal trypanosomiasis are critically evaluated, and possible reasons for their failure in many cases are discussed. Some formerly performed campaigns in specific areas with positive results cannot be taken as examples to solve the main problems. In future, a significant reduction of trypanosomiasis cases will be possible to achieve only if a concerted long-term Pan-African approach, based on financial security, the continuity of expert staff, and a well-planned, ecologically sound land use, is generally accepted.

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

[See also 33: 15411, 15420, 15424, 15449, 15451, 15452, 15453, 15480, 15481, 15496]

15432. **Adams, E. R., Hamilton, P. B., Rodrigues, A. C., Malele, I. I., Delespau, V., Teixeira, M. M. & Gibson, W.,** 2010. New *Trypanosoma (Duttonella) vivax* genotypes from tsetse flies in East Africa. *Parasitology*, **137** (4): 641-650.

School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK.

Salivarian trypanosomes pose a substantial threat to livestock, but their full diversity is not known. To survey trypanosomes carried by tsetse in Tanzania, DNA samples from infected proboscides of *Glossina pallidipes* and *G. swynnertoni* were identified using fluorescent fragment length barcoding (FFLB), which discriminates species by size polymorphisms in multiple regions of the ribosomal RNA locus. FFLB identified the trypanosomes in 65 of 105 (61.9 percent) infected proboscides, revealing nine mixed infections. Of seven different FFLB profiles, two were similar but not identical to reference West African *Trypanosoma vivax*; five other profiles belonged to known species also identified in fly midguts. Phylogenetic analysis of the glycosomal glyceraldehyde phosphate dehydrogenase gene revealed that the Tanzanian *T. vivax* samples fell into two distinct groups, both outside the main clade of African and South American *T. vivax*. These new *T. vivax* genotypes were common and widespread in tsetse in Tanzania. The *T. brucei*-like trypanosome previously described from tsetse midguts was also found in 2 proboscides, demonstrating a salivarian transmission route. Investigation of mammalian host range and pathogenicity will reveal the importance of these new trypanosomes for the epidemiology and control of animal trypanosomiasis in East Africa.

15433. **Alves, F. M., Olifiers, N., Bianchi, R. D., Duarte, A. C., Cotias, P. M., D'Andrea, P. S., Gompper, M. E., Mourao, G. D., Herrera, H. M. & Jansen, A. M.,** 2010. Modulating variables of *Trypanosoma cruzi* and *Trypanosoma evansi* transmission in free-ranging coati (*Nasua nasua*) from the Brazilian Pantanal Region. *Vector Borne Zoonotic Diseases*. e Publication ahead of print, December 13.

1 Laboratório de Biologia de Tripanossomatídeos, FIOCRUZ/RJ, Rio de Janeiro/RJ, Brazil. [jansen@ioc.fiocruz.br].

This is a long-term follow-up of infection by *Trypanosoma cruzi* (TC) and *Trypanosoma evansi* (TE) in the free-ranging coatis (Procyonidae: *Nasua nasua*) from the

Pantanal region (Mato Grosso do Sul, Brazil). We evaluated TC and TE infection by immunofluorescence assay, haemoculture (HC), and microhaematocrit centrifuge techniques (MHCT). We also examined coatis health by quantifying haematological parameters including packed cell volume (PCV), white blood cell (WBC) count, and differential leukocyte count. TC isolates through HC were typed by the miniexon gene. Mixed infections by both parasites and the two main lineages of TC (76 percent TCI, 3 percent TCII, and 14 percent TCI/TCII) were observed. *Trypanosoma rangeli* was also isolated (7 percent). Overall, seroprevalences of TC and TE infection were 53.5 percent and 42.0 percent, respectively. Positive HC (indicating high TC parasitaemia) occurred in 34 percent of seropositive coatis for TC, and positive MHCT (high TE parasitaemia) were observed in 36.4 percent of seropositive coatis for TE. We detected a higher prevalence of positive HC in females (72 percent) than males (43 percent), and also during the dry season, indicating a seasonal potential of this host species for TC transmission. These features did not occur for TE infection. However, the prevalence of TE based on serology and MHCT was higher among adults than subadults. Coatis with positive HC or MHCT displayed a slight decrease in their WBC. In contrast to the animals with positive HC, coatis with positive MHCT displayed a decrease on their PCV. Moreover, concurrent high TC and TE parasitaemias caused a larger decrease of PCV values. This study corroborates the importance of coatis in the maintenance of TC and TE transmission cycles in the southern Pantanal and shows a seasonal character of TC transmissibility to its vector by the coati population from the study area.

15434. **Berlin, D., Nasereddin, A., Azmi, K., Erekat, S., Abdeen, Z. & Baneth, G.,** 2010. Longitudinal study of an outbreak of *Trypanosoma evansi* infection in equids and dromedary camels in Israel. *Veterinary Parasitology*, **174** (3-4): 317-322.

Veterinary Teaching Hospital, Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel. [berlin@agri.huji.ac.il].

An outbreak of trypanosomosis caused by *Trypanosoma evansi* involving horses, camels and donkeys occurred in a farm in Israel. A longitudinal study of two outbreak phases was conducted which included clinical monitoring, blood smears, packed cell volume (PCV), serology and polymerase chain reaction (PCR) followed by reverse dot blot (RDB) for the molecular detection of infection. This was the first reported *T. evansi* outbreak in domestic animals in Israel. Most of the camels on the farm (8/10; 80 percent) were diagnosed with *T. evansi* infection whereas infection was less prevalent in the horses (3/7; 43 percent) and donkeys (6/13; 46 percent). Clinical disease was evident in four camels and one horse exhibiting characteristic clinical signs, anaemia and parasitaemia detected on blood smears and by positive RDB. Six other animals were diagnosed as asymptomatic latent carriers by positive RDB and six additional animals were only seropositive and were considered suspected carriers. A significant difference was found in the mean PCV between symptomatic and latent carriers with severe anaemia observed only in the symptomatic animals. An anaphylactic-like reaction, fatal in one case, was observed in two camels diagnosed with severe trypanosome parasitaemia immediately following treatment with melarsenoxide cysteamine. Furthermore, recurrence of infection was documented in one camel four months post treatment.

15435. **Berrang-Ford, L., Lundine, J. & Breau, S.,** 2010. Conflict and human African trypanosomiasis. *Social Science & Medicine*. Available online, **June 23**.

McGill University, Geography, 805 Sherbrooke Street Ouest, Burnside Hall Rm. 705, Montreal, QC, Canada H3A 2K6. [lea.berrangford@mcgill.ca].

Human African trypanosomiasis (HAT) has reemerged 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.

15436. **Davis, S., Aksoy, S. & Galvani, A.,** 2010. A global sensitivity analysis for African sleeping sickness. *Parasitology*: 1-11. **Published online, November 16**.

Yale School of Public Health, 60 College Street, P.O. Box 208034, New Haven, CT 06520 USA; and School of Mathematics and Geospatial Sciences, RMIT University, GPO Box 2476 V, Melbourne, Victoria 3001, Australia [stephen.davis@rmit.edu.au]

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, R_0 , 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 R_0 , using parameter ranges based on literature, field data and expertise out of Uganda. For West African sleeping sickness, our results indicate that the proportion of bloodmeals 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.

15437. **Dobigny, G., Poirier, P., Hima, K., Cabaret, O., Gauthier, P., Tatard, C., Costa, J. M. & Bretagne, S.**, 2010. Molecular survey of rodent-borne *Trypanosoma* in Niger with special emphasis on *T. lewisi* imported by invasive black rats. *Acta Tropica*. **e Publication ahead of print, November 30.**

Institut de Recherche pour le Développement, Centre de Biologie pour la Gestion des Populations, Centre régional Agrhymet, BP 11011, Niamey, Niger; and Centre de Biologie et de Gestion des Populations (UMR IRD-INRA-Cirad-Montpellier SupAgro), Campus de Baillarguet, CS30016, 34988 Montpellier-sur-Lez, France. [gauthier.dobigny@ird.fr].

Invading rodent species can harbour parasites with potential transmission to native rodents and/or humans. To investigate trypanosomes prevalence in rodents, the spleen of 76 rodents from Niger identified by their karyotype was used as a DNA source for *Trypanosoma* detection using a newly developed qPCR assay. Of the invasive black rat, *Rattus rattus*, 71 percent (10/14) were PCR positive as well as 6 percent (4/62) of native African rodents. Sequences of approximately 400bp of the SSU rDNA gene identified phylogenetically close *Trypanosoma* lineages. *Trypanosoma lewisi* was present in all positive black rats and the sequences displayed 100 percent similarity with *T. lewisi*-infected humans in Senegal. *T. lewisi* was also detected in one *Acomys johannis*, suggesting a possible transmission to native species. In addition to improved knowledge of *Trypanosoma* diversity in rodents, our data underscore the introduction of the potentially pathogenic *T. lewisi* kinetoplastid through the human-mediated invasion of black rats all over West Africa.

15438. **Hoppenheit, A., Steuber, S., Bauer, B., Ouma, E. M., Diall, O., Zessin, K. H. & Clausen, P. H.**, 2010. Host preference of tsetse: an important tool to appraise the Nagana risk of cattle in the cotton zone of Mali. *Wiener Klinische Wochenschrift*, **122** Suppl 3: 81-86.

Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany. [Antje_hoppenheit@yahoo.de].

Nagana, a vector-borne epizootic caused by trypanosomes, severely constrains the use of draught animals in the cotton zone of south-eastern Mali. The disease causes considerable economic losses for the local farmers due to high mortality and morbidity ensuing productivity losses. Nagana is routinely controlled by the use of trypanocides and an overreliance on their use throughout past decades resulted in multiple drug resistance of trypanosomes in most parts of West Africa's cotton belt. Designing alternative, effective vector control strategies requires an identification of the preferred hosts of tsetse flies through blood meal analysis as a prerequisite for estimating infection risk. A survey was therefore

conducted between November 2008 and April 2009, catching 474 *Glossina* species which were dissected. Blood meals were smeared on filter paper (Whatman®-FTA-Cards) for laboratory analysis. DNA extractions and amplification using universal vertebrate cytochrome b primers of 120 assorted samples detected 74 DNA-containing specimens. The subsequent use of cattle-specific primers yielded 52 visible amplicons in the gel electrophoresis. Sequencing and BLASTN® analysis of the remaining samples revealed 19 blood meals matching with existing sequences of the human genome in Genbank®. Two samples originated from crocodiles whereas one was unidentifiable.

15439. **Kohagne Tongue, L., Mengue M'Eyi, P., Mimpfoundi, R. & Louis, F. J.,** 2010. *Glossina* feeding habits and diversity of species of trypanosomes in an active focus of human African trypanosomiasis in Gabon. *Bulletin de la Société de Pathologie Exotique*, **103** (4): 264-271.

Programme sous régional de lutte contre la trypanosomiase humaine africaine, Organisation de coordination pour la lutte contre les endémies en Afrique centrale (OCEAC), BP 15665, Yaoundé, Cameroon. [kohagne_oceac@yahoo.fr].

Feeding host is an important factor determining the *Glossina* infection rate and the proportion of different species of trypanosome. *Glossina* feed both upon animals and humans. In order to identify species of trypanosomes present in the Komo-Mondah focus and to verify whether there is any relationship between the prevalence of sleeping sickness and the feeding habits of *Glossina*, we have carried out an entomological survey in this focus in Gabon. Flies were dissected and organs were analysed by PCR, while the origin of blood meals was determined by ELISA. Three species of trypanosomes were found: *Trypanosoma congolense* "forest type" (14/104; 13.46 percent), *Trypanosoma vivax* (11/104; 10.58 percent) and *Trypanosoma brucei* s.l. (65/104; 62.5 percent) with 13.46 percent (14/104) of mixed infections of *T. brucei* s.l. and *T. congolense*. *Glossina palpalis palpalis* was caught in all biotopes investigated (91.85 percent) and was infected by all these species of trypanosomes. *Glossina caliginea* was not infected and *Glossina fuscipes fuscipes* was infected by *T. brucei* s.l. Tsetse flies feed more on animals than on humans in almost all villages, but there was no significant difference between the number of blood meals taken by these two groups of vertebrates ($\text{Chi}^2 = 7.43$; $p > 0.05$). A negative correlation was found between the zoophylic/anthropophylic index and the prevalence of HAT. This result is insufficient to conclude that this index can be used as an indicator of the degree of prevalence of HAT. In fact, the trypanosomosis risk seems to be an appropriate indicator of the prevalence of HAT in an area. The identification of the reservoir hosts in this focus would be useful for a good understanding of the HAT epidemiology.

15440. **Kone, N., N'Goran, E. K., Sidibe, I., Kombassere, A. W. & Bouyer, J.,** 2010. Spatio-temporal distribution of tsetse and other biting flies in the Mouhoun River basin, Burkina Faso. *Medical & Veterinary Entomology*. **e Publication ahead of print, December 27.**

Centre International de Recherche-Développement sur l'Élevage en Zone Subhumide, Bobo Dioulasso, Burkina Faso; Département de Zoologie & Biologie Animale, UFR Biosciences, University of Cocody-Abidjan, Abidjan,

Ivory Coast; Contrôle des Maladies Animales Exotiques et Émergentes, UMR15, Centre de Coopération Internationale en Recherche Agronomique pour le Développement/Initiative Française pour la Recherche Agronomique (CIRAD/INRA), Montpellier, France; and Service de Parasitologie, Institut Sénégalais de Recherches Agricoles-Laboratoire National d'Élevage et de Recherches Vétérinaires (ISRA-LNERV), Dakar-Hann, Senegal.

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.

15441. **Muzari, M. O., Burgess, G. W., Skerratt, L. F., Jones, R. E. & Duran, T. L.,** 2010. Host preferences of tabanid flies based on identification of blood meals by ELISA. *Veterinary Parasitology*, **174** (3-4): 191-198.

School of Veterinary and Biomedical Sciences, James Cook University, Townsville, Qld 4811, Australia. [Odwell.Muzari@jcu.edu.au].

Tabanid flies in Australia are potential vectors of the parasite *Trypanosoma evansi* which causes the animal disease surra. It is endemic to most of south-east Asia and could enter Australia, but evaluation of the potential impact of a surra incursion requires identification of the major hosts of Australian tabanids. This study investigated the natural pattern of feeding and host preference by tabanid flies of Townsville, north Queensland by identification of ingested blood in trap-caught tabanids using ELISA. The assays were developed for identification of horse, cow, macropod and pig blood meals. Macropods were the most frequent food source for each of six major tabanid species in the area. This did not vary with location for one species, *Tabanus pallipennis*, despite macropod densities being lower than other hosts such as cattle and horses in some locations. Feeding patterns on other hosts generally depended on availability and density of animals. All tabanid species fed on at least three of the host species tested and mixed meals were also commonly encountered, suggesting a level of opportunistic feeding in addition to a preference for macropods. Some

of the blood meals detected were possibly from previous gonotrophic cycles. The results indicate that all tabanid species examined could potentially transmit surra and all the host types investigated could be affected, but macropods face the highest transmission risk.

15442. **Mwangi, S., Murungi, E., Jonas, M. & Christoffels, A.,** 2010. Evolutionary genomics of *Glossina morsitans* immune-related CLIP domain serine proteases and serine protease inhibitors. *Infection, Genetics & Evolution*. **In press; corrected proof.**

South African National Bioinformatics Institute, University of the Western Cape, Private Bag X17, Modderdam Road, Bellville, Cape Town, South Africa. [sarah@sanbi.a.za].

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* remains 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.

15443. **Njitchouang, G. R., Njiokou, F., Nana-Djeunga, H., Asonganyi, T., Fewou-Moundipa, P., Cuny, G. & Simo, G.,** 2010. A new transmission risk index for human African trypanosomiasis and its application in the identification of sites of high transmission of sleeping sickness in the Fontem focus of southwest Cameroon. *Medical & Veterinary Entomology*. **e Publication ahead of print, December 27.**

General Biology Laboratory, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé 1, Yaoundé, Cameroon; Department of Biochemistry, Faculty of Science, University of Yaoundé 1, Yaoundé, Cameroon; Department of Physiological Sciences, Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Yaoundé, Cameroon; and Laboratoire de Recherche et de Coordination sur les Trypanosomoses, Institut de Recherche pour le Développement (IRD), UMR 177, CIRAD, Montpellier, France; and Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon.

A new index for the risk for transmission of human African trypanosomiasis was developed from an earlier index by adding terms for the proportion of tsetse infected with *Trypanosoma brucei gambiense* group 1 and the contribution of animals to tsetse diet. The validity of the new index was then assessed in the Fontem focus of southwest Cameroon. Averages of 0.66 and 4.85 *Glossina palpalis palpalis* (Diptera: Glossinidae) were caught per trap/day at the end of one rainy season (November) and the start of the next (April), respectively. Of 1 596 tsetse flies examined, 4.7 percent were positive for *Trypanosoma brucei* s.l. midgut infections and 0.6 percent for *T. b. gambiense* group 1. Among 184 bloodmeals identified, 55.1 percent were from pigs, 25.2 percent from humans, 17.6 percent from wild animals and 1.2 percent from goats. Of the meals taken from humans, 81.5 percent were taken at sites distant from pigsties. At the end of the rainy season, catches were low and similar between biotopes distant from and close to pigsties, but the risk for transmission was greatest at sites distant from the sties, suggesting that the presence of pigs reduced the risk to humans. At the beginning of the rainy season, catches of tsetse and risk for transmission were greatest close to the sties. In all seasons, there was a strong correlation between the old and new indices, suggesting that both can be used to estimate the level of transmission, but as the new index is the more comprehensive, it may be more accurate.

15444. **Oberle, M., Balmer, O., Brun, R. & Roditi, I.,** 2010. Bottlenecks and the maintenance of minor genotypes during the life cycle of *Trypanosoma brucei*. *PLoS Pathogens*, **6** (7): e1001023.

Swiss Tropical and Public Health Institute, Basel, Switzerland.
[isabel.roditi@izb.unibe.ch].

African trypanosomes are digenetic parasites that undergo part of their developmental cycle in mammals and part in tsetse flies. We established a novel technique to monitor the population dynamics of *Trypanosoma brucei* throughout its life cycle while minimizing the confounding factors of strain differences or variation in fitness. Clones derived from a single trypanosome were tagged with short synthetic DNA sequences in a non-transcribed region of the genome. Infections were initiated with mixtures of tagged parasites and a combination of polymerase chain reaction and deep sequencing were used to monitor the composition of populations throughout the life cycle. This revealed that a minimum of several hundred parasites survived transmission from a tsetse fly to a mouse, or *vice versa*, and contributed to the infection in the new host. In contrast, the parasites experienced a pronounced bottleneck during differentiation and migration from the midgut to the salivary glands of tsetse. In two cases a single tag accounted for > or =99 percent of the population in the glands, although minor tags could be also detected. Minor tags were transmitted to mice together with the dominant tag(s), persisted during a chronic infection, and survived transmission to a new insect host. An important outcome of the bottleneck within the tsetse is that rare variants can be amplified in individual flies and disseminated by them. This is compatible with the epidemic population structure of *T. brucei*, in which clonal expansion of a few genotypes in a region occurs against a background of frequent recombination between strains.

15445. **Rodriguez, N. F., Tejedor-Junco, M. T., Hernandez-Trujillo, Y., Gonzalez, M. & Gutierrez, C.,** 2010. The role of wild rodents in the transmission of *Trypanosoma evansi* infection in an endemic area of the Canary Islands (Spain). *Veterinary*

Parasitology, **174** (3-4): 323-327.

Department of Animal Medicine and Surgery, Veterinary Faculty, University of Las Palmas de Gran Canaria, 35413 Arucas, Las Palmas, Canary Islands, Spain. [mtejedor@dcc.ulpgc.es].

Trypanosoma evansi was diagnosed for the first time in camels in the Canary Islands in 1997. Several sanitary measures including treatment of infected animals were taken; however, nowadays a little area is still infected. In order to determine possible reservoirs 138 wild rodents were trapped, 64 of them in the infected farms and the remaining 74 in other areas. The captured species were *Rattus rattus* (24), *Rattus norvegicus* (69), and *Mus musculus domesticus* (45). Serological (CATT/*T. evansi*), parasitological (micro-haematocrit centrifugation technique and stained smears) and molecular (PCR) methods for *T. evansi* and *T. lewisi* were used as diagnostic methods. None of the examined rodents was positive for *T. evansi*; 18, however, they showed motile trypanosomes using the micro-haematocrit centrifugation technique and were positive for *T. lewisi* by PCR. The results would suggest that the rodent species studied would not play a relevant role in the epidemiology of *T. evansi* infection in the Canary Islands.

15446. **Seck, M. T., Bouyer, J., Sall, B., Bengaly, Z. & Vreysen, M. J.**, 2010. The prevalence of African animal trypanosomoses and tsetse presence in Western Senegal. *Parasite*, **17** (3): 257-265.

Institut Sénégalais de Recherches Agricoles, Laboratoire National d'Élevage et de Recherches Vétérinaires, Service de Parasitologie, BP 2057 Dakar - Hann, Senegal; Direction des Services Vétérinaires, 37, avenue Pasteur, BP 67, Dakar, Sénégal; Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Unité Propre de Recherche Animal et gestion intégrée des risques, Campus International de Baillarguet, F34398, Montpellier, France; and Entomology Unit, Food and Agriculture Organization/International Atomic Energy Agency Agriculture and Biotechnology Laboratory, Joint Food and Agriculture Organization/International Atomic Energy Agency Programme, Vienna, Austria. [mjtseck@hotmail.fr].

In 2005, the Government of Senegal initiated a tsetse eradication campaign in the Niayes and La Petite Côte aiming at the removal of African animal trypanosomosis (AAT), which is one of the main constraints to the development of more effective cattle production systems. The target area has particular meteorological and ecological characteristics that provide great potential for animal production, but it is unfortunately still infested by the riverine tsetse species *Glossina palpalis gambiensis* Vanderplank (Diptera: Glossinidae). The tsetse project in Senegal has adopted an area-wide integrated pest management (AW-IPM) approach that targets the entire tsetse population within a delimited area. During the first phase of the programme, a feasibility study was conducted that included the collection of entomological, veterinary, population genetics, environmental and socioeconomic baseline data. This paper presents the parasitological and serological prevalence data of AAT in cattle residing inside and outside the tsetse-infested areas of the target zone prior to the control effort. At the herd level, a mean parasitological prevalence of 2.4 percent was observed, whereas a serological prevalence of 28.7 percent, 4.4 percent, and 0.3 percent was obtained

for *Trypanosoma vivax*, *T. congolense* and *T. brucei brucei*, respectively. The observed infection risk was three times higher for *T. congolense* and *T. vivax* in the tsetse-infested than in the assumed tsetse-free areas. Moreover, AAT prevalence decreased significantly with distance from the nearest tsetse captured which indicated that cyclical transmission of the parasites by tsetse was predominant over mechanical transmission by numerous other biting flies present. The importance of these results for the development of a control strategy for the planned AW-IPM campaign is discussed.

15447. **Sehgal, R. N., Buermann, W., Harrigan, R. J., Bonneaud, C., Loiseau, C., Chasar, A., Sepil, I., Valkiunas, G., Iezhova, T., Saatchi, S. & Smith, T. B., 2010.** Spatially explicit predictions of blood parasites in a widely distributed African rainforest bird. *Proceedings of the Royal Society B: Biological Sciences*. **Published online before print, September 29.**

Department of Biology, San Francisco State University, 1600 Holloway Avenue, San Francisco, CA 94132, USA. [sehgal@sfsu.edu].

Critical to the mitigation of parasitic vector-borne diseases is the development of accurate spatial predictions that integrate environmental conditions conducive to pathogen proliferation. Species of *Plasmodium* and *Trypanosoma* readily infect humans, and are also common in birds. Here, we develop predictive spatial models for the prevalence of these blood parasites in the olive sunbird (*Cyanomitra olivacea*). Since this species exhibits high natural parasite prevalence and occupies diverse habitats in tropical Africa, it represents a distinctive ecological model system for studying vector-borne pathogens. We used PCR and microscopy to screen for haematozoa from 28 sites in Central and West Africa. Species distribution models were constructed to associate ground-based and remotely sensed environmental variables with parasite presence. We then used machine-learning algorithm models to identify relationships between parasite prevalence and environmental predictors. Finally, predictive maps were generated by projecting model outputs to geographically unsampled areas. Results indicate that for *Plasmodium* spp., the maximum temperature of the warmest month was most important in predicting prevalence. For *Trypanosoma* spp., seasonal canopy moisture variability was the most important predictor. The models presented here visualize gradients of disease prevalence, identify pathogen hot spots and will be instrumental in studying the effects of ecological change on these and other pathogens.

15448. **Wardrop, N. A., Atkinson, P. M., Gething, P. W., Fevre, E. M., Picozzi, K., Kakembo, A. S. & Welburn, S. C., 2010.** Bayesian geostatistical analysis and prediction of Rhodesian human African trypanosomiasis. *PLoS Neglected Tropical Diseases*, **4** (12): e914.

Centre for Infectious Diseases, Division of Pathway Medicine, College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh, UK [Sue.Welburn@ed.ac.uk].

The persistent spread of Rhodesian human African trypanosomiasis (HAT) in Uganda in recent years has increased concerns of a potential overlap with the Gambian form of the disease. Recent research has aimed to increase the evidence base for targeting control measures by focusing on the environmental and climatic factors that control the spatial

distribution of the disease. One recent study used simple logistic regression methods to explore the relationship between prevalence of Rhodesian HAT and several social, environmental and climatic variables in two of the most recently affected districts of Uganda, and suggested the disease had spread into the study area due to the movement of infected, untreated livestock. Here we extend this study to account for spatial autocorrelation, incorporate uncertainty in input data and model parameters, and undertake predictive mapping for risk of high HAT prevalence in future. Using a spatial analysis in which a generalized linear geostatistical model is used in a Bayesian framework to account explicitly for spatial autocorrelation and incorporate uncertainty in input data and model parameters we are able to demonstrate a more rigorous analytical approach, potentially resulting in more accurate parameter and significance estimates and increased predictive accuracy, thereby allowing an assessment of the validity of the livestock movement hypothesis given more robust parameter estimation and appropriate assessment of covariate effects. Analysis strongly supports the theory that Rhodesian HAT was imported to the study area via the movement of untreated, infected livestock from endemic areas. The confounding effect of health care accessibility on the spatial distribution of Rhodesian HAT and the linkages between the disease's distribution and minimum land surface temperature have also been confirmed via the application of these methods. In conclusion, predictive mapping indicates an increased risk of high HAT prevalence in the future in areas surrounding livestock markets, demonstrating the importance of livestock trading for continuing disease spread. Adherence to government policy to treat livestock at the point of sale is essential to prevent the spread of sleeping sickness in Uganda.

5. HUMAN TRYPANOSOMOSIS

(a) SURVEILLANCE

15449. **Frischknecht, F.**, 2010. Imaging parasites at different scales. 2010. *Cell Host & Microbe*, **8** (1): 16-19.

Department of Infectious Diseases, Parasitology, University of Heidelberg Medical School, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany. [freddy.frischknecht@med.uni-heidelberg.de].

Pathogens interact with their hosts at different spatial and temporal scales. Studying these interactions therefore requires a wide range of imaging tools and approaches that bridge physics and biology, as shown by this minireview focusing on recent studies of the causative agents of malaria, toxoplasmosis, and sleeping sickness.

15450. **Hasker, E., Lutumba, P., Mumba, D., Lejon, V., Buscher, P., Kande, V., Muyembe, J. J., Menten, J., Robays, J. & Boelaert, M.**, 2010. Diagnostic accuracy and feasibility of serological tests on filter paper samples for outbreak detection of *T. b. gambiense* human African trypanosomiasis. *American Journal of Tropical Medicine & Hygiene*, **83** (2): 374-379.

Institute of Tropical Medicine Antwerp, Belgium. [ehasker@itg.be].

Control of human African trypanosomiasis (HAT) in the Democratic Republic of Congo is based on mass population screening by mobile teams; a costly and labour-intensive approach. We hypothesized that blood samples collected on filter paper by village health workers and processed in a central laboratory might be a cost-effective alternative. We estimated sensitivity and specificity of micro-card agglutination test for trypanosomiasis (micro-CATT) and enzyme-linked immunosorbent assay (ELISA)/*T. b. gambiense* on filter paper samples compared with parasitology-based case classification and used the results in a Monte Carlo simulation of a lot quality assurance sampling (LQAS) approach. Micro-CATT and ELISA/*T. b. gambiense* showed acceptable sensitivity (92.7 percent [95 percent CI 87.4-98.0 percent] and 82.2 percent [95 percent CI 75.3-90.4 percent]) and very high specificity (99.4 percent [95 percent CI 99.0-99.9 percent] and 99.8 percent [95 percent CI 99.5-100 percent]), respectively. Conditional on high sample size per lot (> or = 60 percent), both tests could reliably distinguish a 2 percent from a zero prevalence at village level. Alternatively, these tests could be used to identify individual HAT suspects for subsequent confirmation.

15451. **Jamonneau, V., Bucheton, B., Kabore, J., Ilboudo, H., Camara, O., Courtin, F., Solano, P., Kaba, D., Kambire, R., Lingue, K., Camara, M., Baelmans, R., Lejon, V. & Buscher, P.,** 2010. Revisiting the immune trypanolysis test to optimize epidemiological surveillance and control of sleeping sickness in West Africa. *PLoS Neglected Tropical Diseases*, **4** (12): e917.

Institut de Recherche pour le Développement (IRD), Unité Mixte de Recherche IRD-CIRAD 177, Montpellier, France. [vincent.jamonneau@ird.fr].

Because of its high sensitivity and its ease of use in the field, the card agglutination test for trypanosomiasis (CATT) is widely used for mass screening of sleeping sickness. However, the CATT exhibits false positive results (i) raising the question of whether CATT-positive subjects who are negative in parasitology are truly exposed to infection and (ii) making it difficult to evaluate whether *Trypanosoma brucei* (*T. b.*) *gambiense* is still circulating in areas of low endemicity. The objective of this study was to assess the value of the immune trypanolysis test (TL) in characterizing the HAT status of CATT-positive subjects and to monitor HAT elimination in West Africa. TL was performed on plasma collected from CATT-positive persons identified within medical surveys in several West African HAT foci in Guinea, Côte d' Ivoire and Burkina Faso with diverse epidemiological statuses (active, latent, or historical). All HAT cases were TL+. All subjects living in a nonendemic area were TL-. CATT prevalence was not correlated with HAT prevalence in the study areas, whereas a significant correlation was found using TL. TL appears to be a marker for contact with *T. b. gambiense*. TL can be a tool (i) at an individual level to identify non-parasitologically confirmed CATT-positive subjects as well as those who had contact with *T. b. gambiense* and should be followed up, (ii) at a population level to identify priority areas for intervention, and (iii) in the context of HAT elimination to identify areas free of HAT.

15452. **Lindner, A. K. & Priotto, G.,** 2010. The unknown risk of vertical transmission in sleeping sickness-a literature review. *PLoS Neglected Tropical Diseases*, **4** (12): e783.

London School of Hygiene & Tropical Medicine, London, UK. [A.K.Lindner@gmx.de].

Children with human African trypanosomiasis (HAT) present with a range of generally non-specific symptoms. Late diagnosis is frequent with often tragic outcomes. Trypanosomes can infect the foetus by crossing the placenta. Unequivocal cases of congenital infection that have been reported include newborn babies of infected mothers who were diagnosed with HAT in the first five days of life and children of infected mothers who had never entered an endemic country themselves. This review systematically summarizes the literature on the vertical transmission of HAT, to our knowledge for the first time. To approach the broader aspects of the subject, articles considering the epidemiology of childhood HAT and HAT in pregnancy were also included. The HAT guidelines and technical reports of the World Health Organization, Médecins Sans Frontières, Institut de Recherche pour le Développement, and of one endemic country were reviewed. Publications describing congenital HAT are very limited and consist only of single case reports and small case series. Generally it is assumed to be a rare event, but it has never been systematically investigated. In two publications, it is hypothesized that congenital HAT occurs more often than suspected. Not all guidelines and not all HAT literature mention this transmission route. It is concluded that the risk of vertical transmission is unknown. Awareness of congenital HAT is insufficient, and as a result opportunities for an early diagnosis in newborns may be missed. All HAT guidelines and local HAT protocols should stress that in endemic areas pregnant women should be systematically checked for HAT and that newborns of HAT infected mothers should be assessed for the disease as soon as possible. Studies on the impact of HAT on fertility and pregnancy and studies on congenital HAT are long overdue.

15453. **Maina, N. W., Kagira, J. M., Oberle, M., Ndung'u, J. M. & Brun, R.,** 2010. Co-infection of sleeping sickness patients with malaria and loiasis in southern Sudan. *Journal of Protozoology Research*, **20**: 12-19.

Institute of Primate Research, Nairobi, Kenya [m.ngotho@primateresearch.org].

A cross-sectional study was conducted in 2003 on sleeping sickness (SS) patients in the Ibba hospital in Maridi County, western Equatorial province of southern Sudan. The occurrences of co-infection with bloodborne infections and haematological profiles were investigated in SS patients. Fifty SS patients (23 males and 27 females) were included in the study. Most (49) of the patients were in second stage disease, but trypanosomes could be demonstrated in the cerebrospinal fluid (CSF) of only eight of them. Majority of the patients had co-infection with loiasis (36 percent), malaria (30 percent) or both loiasis and malaria (10 percent), and only 24 percent were free from other infections. Other parasitic infections observed from symptomatic patients were onchocerciasis (2), giardiasis (2), trichomoniasis (2), helminthosis (2) and amoebiasis (2). Co-infection was more common in female (85 percent) than in male patients (65 percent), which may be attributed to occupational activities by females. The patients had various disease symptoms including headache (96 percent), arthralgia/myalgia (88 percent), pruritus (82 percent), fever (52 percent), insomnia (26 percent) and mental disturbance (20 percent). The nutritional status of most of them (84 percent) was below normal Body Mass Index (20-13), and anaemia was common (79 percent). Despite most patients having either normal (66 percent) or low (12 percent) white blood cell (WBC) counts, further analysis in 23 of them revealed that majority had lymphocytosis (83 percent), eosinophilia (96 percent) and neutropaenia (61 percent), some of which are indicative of a depressed or exhausted immune system. *Loa loa* parasites obstructed microscopic observation of trypanosomes at the buffy coat interphase of a

microhaematocrit tube. This study has shown that co-infections of malaria and loiasis are common in SS patients in this region. Apart from the SS and malaria control it would be important to also introduce control programmes against nematode infections and particularly loiasis in this region. Combined control of the three diseases would decrease morbidity and co-morbidity due to multiple parasitic diseases.

15454. **Matovu, E., Mugasa, C. M., Ekangu, R. A., Deborggraeve, S., Lubega, G. W., Laurent, T., Schoone, G. J., Schallig, H. D. & Buscher, P.,** 2010. Phase II evaluation of sensitivity and specificity of PCR and NASBA followed by oligochromatography for diagnosis of human African trypanosomiasis in clinical samples from D.R. Congo and Uganda. *PLoS Neglected Tropical Diseases*, **4** (7): e737.

Department of Veterinary Parasitology and Microbiology, Makerere University, Kampala, Uganda. [matovue@vetmed.mak.ac.ug].

The polymerase chain reaction (PCR) and nucleic acid sequence-based amplification (NASBA) have been recently modified by coupling to oligochromatography (OC) for easy and fast visualization of products. In this study we evaluate the sensitivity and specificity of the PCR-OC and NASBA-OC for diagnosis of *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* human African trypanosomiasis (HAT). Both tests were evaluated in a case-control design on 143 HAT patients and 187 endemic controls from the Democratic Republic of Congo (DRC) and Uganda. The overall sensitivity of PCR-OC was 81.8 percent and the specificity was 96.8 percent. The PCR-OC showed a sensitivity and specificity of 82.4 percent and 99.2 percent on the specimens from DRC and 81.3 percent and 92.3 percent on those from Uganda. NASBA-OC yielded an overall sensitivity of 90.2 percent, and a specificity of 98.9 percent. The sensitivity and specificity of NASBA-OC on the specimens from DRC was 97.1 percent and 99.2 percent, respectively. On the specimens from Uganda we observed a sensitivity of 84.0 percent and a specificity of 98.5 percent. The tests showed good sensitivity and specificity for the *T. b. gambiense* HAT in DRC but a rather low sensitivity for *T. b. rhodesiense* HAT in Uganda.

15455. **Wastling, S. L., Picozzi, K., Kakembo, A. S. & Welburn, S. C.,** 2010. LAMP for human African trypanosomiasis: a comparative study of detection formats. *PLoS Neglected Tropical Diseases*, **4** (11): e865.

Centre for Infectious Diseases, Division of Pathway Medicine, School of Biomedical Sciences, College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh, UK. [sue.welburn@ed.ac.uk].

Loop-mediated isothermal amplification (LAMP) is at the forefront of the search for innovative diagnostics for human African trypanosomiasis (HAT). Several simple endpoint detection methods have been developed for LAMP and here we compare four of these: (i) visualization of turbidity; (ii) addition of hydroxynaphthol blue before incubation; (iii) addition of calcein with MnCl before incubation and (iv) addition of Quant-iT PicoGreen after incubation. These four methods were applied to four LAMP assays for the detection of human African trypanosomiasis, including two Trypanozoon specific and two *Trypanosoma brucei rhodesiense* specific reactions using DNA extracted from cryo-preserved procyclic

form *T. b. rhodesiense*. A multi-observer study was performed to assess inter-observer reliability of two of these methods: hydroxynaphthol blue and calcein with MnCl₂, using DNA prepared from blood samples stored on Whatman FTA cards. Results showed that hydroxynaphthol blue was the best of the compared methods for easy, inexpensive, accurate and reliable interpretation of LAMP assays for HAT. Hydroxynaphthol blue generates a violet to sky blue colour change that was easy to see and was consistently interpreted by independent observers. Visible turbidity detection is not possible for all currently available HAT LAMP reactions; Quant-iT PicoGreen is expensive and addition of calcein with MnCl₂ adversely affects reaction sensitivity and was unpopular with several observers.

(b) PATHOLOGY AND IMMUNOLOGY

[See also 33: 15408, 15409, 15502]

15456. **Freedman, B. I., Kopp, J. B., Langefeld, C. D., Genovese, G., Friedman, D. J., Nelson, G. W., Winkler, C. A., Bowden, D. W. & Pollak, M. R., 2010.** The apolipoprotein L1 (APOL1) gene and nondiabetic nephropathy in African Americans. *Journal of the American Society of Nephrology*, **21** (9): 1422-1426.

Section on Nephrology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1053, USA.
[bfreedma@wfubmc.edu].

Mapping by admixture linkage disequilibrium (LD) detected strong association between nonmuscle myosin heavy chain 9 gene (MYH9) variants on chromosome 22 and nondiabetic nephropathy in African Americans. MYH9-related variants were posited to be the probable, but not necessarily the definitive, causal variants as a result of impressive statistical evidence of association, renal expression, and a role in autosomal dominant MYH9 disorders characterized by progressive glomerulosclerosis (Epstein and Fechtner syndromes). Dense mapping within MYH9 revealed striking LD patterns and racial variation in risk allele frequencies, suggesting population genetic factors such as selection may be operative in this region. Genovese and colleagues examined large chromosomal regions adjacent to MYH9 using genome-wide association methods and non-HapMap single nucleotide polymorphisms identified in Yoruba from the 1 000 Genomes project. Statistically stronger associations were detected between two independent sequence variants in the Apolipoprotein L1 gene (APOL1) and nondiabetic nephropathy in African Americans, with odds ratios of 10.5 in idiopathic FSGS and 7.3 in hypertension-attributed ESRD. These kidney disease risk variants likely rose to high frequency in Africa because they confer resistance to trypanosomal infection and protect from African sleeping sickness. Risk variants in MYH9 and APOL1 are in strong LD, and the genetic risk that was previously attributed to MYH9 may reside, in part or in whole, in APOL1, although more complex models of risk cannot be excluded. This association likely explains racial disparities in nondiabetic nephropathy as a result of the high prevalence of risk alleles in individuals of African ancestry.

15457. **Frost, L., 2010.** Dental management of the tropical disease human African trypanosomiasis: an unusual case of pseudobulbar palsy. *British Dental Journal*, **210** (1): 13-16.

Dental Officer, Solent Healthcare Dental Service, Dental Department, Bitterne Health Centre, Commercial Street, Southampton, Hampshire, SO18 6BT, UK.

Human African trypanosomiasis (sleeping sickness) is a parasitic tropical disease endemic to sub-Saharan Africa. Due to migration and holiday travel patterns cases are increasing in the United Kingdom. The neurological sequelae have dental management implications both directly from the consequent physical disability and indirectly from the oral side-effects of the medications used to manage symptoms. Changes in disease demographics require the dental profession to increase its awareness of migration medicine and the appropriate dental management of such diseases.

15458. **Genovese, G., Friedman, D. J., Ross, M. D., Lecordier, L., Uzureau, P., Freedman, B. I., Bowden, D. W., Langefeld, C. D., Oleksyk, T. K., Uscinski Knob, A. L., Bernhardt, A. J., Hicks, P. J., Nelson, G. W., Vanhollenbeke, B., Winkler, C. A., Kopp, J. B., Pays, E. & Pollak, M. R.**, 2010. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science*, **329** (5993): 841-845.

Division of Nephrology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA.

African Americans have higher rates of kidney disease than European Americans. Here, we show that, in African Americans, focal segmental glomerulosclerosis (FSGS) and hypertension-attributed end-stage kidney disease (H-ESKD) are associated with two independent sequence variants in the APOL1 gene on chromosome 22 {FSGS odds ratio = 10.5 [95 percent confidence interval (CI) 6.0 to 18.4]; H-ESKD odds ratio = 7.3 (95 percent CI 5.6 to 9.5)}. The two APOL1 variants are common in African chromosomes but absent from European chromosomes, and both reside within haplotypes that harbour signatures of positive selection. ApoL1 (apolipoprotein L-1) is a serum factor that lyses trypanosomes. *In vitro* assays revealed that only the kidney disease-associated ApoL1 variants lysed *Trypanosoma brucei rhodesiense*. We speculate that evolution of a critical survival factor in Africa may have contributed to the high rates of renal disease in African Americans.

15459. **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.**, 2010. Matrix metalloproteinase-9 and intercellular adhesion molecule 1 are powerful staging markers for human African trypanosomiasis. *Tropical Medicine & International Health*. **Published online, 18 October.**

Biomedical Proteomics Research Group, Medical University Centre, Geneva, Switzerland; Institut National de Recherche Biomédicale, Kinshasa, Congo; Department of Veterinary Parasitology and Microbiology, Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda; Department of Biochemistry, Faculty of Science, Makerere University, Kampala, Uganda; Swiss Institute of Bioinformatics, Medical University Centre, Geneva, Switzerland; Foundation for Innovative New Diagnostics (FINN), Geneva, Switzerland; and Department of Parasitology, Institute of Tropical Medicine, Antwerp, Belgium.

A critical step before treatment of human African trypanosomiasis (HAT) is the correct staging of the disease. As 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.

15460. **MacLean, L. M., Odiit, M., Chisi, J. E., Kennedy, P. G. & Sternberg, J. M.,** 2010. Focus-specific clinical profiles in human African trypanosomiasis caused by *Trypanosoma brucei rhodesiense*. *PLoS Neglected Tropical Diseases*, **4** (12): e906.

Department of Biology, Hull York Medical School, Centre for Immunology and Infection, University of York, York, UK. [lorna.maclea@york.ac.uk].

Diverse clinical features have been reported in human African trypanosomiasis (HAT) foci caused by *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) giving rise to the hypothesis that HAT manifests as a chronic disease in south East African countries and increased in virulence towards the north. Such variation in disease severity suggests there are differences in host susceptibility to trypanosome infection and/or genetic variation in trypanosome virulence. Our molecular tools allow us to study the role of host and parasite genotypes, but obtaining matched extensive clinical data from a large cohort of HAT patients has previously proved problematic. We present a retrospective cohort study providing detailed clinical profiles of 275 HAT patients recruited in two northern foci (Uganda) and one southern focus (Malawi) in East Africa. Characteristic clinical signs and symptoms of *T. b. rhodesiense* infection were recorded and the degree of neurological dysfunction determined on admission. Clinical observations were mapped by patient estimated post-infection time. We have identified common presenting symptoms in *T. b. rhodesiense* infection; however, marked differences in disease progression and severity were identified between foci. HAT was characterised as a chronic haemo-lymphatic stage infection in Malawi, and as an acute disease with marked neurological impairment in Uganda. Within Uganda, a more rapid progression to meningo-encephalitic stage of infection was observed in one focus (Soroti) where HAT was characterized by early onset neurodysfunction; however, severe neuropathology was more frequently observed in patients in a second focus (Tororo). We have established focus-specific HAT clinical phenotypes showing dramatic variations in disease severity and rate of stage progression both between northern and southern East

African foci and between Ugandan foci. Understanding the contribution of host and parasite factors in causing such clinical diversity in *T. b. rhodesiense* HAT has much relevance for both improvement of disease management and the identification of new drug therapy.

15461. **Rodgers, J.**, 2010. Trypanosomiasis and the brain. *Parasitology*, **137**(14): 1995-2006.

Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Bearsden Road, Glasgow G61 1QH, UK. [Jean.Rodgers@vet.gla.ac.uk].

Neurological involvement following trypanosome infection has been recognised for over a century. However, there are still many unanswered questions concerning the mechanisms used by the parasite to gain entry to the CNS and the pathogenesis of the resulting neuroinflammatory reaction. There is a paucity of material from human cases of the disease therefore the majority of current research relies on the use of animal models of trypanosome infection. This review reports contemporary knowledge, from both animal models and human samples, regarding parasite invasion of the CNS and the neuropathological changes that accompany trypanosome infection and disease progression. The effects of trypanosomes on the blood-brain barrier are discussed and possible key molecules in parasite penetration of the barrier highlighted. Changes in the balance of CNS cytokines and chemokines are also described. The article closes by summarising the effects of trypanosome infection on the circadian sleep-wake cycle, and sleep structure, in relation to neuroinflammation and parasite location within the CNS. Although a great deal of progress has been made in recent years, the advent and application of sophisticated analysis techniques, to decipher the complexities of HAT pathogenesis, herald an exciting and rewarding period for advances in trypanosome research.

15462. **Sternberg, J. M. & Maclean, L.**, 2010. A spectrum of disease in human African trypanosomiasis: the host and parasite genetics of virulence. *Parasitology*, **137** (14): 2007-2015.

Institute of Biological and Environmental Sciences, University of Aberdeen, Zoology Building, Aberdeen AB242TZ, UK. [j.sternberg@abdn.ac.uk].

For over 50 years it has been known that there are considerable differences in the severity and rate of progression of both *Trypanosoma brucei rhodesiense* and *T. b. gambiense* infection between individuals. Yet research into the factors, whether parasite or host, which control virulence in human African trypanosomiasis is in its infancy. In this paper we review the clinical evidence for virulence variation and the epidemiological and experimental data that give clues as to the mechanisms involved. Evidence will be presented for both asymptomatic forms of *T. b. gambiense* infection and low virulence forms of *T. b. rhodesiense* infection in humans. While in both cases the mechanisms remain to be elucidated, the overall infection virulence phenotype is determined by both parasite and host genotype.

15463. **Tiberti, N., Hainard, A., Lejon, V., Robin, X., Ngoyi, D. M., Turck, N., Matovu, E., Enyaru, J., Ndung'u, J. M., Scherl, A., Dayon, L. & Sanchez, J. C.**, 2010. Discovery and verification of osteopontin and beta-2-microglobulin as promising

markers for staging human African trypanosomiasis. *Molecular & Cellular Proteomics*, **9** (12): 2783-2795.

Biomedical Proteomics Research Group, Medical University Centre, Geneva, Switzerland. [jean-charles.sanchez@unige.ch].

Human African trypanosomiasis, or sleeping sickness, is a parasitic disease endemic in sub-Saharan Africa, transmitted to humans through the bite of a tsetse fly. The first or haemolympathic stage of the disease is associated with presence of parasites in the bloodstream, lymphatic system, and body tissues. If patients are left untreated, parasites cross the blood-brain barrier and invade the cerebrospinal fluid and the brain parenchyma, giving rise to the second or meningoencephalitic stage. Stage determination is a crucial step in guiding the choice of treatment, as drugs used for S2 are potentially dangerous. Current staging methods, based on counting white blood cells and demonstrating trypanosomes in cerebrospinal fluid, lack specificity and/or sensitivity. In the present study, we used several proteomic strategies to discover new markers with potential for staging human African trypanosomiasis. Cerebrospinal fluid (CSF) samples were collected from patients infected with *Trypanosoma brucei gambiense* in the Democratic Republic of Congo. The stage was determined following the guidelines of the national control programme. The proteome of the samples was analyzed by two-dimensional gel electrophoresis (n = 9), and by sixplex tandem mass tag (TMT) isobaric labelling (n = 6) quantitative mass spectrometry. Overall, 73 proteins were overexpressed in patients presenting the second stage of the disease. Two of these, osteopontin and beta-2-microglobulin, were confirmed to be potential markers for staging human African trypanosomiasis (HAT) by Western blot and ELISA. The two proteins significantly discriminated between S1 and S2 patients with high sensitivity (68 percent and 78 percent, respectively) for 100 percent specificity, and a combination of both improved the sensitivity to 91 percent. The levels of osteopontin and beta-2-microglobulin in CSF of S2 patients (mug/ml), as well as the fold increased concentration in S2 compared with S1 (3.8 and 5.5 respectively) make the two markers good candidates for the development of a test for staging HAT patients.

(c) TREATMENT

[See also **33**: 15406]

15464. **Barrett, M. P.**, 2010. Potential new drugs for human African trypanosomiasis: some progress at last. *Current Opinion in Infectious Diseases*, **23** (6): 603-608.

Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow, UK. [michael.barrett@glasgow.ac.uk].

This review covers recent developments towards novel treatments for human African trypanosomiasis (HAT). Within the past decade, some important advances in the treatment of HAT have been made. One old drug, melarsoprol, previously administered over a period of a month or more, is now given in a 10-day regimen greatly reducing hospital costs. A combination chemotherapy, eflornithine alongside nifurtimox, has been introduced to decrease the time frame and overall dosing of eflornithine and reducing the risk of drug

resistance emerging. One new, orally available diamidine prodrug, pafuramidine, that recently completed phase III clinical trials, disappointingly was halted in its progress to clinic when unforeseen toxicity issues emerged. The diamidine series, however, has recently yielded representatives that cure second-stage central nervous system (CNS)-involved infections in experimental animals while showing less tissue accumulation in mammals and thus offer considerable promise. A nitroheterocycle, fexinidazole, whose trypanocidal activity was first shown nearly 30 years ago, has entered clinical trials. Another approach, grounded in the use of pharmacokinetic data, has brought another new class of compound based on the oxaborole scaffold forward for clinical candidacy. Furthermore, several target-based and whole organism-based chemical compound screening campaigns have identified promising hits for lead development. The new developments in trypanocidal drug discovery mean that new compounds could become available within the next five years to support the WHO declared campaign to eliminate HAT.

15465. **Burri, C.**, 2010. Chemotherapy against human African trypanosomiasis: is there a road to success? *Parasitology*, **137** (14): 1987-1994.

Swiss Tropical and Public Health Institute, Department of Medicines Research, Basel, Switzerland. [Christian.Burri@unibas.ch].

For over fifty years, human African trypanosomiasis (HAT, sleeping sickness) has been treated with suramin, pentamidine and the very toxic organo-arsenical melarsoprol that was the only drug available for effective treatment of the second stage of the disease. Recently, there have been significant efforts using molecular and biochemical approaches to drug design, including high-throughput screening, but the number of lead compounds with promising activity against *T. brucei* spp. and an acceptable toxicity index has remained astonishingly small. Clinical research continues to be difficult due to the economic constraints and the complexity of trials on a low prevalence disease in remote and impoverished African regions. Despite those limitations the situation for the patients is improving thanks to the combination of a number of critical factors. By the late 1990s the disease had reached epidemic levels that triggered political support. WHO would sign a donation agreement with the manufacturers for all drugs to treat HAT. A result of this agreement was that eflornithine which is much safer than melarsoprol became available and widely used by non-governmental organizations. The Impamel I and II programmes demonstrated that against all odds the conduct of clinical trials on HAT was feasible. This allowed the initiation of trials on combination therapies which eventually resulted in the nifurtimox-eflornithine combination treatment (NECT). This combination is currently being introduced as first line treatment, and there is even the prospect of having a new compound, fexinidazole, in the development pipeline. This review summarizes the key information about the existing drugs and gives a comprehensive summary about the recent and currently ongoing efforts towards new drugs.

15466. **Castillo, E., Dea-Ayuela, M. A., Bolas-Fernandez, F., Rangel, M. & Gonzalez-Rosende, M. E.**, 2010. The kinetoplastid chemotherapy revisited: current drugs, recent advances and future perspectives. *Current Medicinal Chemistry*, **17** (33): 4027-4051.

Department of Physiology, Pharmacology and Toxicology, University CEU-Cardenal Herrera, Edificio Seminario s/n, 46113-Moncada, Valencia, Spain.

Leishmaniasis, African sleeping sickness and Chagas disease, caused by the kinetoplastid parasites *Leishmania* spp, *Trypanosoma brucei* and *Trypanosoma cruzi*, respectively, are among the most important parasitic diseases, affecting millions of people and considered to be within the most relevant group of neglected tropical diseases. The main alternative to control such parasitosis is chemotherapy. Nevertheless, the current chemotherapeutic treatments are far from being satisfactory. This review outlines the current understanding of different drugs against leishmaniasis, African sleeping sickness and Chagas disease, their mechanism of action and resistance. Recent approaches in the area of anti-leishmanial and trypanocidal therapies are also enumerated, as well as new modulators from the mode of action, development of new formulations of old drugs, therapeutic switching and "in silico" drug design.

15467. **Hannaert, V.**, 2010. Sleeping sickness pathogen (*Trypanosoma brucei*) and natural products: therapeutic targets and screening systems. *Planta Medica*. **e Publication ahead of print, October 13.**

Research Unit for Tropical Diseases, de Duve Institute and Laboratory of Biochemistry, Université catholique de Louvain, Brussels, Belgium.

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.

15468. **Johanson, C., Stopa, E., McMillan, P., Roth, D., Funk, J. & Krinke, G.**, 2010. The distributional nexus of choroid plexus to cerebrospinal fluid, ependyma, and brain: toxicologic/pathologic phenomena, periventricular destabilization, and lesion spread. *Toxicologic Pathology*. **e Publication ahead of print, December 28.**

Department of Neurosurgery, Brown Medical School, Providence, RI 02903, USA. [Conrad_Johanson@brown.edu].

Bordering the ventricular cerebrospinal fluid (CSF) are epithelial cells of the 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.

15469. **Lutje, V., Seixas, J. & Kennedy, A., 2010.** Chemotherapy for second-stage human African trypanosomiasis. *Cochrane Database Systemic Reviews*, **8**: CD006201.

International Health Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, UK, L3 5QA.

Human African trypanosomiasis, or sleeping sickness, is a painful and protracted disease affecting people in the poorest parts of Africa and is fatal without treatment. Few drugs are currently available for second-stage sleeping sickness, with considerable adverse events and variable efficacy. This study set out to evaluate the effectiveness and safety of drugs for treating second-stage human African trypanosomiasis. We searched the Cochrane Infectious Diseases Group Specialized Register (May 2010), CENTRAL (The Cochrane Library Issue 3 2010), MEDLINE (1966 to May 2010), EMBASE (1974 to May 2010), LILACS (1982 to May 2010), BIOSIS (1926-May 2010), mRCT (May 2010) and reference lists. We contacted researchers working in the field and organizations. Randomized and quasi-randomized controlled trials were used as selection criteria, while two authors (VL and AK) extracted data and assessed methodological quality; a third author (JS) acted as an arbitrator. Included trials only reported dichotomous outcomes, and we present these as risk ratio (RR) with 95 percent confidence intervals (CI). Nine trials with 2 577 participants, all with *Trypanosoma brucei gambiense* HAT, were included. Seven trials tested currently available drugs: melarsoprol, eflornithine, nifurtimox, alone or in combination; one trial tested pentamidine, and one trial assessed the addition of prednisolone to melarsoprol. Fixed 10-day regimens of melarsoprol were found to be as effective as those of 26 days, with similar numbers of adverse events. Melarsoprol monotherapy gave fewer relapses than pentamidine or nifurtimox, but resulted in more adverse events. Later trials evaluate nifurtimox combined with eflornithine (NECT), showing this gives few relapses and is well tolerated. It also has practical advantages in reducing the burden on health personnel and patients, when compared to eflornithine monotherapy. It is concluded that the choice of therapy for second stage *gambiense* HAT will continue to be determined by what is locally

available, but eflornithine and NECT are likely to replace melarsoprol, with careful parasite resistance monitoring. We need research on reducing adverse effects of currently used drugs, testing different regimens, and experimental and clinical studies of new compounds, effective for both stages of the disease.

15470. **Mukhopadhyay, R. & Beitz, E.,** 2010. Metalloid transport by aquaglyceroporins: consequences in the treatment of human diseases. *Advances in Experimental Medicine & Biology*, **679**: 57-69.

Department of Pharmaceutical and Medicinal Chemistry University of Kiel, Gutenbergstrasse 76, 24118 Kiel, Germany. [rmukhop@fiu.edu].

Metalloids can severely harm human physiology in a toxicological sense if taken up from the environment in acute high doses or chronically. However, arsenic or antimony containing drugs are still being used as treatment and are often the sole regime for certain forms of cancer, mainly types of leukaemia and diseases caused by parasites, such as sleeping sickness or leishmaniasis. In this chapter, we give an outline of the positive effects of arsenicals and antimonials against such diseases, we summarize data on uptake pathways through human and parasite aquaglyceroporins, and we discuss the progress and options in the development of therapeutic aquaporin and aquaglyceroporin inhibitor compounds.

15471. **Paine, M. F., Wang, M. Z., Generaux, C. N., Boykin, D. W., Wilson, W. D., De Koning, H. P., Olson, C. A., Pohligh, G., Burri, C., Brun, R., Murilla, G. A., Thuita, J. K., Barrett, M. P. & Tidwell, R. R.,** 2010. Diamidines for human African trypanosomiasis. *Current Opinion in Investigational Drugs*, **11** (8): 876-883.

University of North Carolina at Chapel Hill, Eshelman School of Pharmacy, Campus Box 7569; and Department of Pathology and Laboratory Medicine, Campus Box 7525, Chapel Hill, NC 27599, USA.

Aromatic diamidines are potent trypanocides. Pentamidine, a diamidine, has been used for more than 60 years to treat human African trypanosomiasis (HAT); however, the drug must be administered parenterally and is active against first-stage HAT only, prior to the parasites causing neurological deterioration through invasion of the CNS. A major research effort to design novel diamidines has led to the development of orally active prodrugs and, remarkably, a new generation of compounds that can penetrate the CNS. In this review, progress in the development of diamidines for the treatment of HAT is discussed.

15472. **Pepin, J., Labbe, A. C., Mamadou-Yaya, F., Mbelesso, P., Mbadingai, S., Deslandes, S., Locas, M. C. & Frost, E.,** 2010. Iatrogenic transmission of human T cell lymphotropic virus type 1 and hepatitis C virus through parenteral treatment and chemoprophylaxis of sleeping sickness in colonial Equatorial Africa. *Clinical Infectious Diseases*, **51** (7): 777-784.

Department of Microbiology and Infectious Diseases, Université de Sherbrooke, Sherbrooke, Quebec, Canada. [jacques.pepin@usherbrooke.ca].

The simultaneous emergence of human immunodeficiency virus (HIV)-1 group M and HIV-2 into human populations, around 1921-1940, is attributed to urbanization and changes in sexual behaviour. We hypothesized that the initial dissemination of HIV-1, before sexual transmission predominated, was facilitated by the administration, via reusable syringes and needles, of parenteral drugs against tropical diseases. As proxies for highly lethal HIV-1, we investigated risk factors for hepatitis C virus (HCV) and human T cell lymphotropic virus 1 (HTLV-1) infections, blood-borne viruses compatible with prolonged survival, in an area known in 1936-1950 as the most virulent focus of African trypanosomiasis. A cross-sectional survey was carried out based on individuals 55 years and older in Mbimou land and Nola, Central African Republic. Dried blood spots were used for HCV and HTLV-1 serologic testing and nucleic acid detection. Adjusted odds ratios (ORs) and 95 percent confidence intervals (CIs) were measured by logistic regression. The results indicated that the only risk factor for HCV genotype 4 infection was treatment of trypanosomiasis before 1951 (OR, 3.13; 95 percent CI, 1.38-7.09). HTLV-1 infection was associated with having received two injections of pentamidine for trypanosomiasis chemoprophylaxis (adjusted OR, 2.03; 95 percent CI, 1.01-4.06) and with transfusions (adjusted OR, 2.82; 95 percent CI, 1.04-7.67). From historical data, we predicted that 59 percent of Mbimous 65 years and older would report treatment for trypanosomiasis before 1951; only 11 percent did so. It is concluded that treatment of trypanosomiasis before 1951 may have caused iatrogenic HCV transmission. Population-wide half-yearly intramuscular pentamidine for trypanosomiasis chemoprophylaxis in 1947-1953 may have caused iatrogenic HTLV-1 transmission. These and other interventions against tropical diseases could have iatrogenically transmitted SIV(cpz), jump-starting the HIV-1 epidemic. The excess mortality among patients with trypanosomiasis treated before 1951 supports this hypothesis.

15473. **Torrele, E., Bourdin Trunz, B., Tweats, D., Kaiser, M., Brun, R., Mazue, G., Bray, M. A. & Pecoul, B.,** 2010. Fexinidazole - a new oral nitroimidazole drug candidate entering clinical development for the treatment of sleeping sickness. *PLoS Neglected Tropical Diseases*, **4** (12): e923.

Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland.
[e.torrele@gmail.com].

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a fatal parasitic disease caused by trypanosomes. Current treatment options for HAT are scarce, toxic, no longer effective, or very difficult to administer, in particular for the advanced, fatal stage of the disease (stage 2, chronic HAT). New safe, effective and easy-to-use treatments are urgently needed. Here it is shown that fexinidazole, a 2-substituted 5-nitroimidazole rediscovered by the Drugs for Neglected Diseases initiative (DNDi) after extensive compound mining efforts of more than 700 new and existing nitroheterocycles, could be a short-course, safe and effective oral treatment curing both acute and chronic HAT and that could be implemented at the primary health care level. To complete the preclinical development and meet the regulatory requirements before initiating human trials, the anti-parasitic properties and the pharmacokinetic, metabolic and toxicological profile of fexinidazole have been assessed. Standard *in vitro* and *in vivo* anti-parasitic activity assays were conducted to assess drug efficacy in experimental models for HAT. In parallel, a full range of preclinical pharmacology and safety studies, as required by international regulatory guidelines before initiating human studies, have been conducted. Fexinidazole is moderately

active *in vitro* against African trypanosomes (IC₅₀ against laboratory strains and recent clinical isolates ranged between 0.16 and 0.93 µg/mL) and oral administration of fexinidazole at doses of 100 mg/kg/day for four days or 200 mg/kg/day for five days cured mice with acute and chronic infection respectively, the latter being a model for the advanced and fatal stage of the disease when parasites have disseminated into the brain. In laboratory animals, fexinidazole is well absorbed after oral administration and readily distributes throughout the body, including the brain. The absolute bioavailability of oral fexinidazole was 41 percent in mice, 30 percent in rats, and 10 percent in dogs. Furthermore, fexinidazole is rapidly metabolized *in vivo* to at least two biologically active metabolites (a sulphoxide and a sulphone derivative) that likely account for a significant portion of the therapeutic effect. Key pharmacokinetic parameters after oral absorption in mice for fexinidazole and its sulphoxide and sulphone metabolites are a C_{max} of 500, 14 171 and 13 651 ng/mL respectively, and an AUC₀₋₂₄ of 424, 45 031 and 96 286 h.ng/mL respectively. Essentially similar PK profiles were observed in rats and dogs. Toxicology studies (including safety pharmacology and four-weeks repeated-dose toxicokinetics in rat and dog) have shown that fexinidazole is well tolerated. The No Observed Adverse Event Levels in the four-weeks repeated dose toxicity studies in rats and dogs was 200 mg/kg/day in both species, with no issues of concern identified for doses up to 800 mg/kg/day. While fexinidazole, like many nitroheterocycles, is mutagenic in the Ames test due to bacterial specific metabolism, it is not genotoxic to mammalian cells *in vitro* or *in vivo* as assessed in an *in vitro* micronucleus test on human lymphocytes, an *in vivo* mouse bone marrow micronucleus test, and an *ex vivo* unscheduled DNA synthesis test in rats. In conclusion, the results of the preclinical pharmacological and safety studies indicate that fexinidazole is a safe and effective oral drug candidate with no untoward effects that would preclude evaluation in man. The drug entered first-in-human phase I studies in September 2009. Fexinidazole is the first new clinical drug candidate with the potential for treating advanced-stage sleeping sickness in thirty years.

15474. **Wainwright, M.**, 2010. Dyes, trypanosomiasis and DNA: a historical and critical review. *Biotechnic & Histochemistry*, **85** (6): 341-354.

School of Pharmacy & Biomolecular Science, Liverpool John Moores University, Liverpool, United Kingdom. [mark_wainwright@hotmail.com].

Trypanosomiasis, a group of diseases including sleeping sickness in humans and Nagana in cattle in Africa, and Chagas disease in South America, remains a considerable problem in the 21st century. The therapies that are available, however, usually have their roots in the "dye therapy" of a century ago, knowledge gained at the microscope from parasite staining procedures and converted to chemotherapy based on compounds closely related to the laboratory reagents. Dyes such as trypan red and trypan blue led to the development of suramin, while cationic nitrogen heterocyclic dyes furnished examples of the phenanthridinium class, such as ethidium (homidium) and isometamidium. Both suramin and isometamidium remain in use. Owing to mutagenicity issues, the presence of ethidium among the phenanthridinium dyes has led to concerns over the clinical use of related derivatives. There are several mechanisms for dye-DNA interaction, however, including possible hydrogen bonding of dye to the polymer, and these are discussed together with structure-activity relations and cellular localization of the phenanthridine and isomeric acridines involved. Better understanding of nucleic acid binding properties has allowed the preparation

of more effective phenanthridinium analogues intended for use as anticancer/antiviral therapy.

6. ANIMAL TRYPANOSOMOSIS

(a) SURVEY AND DISTRIBUTION

[See also 33: 15447, 15496]

15475. **Amer, S., Ryu, O., Tada, C., Fukuda, Y., Inoue, N. & Nakai, Y.,** 2010. Molecular identification and phylogenetic analysis of *Trypanosoma evansi* from dromedary camels (*Camelus dromedarius*) in Egypt: a pilot study. *Acta Tropica*, **117** (1): 39-46.

Laboratory of Sustainable Environmental Biology, Field Centre Studies, Graduate School of Agricultural Science, Tohoku University, 232-3 Yomogida, Naruko-Onsen, Osaki, Miyagi 989-6711, Japan.

Animal trypanosomiasis is one of the major constraints of livestock industry in developing countries. In the present study, the prevalence of *Trypanosoma evansi* was assessed in the blood of dromedary camels (*Camelus dromedarius*) brought to Al Bassatein abattoir, Cairo, Egypt, by the mouse inoculation test. Out of 84 tested camels, four animals (4.7 percent) were infected. Molecular analysis was achieved by PCR amplification and sequence analysis of part of the ribosomal RNA gene including 18S, ITS1, 5.8S and ITS2 regions. Despite the conserved nature of 18S region, the ITS region showed obvious heterogeneity compared to analogous database sequences. Analysis of the transferrin receptor encoding gene (ESAG6) showed a variable repertoire in the studied isolates, which may indicate a novel structure of the *T. evansi* population from Egypt and/or a difference in host range. Furthermore, analysis of the variable surface glycoprotein RoTat 1.2 gene marker revealed some heterogeneity at this gene locus. To our knowledge, this is the first molecular analysis of *T. evansi* in Egypt.

15476. **Cox, A. P., Tosas, O., Tilley, A., Picozzi, K., Coleman, P., Hide, G. & Welburn, S. C.,** 2010. Constraints to estimating the prevalence of trypanosome infections in East African zebu cattle. *Parasite Vectors*, **3**: 82.

Centre for Infectious Diseases, School of Biomedical Sciences, College of Medicine and Veterinary Medicine, University of Edinburgh, 1 Summerhall Square, Edinburgh, EH9 1QH, UK. [andrew.cox@lshtm.ac.uk].

In East Africa, animal trypanosomiasis is caused by many tsetse transmitted protozoan parasites including *Trypanosoma vivax*, *T. congolense* and subspecies of *T. brucei* s.l. (*T. b. brucei* and zoonotic human infective *T. b. rhodesiense*) that may co-circulate in domestic and wild animals. Accurate species-specific prevalence measurements of these parasites in animal populations are complicated by mixed infections of trypanosomes within individual hosts, low parasite densities, and difficulties in conducting field studies. Many polymerase chain reaction (PCR)-based diagnostic tools are available to characterize and quantify infection in animals. These are important for assessing the contribution of infections in animal reservoirs and the risk posed to humans from zoonotic trypanosome species. New matrices for DNA

capture have simplified large scale field PCR analyses but few studies have examined the impact of these techniques on prevalence estimations. The Whatman FTA matrix was evaluated using a random sample of 35 village zebu cattle from a population naturally exposed to trypanosome infection. Using a generic trypanosome-specific PCR, prevalence was systematically evaluated. Multiple PCR samples taken from single FTA cards demonstrated that a single punch from an FTA card is not sufficient to confirm the infectivity status of an individual animal as parasite DNA is unevenly distributed across the card. At low parasite densities in the host, this stochastic sampling effect results in underestimation of prevalence based on single punch PCR testing. Repeated testing increased the estimated prevalence of all *Trypanosoma* spp. from 9.7 percent to 86 percent. Using repeat testing, a very high prevalence of pathogenic trypanosomes was detected in these local village cattle: *T. brucei* (34.3 percent), *T. congolense* (42.9 percent), and *T. vivax* (22.9 percent). These results show that, despite the convenience of Whatman FTA cards and specific PCR-based detection tools, the chronically low parasitaemias in indigenous African zebu cattle make it difficult to establish true prevalence. Although this study specifically applies to FTA cards, a similar effect would be experienced with other approaches using blood samples containing low parasite densities. For example, using blood film microscopy or PCR detection from liquid samples where the probability of detecting a parasite or DNA molecule in the required number of fields of view or PCR reaction, is less than one.

15477. **De, S. P. A. G., Schubach, T. M., Figueiredo, F. B., Baptista, C., Fagundes, A., Da, S. B. J. H., De Paula, C. C., Toma, H. K. & Madeira, M. F.,** 2010. Isolation of *Trypanosoma caninum* in domestic dogs in Rio de Janeiro, Brazil. *Parasitology*, **137** (11): 1653-1660.

Laboratorio de Vigilancia em Leishmanioses, Instituto de Pesquisa Clinica Evandro Chagas, Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil. [addressa.guimaraes@ipecc.fiocruz.br].

The domestic dog's involvement with different members of the Trypanosomatidae family has been the focus of several studies due to this animal's close proximity to man. Recently this animal has been infected by a new *Trypanosoma* species (*T. caninum*), described in Rio de Janeiro and 19 similar isolates were later obtained. The objective of this study was to identify these isolates. All samples were isolated from intact skin cultures and analysed morphologically, by biochemical isoenzyme electrophoresis assays and by several molecular PCR assays. Additionally, anti-*Leishmania* sp. antibodies were assessed using the indirect immunofluorescence antibody test (IFAT) in all animals. The methodologies employed to identify the isolates, including partial nucleotide sequences of 18S rRNA gene, indicated patterns identical to *T. caninum* and patterns different from the other species, including *T. cruzi* and *T. rangeli* samples. A phylogenetic tree constructed with the partial 18S ribosomal sequence showed that *T. caninum* is clustered with *T. pestanaei*. Ten (52.6 percent) animals presented anti-*Leishmania* sp. antibodies with titres varying from 1:40 to 1:320. Thus, the hypothesis that this protozoan has disseminated among the dogs in Rio de Janeiro must be considered. The importance of a correct diagnosis in those animals and the possible consequences in the areas where visceral leishmaniasis is found are discussed here.

15478. **Garamszegi, L. Z.,** 2010. The sensitivity of microscopy and PCR-based detection methods affecting estimates of prevalence of blood parasites in birds. *Journal of*

Parasitology, **96** (6): 1197-1203.

Department of Evolutionary Ecology, Estacion Biologica de Donana-CSIC, c/
Americo Vespucio, s/n, 41092, Sevilla, Spain. [laszlo.garamszegi@ebd.csic.es].

Inferences about the evolution of host-parasitic relationships are often made based on the prevalence of avian malaria, which is usually estimated in a large sample of birds using either microscopic or molecular screening of blood samples. However, different techniques often have variable accuracy; thus, screening methodology can raise issues about statistical bias if method sensitivity varies systematically across parasites or hosts. To examine this possibility, published information was collected on the prevalence of species in four genera of avian blood parasites (*Plasmodium*, *Haemoproteus*, *Leucocytozoon*, and *Trypanosoma*) from various sources that used different tools. The data were tested to determine if the application of different methods provided different estimates for the same hosts. In these comparisons between the main methodologies, the PCR-based molecular methods were generally found to provide higher estimates for *Plasmodium* spp. prevalence than microscopic tools, while there was no significant tendency for such a trend in species of *Haemoproteus* and *Leucocytozoon*. When analysing intraspecific variance of prevalence within molecular studies, some studies provided consistently higher estimates for the prevalence of *Haemoproteus* spp. than others, indicating that differences between studies can affect detected estimates. Within microscopic studies, surveys that examined more microscopic fields were more likely to report higher prevalence for *Plasmodium* spp. than those relying on fewer microscopic fields. Consequently, studies making comparisons across parasite genera and/or host species from different sources need to consider several types of bias originating from variation in method sensitivity.

15479. **McInnes, L. M., Hanger, J., Simmons, G., Reid, S. A. & Ryan, U. M.**, 2010. Novel trypanosome *Trypanosoma gilletti* sp. (Euglenozoa: Trypanosomatidae) and the extension of the host range of *Trypanosoma copemani* to include the koala (*Phascolarctos cinereus*). *Parasitology*, **138** (1): 59-70.

Division of Health Sciences, School of Veterinary and Biomedical Sciences,
Murdoch University, Murdoch, Perth, WA 6150, Australia.
[LindaMMcInnes@gmail.com].

Trypanosoma irwini was previously described from koalas and we now report the finding of a second novel species, *T. gilletti*, as well as the extension of the host range of *Trypanosoma copemani* to include koalas. Phylogenetic analysis at the 18S rDNA and gGAPDH loci demonstrated that *T. gilletti* was genetically distinct with a genetic distance (+/- s.e.) at the 18S rDNA locus of 2.7 +/- 0.5 percent from *T. copemani* (wombat). At the gGAPDH locus, the genetic distance (+/- s.e.) of *T. gilletti* was 8.7 +/- 1.1 percent from *T. copemani* (wombat). *Trypanosoma gilletti* was detected using a nested trypanosome 18S rDNA PCR in 3/139 (approximately 2 percent) blood samples and in 2/29 (approximately 7 percent) spleen tissue samples from koalas whilst *T. irwini* was detected in 72/139 (approximately 52 percent) blood samples and *T. copemani* in 4/139 (approximately 3 percent) blood samples from koalas. In addition, naturally occurring mixed infections were noted in 2/139 (approximately 1.5 percent) of the koalas tested.

15480. **Mekuria, S. & Gadissa, F.**, 2010. Survey on bovine trypanosomosis and its vector in Metekel and Awi zones of Northwest Ethiopia. *Acta Tropica*, **117** (2): 146-151.

Hawassa University, Department of Veterinary Medicine, P.O. Box 05, Hawassa, Ethiopia. [solmk2000@Yahoo.com].

A cross-sectional study was conducted between November 2009 and December 2009 in the riverbank of Abay river tributaries, located in three districts of Awi and Metekel zones, Northwest Ethiopia. The prevalence of bovine trypanosomosis, associated risk factors and distribution as well as vector identification in the study area were considered. Blood samples were collected from 540 randomly selected local (zebu) breed of cattle in nine peasant associations of three districts and the assumed risk factors were recorded. The collected samples were examined using haematological and parasitological techniques. In this study, 67 animals (12.42 percent) were infected with different species of trypanosomes. Most of the infections were due to *T. congolense* (77.6 percent) followed by *T. vivax* (14.9 percent), *T. brucei* (6.0 percent) and mixed infection of *T. congolense* and *T. vivax* (1.5 percent). There was no statistical significance ($p>0.05$) between sex, age and coat colour of skin, but significant differences were observed in body condition, altitude and districts ($p<0.05$). Mean PCV values of infected (19.42 percent) and non-infected (24.13 percent) group of animals showed significant variation; and mean PCV value of animals with poor body condition was significantly different ($p<0.001$) from those with good body condition. A total of 3 072 tsetse flies of riverine species or *palpalis* group (*Glossina tachinoides*) and biting flies were caught, of which 2 792 (90.9 percent) were tsetse flies and the remaining were *Stomoxys* and *Tabanus*. The overall apparent densities of tsetse and biting flies were 6.49 and 0.65flies/trap/day, respectively and the difference was significant ($p<0.05$). The study revealed that bovine trypanosomosis is more prevalent in low lands and in poor body condition animals in the study area. Tsetse distribution also coincided with altitude, with high tsetse catches being associated with low lands, but none in mid land. Control strategies have to be designed and implemented in the area to minimize the distribution of tsetse as well as trypanosomosis prevalence.

15481. **Shahzad, W., Munir, R., Khan, M. S., Ahmad, M. D., Ijaz, M., Ahmad, A. & Iqbal, M.**, 2010. Prevalence and molecular diagnosis of *Trypanosoma evansi* in Nili-Ravi buffalo (*Bubalus bubalis*) in different districts of Punjab (Pakistan). *Tropical Animal Health & Production*, **42** (8): 1597-1599.

Livestock Production Research Institute Bahadurnagar, Okara, Punjab, Pakistan. [waseem1971@hotmail.com].

The prevalence of *Trypanosoma evansi* was investigated in 1 250 Nili-Ravi buffaloes of mixed age and sex by the polymerase chain reaction (PCR) for the first time in Pakistan. DNA of the trypanosomes was isolated with TRIAGENT®. The assay was employed using primers ESAG 6/7, specific for a 237-bp fragment from *T. evansi* genomic DNA. The samples were screened for the presence of *T. evansi* also by stained thin smear. Forty-four (3.5 percent) samples were positive by microscopy, while 97 (7.7 percent) samples were

identified by PCR, indicating the high sensitivity of PCR for surveying the disease in epidemiological studies.

15482. **Tamarit, A., Tejedor-Junco, M. T., Gonzalez, M., Alberola, J. & Gutierrez, C.,** 2010. Morphological and biometrical features of *Trypanosoma evansi* isolates from an outbreak in mainland Spain. *Veterinary Parasitology*. **In press, corrected proof.**

Animal Health Laboratory, Council of Agriculture, Fishing and Nourishment, Generalitat Valenciana, Av. Manuel Soto 18, 46024 Valencia, Spain. [atamaritsoler@gmail.com].

According to several authors, *Trypanosoma evansi* is a monomorphic trypanosome found exclusively in slender intermediate forms, although additional studies have revealed that many strains present stumpy forms on rare occasions. In a recent *T. evansi* outbreak in mainland Spain, several atypical forms were observed in blood smear examinations. Molecular procedures were then necessary to confirm the causal agent. Morphological and biometric measures were taken to characterize the different forms of *T. evansi*. In contrast to published information, the results of this study would indicate that biometrically distinct *T. evansi* could also be found in the same farm and even in the same animal species. These data could be useful for many trypanosome endemic areas of the world where molecular methods are not commonly available.

15483. **Yusufmia, S. B., Collins, N. E., Nkuna, R., Troskie, M., Van den Bossche, P. & Penzhorn, B. L.,** 2010. Occurrence of *Theileria parva* and other haemoprotozoa in cattle at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. *Journal of the South African Veterinary Association*, **81** (1): 45-49.

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. [banie.penzhorn@up.ac.za].

Theileria parva, the most important bovine theilerial species in sub-Saharan Africa, causes widespread mortality and morbidity in endemic areas. A survey was conducted using buffy-coat specimens from 60 apparently healthy adult communally herded Nguni-type cattle at the northeastern edge of the Hluhluwe-iMfolozi Park to determine, by means of PCR and reverse line blot (RLB) hybridization, the occurrence of *Theileria* and *Babesia* species. The presence of *Trypanosoma* species was determined using PCR-RFLP. Results showed that 6.7 percent of the specimens were positive for *Theileria parva*. This significant finding suggests that cattle in South Africa, and not only African buffaloes (*Syncerus caffer*), may be subclinical carriers of *T. parva*. Other species identified were *T. mutans* (83.3 percent), *T. velifera* (70.0 percent), *Theileria* sp. (sable) (46.8 percent) and *T. taurotragi* (1.7 percent). Two specimens (3.3 percent) were positive for *Babesia bovis* and single specimens (1.7 percent) positive for *B. bigemina* and *B. rossi*, respectively. Mixed infections, of up to four species, were common (65.0 percent). Only one specimen was found to be positive for *Trypanosoma vivax*, and two for *T. theileri*, of which only the first species is pathogenic.

(b) PATHOLOGY AND IMMUNOLOGY

[See also 33: 15434, 15480, 15501, 15517]

15484. **Aquino, L. P., Machado, R. Z., Lemos, K. R., Marques, L. C., Garcia, M. V. & Borges, G. P.**, 2010. Antigenic characterization of *Trypanosoma evansi* using sera from experimentally and naturally infected bovines, equines, dogs, and coatis. *Revista Brasileira de Parasitologia Veterinaria*, **19** (2): 112-118.

Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista -
- UNESP - SP, Brazil. [zacarias@fcav.unesp.br].

The present research investigated the presence of *T. evansi* antibodies in animals from the sub region of Nhecolândia, in the Pantanal Sul-mato-grossense, by means of an enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescence antibody test (IFAT), and the pattern of polypeptide recognition by sera from experimentally and naturally infected hosts using Western blotting. Serum samples were obtained from bovines (n = 102), horses (n = 98), and dogs (n = 55), and from 32 free-ranging coatis (*Nasua nasua*). None of the bovines was found positive, while sera from 16 dogs (29 percent) and 23 horses (23.4 percent) were positive by ELISA. Sera from eight coatis (25 percent) were found positive using IFAT. Western blotting revealed major polypeptides of *T. evansi* with molecular weight ranging from 74 to 38 kDa. The polypeptides of 66, 48-46, and 38 kDa were identified by sera from experimentally infected bovines, donkeys, dogs, and coatis. The 48-46 and 38 kDa bands were mainly recognized in chronic phase of infection. The antigen with apparent molecular weight of 66 kDa, revealed by antibodies from all experimental animals, was also recognized in sera of horses and dogs from the Pantanal. The 48-46 kDa polypeptide was identified by antibodies from all naturally infected animals and must be further evaluated for use in specific diagnosis of *T. evansi* infection.

15485. **Chamond, N., Cosson, A., Blom-Potar, M. C., Jouvion, G., D'Archivio, S., Medina, M., Droin-Bergere, S., Huerre, M., Goyard, S. & Minoprio, P.**, 2010. *Trypanosoma vivax* infections: pushing ahead with mouse models for the study of Nagana. I. Parasitological, haematological and pathological parameters. *PLoS Neglected Tropical Diseases*, **4** (8): e792.

Laboratoire d'Immunobiologie des Infections à Trypanosoma, Département
d'Immunologie, Institut Pasteur, Paris, France. [pmm@pasteur.fr].

African trypanosomiasis is a severe parasitic disease that affects both humans and livestock. Several different species may cause animal trypanosomosis and although *Trypanosoma vivax* (sub-genus *Duttonella*) is currently responsible for the vast majority of debilitating cases causing great economic hardship in West Africa and South America, little is known about its biology and interaction with its hosts. Relatively speaking, *T. vivax* has been more than neglected despite an urgent need to develop efficient control strategies. Some pioneering rodent models were developed to circumvent the difficulties of working with livestock, but disappointingly were for the most part discontinued decades ago. To gain more insight into the biology of *T. vivax*, its interactions with the host and consequently its pathogenesis, we have developed a number of reproducible murine models using a parasite

isolate that is infectious for rodents. Firstly, we analysed the parasitological characteristics of the infection using inbred and outbred mouse strains to compare the impact of host genetic background on the infection and on survival rates. Haematological studies showed that the infection gave rise to severe anaemia, and histopathological investigations in various organs showed multifocal inflammatory infiltrates associated with extramedullary haematopoiesis in the liver, and cerebral oedema. The models developed are consistent with field observations and pave the way for subsequent in-depth studies into the pathogenesis of *T. vivax* - trypanosomosis.

15486. **Da Silva, A. S., Garcia Perez, H. A., Costa, M. M., Franca, R. T., De Gasperi, D., Zanette, R. A., Amado, J. A., Lopes, S. T., Teixeira, M. M. & Monteiro, S. G.,** 2010. Horses naturally infected by *Trypanosoma vivax* in southern Brazil. *Parasitology Research*, **108** (1): 23-30.

Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Faixa de Camobi-Km 9, Campus Universitario, Predio 20, Sala 4232, Santa Maria, RS, Brazil, 97105-900. [aleksandro_ss@yahoo.com.br].

In this study, we report the first outbreak of the infection by *Trypanosoma vivax* in horses in southern Brazil, a non-endemic region where bovines have only recently been found infected by this trypanosome species. We evaluated 12 horses from a farm in southern Brazil, where four horses displayed pale mucous membranes, fever, weight loss, and swelling of abdomen, prepuce, or vulva. The diagnosis of *T. vivax* was confirmed in four horses by morphological parameters of trypomastigotes in blood smears and species-specific PCR. All *T. vivax*-infected animals showed anaemia, and most showed increased levels of beta-1, beta-2, and gamma globulins. Horses were treated with diminazene aceturate, but cure was not achieved, and the disease relapsed after therapy. These findings demonstrated that Brazilian *T. vivax* isolates, which were already reported infecting cattle, buffaloes, goats, and sheep, can be highly pathogenic for horses, causing severe disease and even death of the animals due to the recurrence of the infection.

(c) TRYPANOTOLERANCE

[See also **33**: 15413, 15576]

15487. **Stein, J., Ayalew, W., Rege, E., Mulatu, W., Lemecha, H., Tadesse, Y., Tekle, T. & Philipsson, J.,** 2010. Trypanosomosis and phenotypic features of four indigenous cattle breeds in an Ethiopian field study. *Veterinary Parasitology*. **In press, corrected proof.**

Dept of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, P.O. Box 7023, Uppsala, Sweden; and 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, Garage, 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 analyzed 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 percent) and Sheko (25.1 percent) cattle whereas Abigar had the lowest PCV (20.0 percent). 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 percent). 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

[See also 33: 15486, 15531, 15539, 15557]

15488. **Affognon, H., Coulibaly, M., Diall, O., Grace, D., Randolph, T. & Waibel, H.,** 2009. Policy study for strategies to manage drug resistance in the context of trypanosomosis control in West Africa: the case of Mali. *ILRI Research Report No. 17*.

International Livestock Research Institute, Nairobi, Kenya.

In Mali, livestock production is practiced by at least 80 percent of the rural population and constitutes the principal source of subsistence for 30 percent of the whole population. However, animal diseases remain a major constraint to livestock development. African Animal Trypanosomosis (AAT) is among the most important constraints to improving livestock productivity and increasing agricultural production nationally. Several strategies are used to control the disease: controlling the vector, the tsetse fly; use of trypanotolerant breeds of cattle; and treatment with trypanocidal drugs. Of these options, drug treatment is the most important, and often the only, strategy applied by cattle farmers in Mali. However, the frequent use of the low-priced trypanocides is contributing to the development and propagation of resistance to the drugs, which is emerging as a major obstacle to their continued use. This study used a stakeholder analysis approach to assess policy constraints that hinder control strategies for TAA and trypanocide resistance. The objective of the study was to characterise the policy environment that influences the ability of farmers to control

trypanosomosis sustainably, and to identify policies needed to support sustainable control strategies and protect the efficacy of trypanocides. To achieve this objective, an initial national-level workshop was held with a wide range of stakeholders. During the workshop, problems associated with trypanosomosis control and resistance were explored, as were the roles played by existing policies. A stakeholder analysis was then conducted to evaluate the incentives facing the various actors to adopt or resist more appropriate policies. Based on the preliminary findings from the national workshop, an in-depth policy study was undertaken, the results of which were presented and discussed with stakeholders. Analyses of policies concerning animal disease control and use of veterinary products revealed a major gap between regulation and reality which was encouraging misuse of veterinary drugs generally, and trypanocides more specifically. The stakeholder analysis approach clarified the various interests and incentives that maintain this suboptimal situation. The findings clearly demonstrate the important role of policy in influencing the environment in which farmers attempt to manage trypanosomosis in their herds. Opportunities for improving the quality of trypanocide products, services, and information made available to farmers to support best-bet control strategies were identified. Some of the identified opportunities, however, are perceived as threats to veterinary professionalism by certain stakeholders, hampering their uptake. Continued engagement with policy makers will be required to raise awareness about the extent and potential impact of drug resistance, and additional evidence will be needed to permit policy makers to understand the trade-offs between maintaining a focus on government-regulated formal-sector systems and recognizing the reality on the ground in terms of reliance by livestock keepers on the informal sector and their own treatment of infected animals. In other words, what are the social costs and benefits of continuing to promote a professional formal sector which is limited in both coverage and quality by the inability of poor farmers to pay for veterinary services, versus providing information to the informal sector and farmers themselves to promote Rational Drug Use? Taking into account divergent views and conflicting interests of stakeholders, ways to promote and disseminate information and messages on Rational Drug Use (RDU) need to be identified that will make best-bet control strategies for trypanosomosis and trypanocide resistance acceptable to all.

15489. **Affognon, H. D., Randolph, T. F. & Waibel, H.,** 2010. Economic analysis of animal disease control inputs at farm level: the case of trypanocide use in villages under risk of drug resistance in West Africa. *Livestock Research for Rural Development*, **22**(12): Article 224.

International Livestock Research Institute (ILRI), P. O. Box 30709, 00100 Nairobi, Kenya; and Development and Agricultural Economics, Faculty of Economics and Management, Leibniz University of Hanover, Koenigsworther Platz 1, 30167, Hanover Germany [h.affognon@cgiar.org].

This paper presents an economic analysis of the use of drugs (isometamidium and diminazene) in controlling African Animal Trypanosomosis (AAT), a serious disease of cattle and small ruminants in villages that exhibit resistance to isometamidium in Burkina Faso and Mali in West Africa. The study applies a production function framework integrating a damage control function to assess the short term productivity effect of trypanocide use under different epidemiological conditions. We found that the marginal value products of isometamidium in all epidemiological conditions, and the marginal value product of

diminazene in high-prevalence-high-resistance conditions are positive and greater than one revealing an underuse of trypanocidal drugs in those conditions. The economical optimum level of isometamidium is far larger than the current use level. In a strict economic interpretation, this implies that in the short term cattle farmers could increase the profitability if they increase trypanocide input beyond current levels. On the other hand, if the use of trypanocide increases, cattle farmers will also be more likely to experience future losses from trypanocide resistance. In this paper we demonstrated the feasibility of applying the damage control framework for measuring the productivity of veterinary therapeutic drugs at farm level in poor African countries.

15490. **Allegye–Cudjoe, E.**, 2009. Field detection and evaluation of trypanocidal drug resistance in Ghana and Benin. *MSc thesis*.

University of Edinburgh, Scotland, UK.

Field studies were conducted to detect and assess trypanocidal drug resistance of trypanosomes infecting cattle in the Sissala East District of northern Ghana and the Banikoara Community of northern Benin. An initial cross-sectional survey of trypanosomosis prevalence and tsetse densities in 14 villages in both Ghana and Benin was carried out to identify high prevalence sites (suspected “hot spots”). The trypanosome prevalence varied considerably between and within countries, with two sites in northern Ghana, Kunchogu (22 percent) and Yugantu (8 percent) recording a relatively high prevalence based on the phase-contrast buffy coat technique (BCT). At Sissala East District, 83.3 percent of all trypanosome infections were due to *T. vivax* and 16.7 percent due to *T. congolense*. At Banikoara Community all trypanosome infections were due to *T. vivax*. Almost all the tsetse flies caught along the Sissili River and its tributaries in northern Ghana and the Alibori River and its tributaries in northern Benin were *Glossina tachinoides*, except 6 flies caught at a tributary of the Sissili River that were unidentified tsetse flies. Tsetse densities were much higher in the Sissala East District (zero to 6.8 flies per trap per day) than in the Banikoara Community (zero to 0.8 flies per trap per day). In the highest risk site, a longitudinal study was conducted from May to July 2008. A total of 100 cattle were randomly selected from the village herd, these cattle were ear-tagged and assigned into two groups, 50 were treated with isometamidium chloride (ISMM) at 1 mg/Kg body weight and 50 were left to serve as controls. Both groups of cattle were screened at intervals of 14 days until 56 days for trypanosome parasites using BCT. During this follow up period eight animals (16 percent) became positive in the control while six animals (12 percent) were found positive in the treated group. A statistical analysis using the relative risk (RR) test showed a minimal protection failure rate of 28 percent with ISMM treatments. This study shows together with evidence from neighbouring countries that trypanocide resistance is a threat which must be taken seriously in Ghana. Livestock farmers in the Sissala East District are particularly vulnerable because most of the cattle are trypano-sensitive Zebus and trypanocidal drugs remain the main strategy for the control of cattle trypanosomosis.

15491. **Clausen, P. H., Bauer, B., Zessin, K. H., Diall, O., Bocoum, Z., Sidibe, I., Affognon, H., Waibel, H., Grace, D. & Randolph, T.**, 2010. Preventing and containing trypanocide resistance in the cotton zone of West Africa. *Transboundary & Emerging Diseases*, **57** (1-2): 28-32.

Institute for Parasitology and Tropical Veterinary Medicine, Freie Universitaet Berlin, Berlin, Germany. [yahyatahamtan@yahoo.com].

Trypanocidal drugs are the most commonly purchased and used livestock input by resource-poor farmers in sub-Saharan Africa. The effective use of trypanocidal drugs by smallholder farmers is threatened by the development of widespread resistance. This is a particular concern for smallholder crop-livestock farmers in the cotton zone of West Africa. A recent project funded by the Germany Ministry for Economic Cooperation and Development (BMZ) confirmed significant resistance to trypanocidal drugs in villages with high trypanosomosis risk in Burkina Faso and Mali. Strategies for resistance prevention were investigated. Keeping trypanotolerant cattle was found to be an effective disease management strategy, but farmers' preference for trypano-susceptible breeds, for reasons unrelated to animal health, suggests that the introgression of zebu genotype will continue. Community vector control was found to be effective in managing trypanosomosis in the presence of resistance and the high-level participatory approach tested was found to be more sustainable than low-level approaches previously used in the region. This suggests that participatory vector control with appropriate external support is likely to be a viable option for implementing resistance "clean-up". Promoting rational drug use (RDU) emerged as a promising prevention strategy, with clear improvements in farmer knowledge, farmer practise, and animal health outcomes. However, policy studies showed low understanding of the problem of resistance and the absence of an enabling environment for RDU. Engagement was initiated with actors involved in the problem of resistance and for its solution, including manufacturers, sellers, and users of drugs, regulators and extension providers.

15492. **Dao, D.**, 2005. Determinism of human factors in the control of Animal African Trypanosomosis: the case of the agro-pastoralists from the Department of Mandiana, Upper Guinea. *MSc., University of Ouagadougou*.

This study, conducted as a MSc. thesis project, contributes to improving the control of African Animal Trypanosomosis (AAT) which is currently one of the major constraints to livestock development in sub-Saharan Africa. The target population was agro-pastoralists in Mandiana Department, Upper Guinea. The main objective was to understand the socio-cultural practices of agro-pastoralists and the influence of service providers in the control of AAT, and to analyse farmers' relevant knowledge, attitudes and practices. The following hypothesis emerged: the perception of AAT and its representation vary as a function of knowledge, attitudes and practices of the agro-pastoralists in Mandiana, especially with respect to whether they are small, medium or large producers and have an empirical experience in managing this disease. This study revealed that agro-pastoralists have partial knowledge about the causes of AAT. Of the surveyed farmers, 68 percent believe that tsetse flies are the main cause of trypanosomosis. More than 50 percent of farmers know some of the typical symptoms of AAT and 86 percent of treatments are with trypanocidal drugs exclusively. Farmer behaviour towards the disease is influenced by their experience, their cattle numbers, their level of education and access to service providers. Agro-pastoralists prefer using service providers from the formal sector, but their non-availability and the high cost of their services lead the agro-pastoralists to seek drugs and services from the informal sector or to treat animals themselves. Of the surveyed farmers, 54 percent treat their animals themselves and 21 percent seek the services of non professionals. Nearly all treatments (99

percent) made by qualified service providers (veterinarians, technicians and paravets) were performed correctly. However, nearly half of the treatments (47 percent) performed by non professionals and by farmers were not successful. The study confirms that there is a common effort to control AAT in this area, but many of the treatments made by non professionals are not successful. Direct training of non professionals and paravets is a crucial aspect of the problem that has not yet been addressed.

15493. **Gutierrez, C., Desquesnes, M., Touratier, L. & Buscher, P.,** 2010. *Trypanosoma evansi*: recent outbreaks in Europe. *Veterinary Parasitology*, **174** (1-2): 26-29.

University of Las Palmas de Gran Canaria, Department of Animal Medicine, 35413 Arucas, Las Palmas, Canary Islands, Spain. [cgutierrez@dpat.ulpgc.es].

Here, two recent outbreaks of *Trypanosoma evansi* infection in mainland France and Spain associated with the importation of dromedary camels from the Canary Islands, are reported. The disease is well known on the Archipelago since 1997 and many efforts have been made towards control and eventual eradication, but some areas still remain affected. Both mainland outbreaks were controlled by means of massive treatments and monthly serological, parasitological, and molecular (PCR) evaluations carried out by Valencian Regional Animal Health laboratory and by CIRAD, Montpellier, respectively. Possible causes for the persistence of the parasite in a small area of the Canaries are discussed. *T. evansi* must be included among the animal health conditions for international trade within the European Union as well as many other countries. Moreover, procedures including diagnosis, curative or preventive treatment, and quarantine should be established to ensure the status of the animals moving from a country to another.

15494. **Liebenehm, S.,** 2008. Economic impact of livestock research on farmers' knowledge and productivity: The case of trypanosomosis in West Africa. *MSc thesis*.

Leibniz University of Hannover, Germany.

In tropical Africa livestock play an important role not only for income, food and fertilizer but also for sustaining the livelihoods, security and health of the poor. African Animal Trypanosomosis (AAT) is one of the most severe cattle diseases in sub-Saharan Africa. The transmission of the disease by the tsetse fly causes anaemia, fever and diarrhoea, poor condition and finally terminates in death. For the farmer, trypanosomosis has an incisive economic impact both in terms of output loss and capability deprivation. Because of the disease, approximately three million cattle die each year – that corresponds to a production loss of USD 1.0 to 1.2 billion. Moreover, poor disease management, i.e. the misuse of preventive and curative medicines, generates resistance of the pathogen. The objective of the study was to assess the impact of livestock research activities on farmers' knowledge and productivity. Data were collected in 2007 from 508 cattle farmers in the cotton zone of Kéné Dougou - common to south-eastern Mali and south-western Burkina Faso - to study the knowledge, attitudes and practices concerning AAT and its control. Since the pathogen's resistance to recent treatments has grown, it is important to monitor the current levels of farmers' know-how and action in order to create a baseline for further impact assessments. It is hypothesized that improvements in knowledge due to former survey interventions on

trypanosomosis change behaviour; and actual practices will become more effective leading to a reduction of treatment failures that will thereby increase cattle productivity due to a decline in output loss. Applying Propensity Score Matching to assess the impact of former public survey interventions on specific disease knowledge and management techniques – measured in knowledge scores - indicates that participants reach generally higher scores in all knowledge categories than their counterparts. Moreover, the acquisition of additional knowledge and the application of improved control and preventive strategies significantly increase levels of added value. In addition, the enhanced knowledge results in a reduction of trypanocide expenditures in favour of less expensive inputs like forage or other veterinary inputs.

15495. **Ouma, E. M.**, 2010. Management of trypanocidal drug resistance in cattle in identified chemo-resistance hot spots in the administrative District of Sikasso, south-east Mali. *PhD dissertation*.

Freie Universität Berlin, Germany.

Animal trypanosomosis still remains a major disease constraining livestock production across sub-Saharan Africa. Additionally, the development and spread of chemoresistance further severely threaten the cattle-based livelihoods of the rural poor in the cotton belt of West Africa. If not addressed, it will exacerbate rural poverty. Best-bet strategies, including tsetse control and strategic helminth control were tested for their efficacy to contain and or reverse trypanocide resistance in Sikasso, south-east Mali, where drug resistance had earlier been detected. The study was implemented in three phases: pre-intervention, intervention and post-intervention. Two areas of Sikasso, the eastern sector and the western sector, with comparable ecology and production systems were covered. The study was conducted in four villages from each sector. The pre-intervention phase, conducted between November and December 2007, involved a cross-sectional (tsetse catches, trypanosome prevalence and drug use practices) and a longitudinal (drug sensitivity testing) survey. Two tsetse species, *Glossina palpalis gambiensis* and *G. tachinoides* occurred in the study area. The eastern sector had a mean trypanosome prevalence of 13.9 percent which was not significantly different ($p > 0.05$) from 17.5 percent in the western sector. Two trypanocidal drugs, isometamidium chloride (ISMM) for prophylaxis and diminazene aceturate (DIM) for therapy, were found to be commonly used. Multiple drug-resistant *Trypanosoma congolense* were prevalent in both areas while *T. vivax* were sensitive to 3.5 mg/kg bodyweight DIM. Effects of tsetse control and de-worming to contain and reverse trypanocide resistance were assessed during in the intervention phase. In the intervention area (eastern sector), targets ($n=957$) impregnated in 0.4 percent deltamethrin (DECIS®, Roussel-Uclaf, France) (dry season) and targeted treatment of cattle (spraying on the limbs, lower abdominal area, brisket and perineal area) with 0.05 percent deltamethrin (Butox®, Intervet, the Netherlands) (rainy season) were used to control tsetse between March 2008 and November 2009. Additionally, strategic helminth treatment of risk group cattle (3-12 months) with 10mg/kg bodyweight albendazole (10 percent Albendazole®, Kela, Belgium) was conducted in the intervention and control areas between June 2009 and November 2009. Albendazole was first used in June 2008 and repeated during the November 2008, June 2009 and November 2009 monitoring visits after randomly allocating the animals at risk into an albendazole treatment group and a control group. Five epidemiological visits (June 2008, November 2008, February 2009, June

2009 and November 2009) were conducted to estimate the resultant dynamics in tsetse catches, trypanosome infections, and helminth infections in the two areas. All trypanosome positive risk cattle and those with PCV \leq 20 percent were treated with 3.5mg/kg bodyweight DIM. Tsetse control resulted, respectively, in 91.3 percent and 97.3 percent reductions of *G. p. gambiensis* and *G. tachinoides* catches in the intervention area. A reduction of 18.3 percent for the former and of 37.1 percent for the latter fly species also occurred in the control area. Significantly ($p < 0.001$) lower trypanosome infections in the intervention area than in the control area were observed at herd, village, and area levels. Strongyle eggs, *Strongyloides* species eggs, *Toxocara* species eggs and *Moniezia* species eggs, among others, were detected. No *Fasciola* species eggs were detected in cattle of both areas. In the intervention area, the probability of albendazole treated cattle to acquire new trypanosome infections was calculated as 0.003 which was not significantly ($p > 0.05$) different from 0.007 for the control group. The post-intervention phase involved a cross-sectional and a longitudinal survey and was conducted from November to December 2009. As for the pre-intervention phase, trypanosome prevalence and drug sensitivity investigations were undertaken. There was a significant ($p < 0.05$) reduction in trypanosome prevalence in the intervention area from 13.9 percent during the pre-intervention phase to < 1 percent after intervention. In contrast, trypanosome prevalence remained unchanged in the control area. Only three *T. vivax* cases remained in the intervention area of which one was resistant to 0.5mg/kg bodyweight ISMM but was cleared by 3.5 mg/kg bodyweight DIM. Trypanocidal resistance patterns in the control area did not change. A faecal egg count reduction test (FECRT) to estimate the efficacy of albendazole revealed a 55.6 percent reduction of strongyle faecal egg counts. The low FECRT warrants further evaluation before concluding that resistance in strongyle helminths does exist. This investigation has shown that in a region of high trypanosomosis risk and trypanocidal drug resistance, tsetse control undertaken simultaneously with strategic helminth control and targeted treatment with trypanocidal drugs is likely to reduce trypanosomosis risk to negligible levels.

15496. Talaki, E., Sidibé, I., Diall, O., Affognon, H., Grace, D., Djiteye, A., Bocoum, Z., Diarra, B., Belem, A. M. G. & Pangui, L. J., 2009. Seasonal variations and risk factors of animal trypanosomosis in a context of drug resistance in the Sikasso zone in Mali. *Bulletin of Animal Health and Production in Africa*, **57** (2): 149-160.

International Livestock Research Institute (ILRI), P. O. Box 30709, 00100 Nairobi, Kenya.

A series of three longitudinal surveys focused on cattle were conducted between August 2003 and April 2004 at 11 sites in the Sikasso District in Mali. They were part of an epidemiological seasonal study of animal trypanosomosis in a context of trypanocide resistance. From a parasitological perspective, there is heterogeneity in the evolution of the disease. Very strong parasitological prevalences were recorded during the August period, which corresponds to the rainy season, in Diassadiè (34 percent) and Wahibéra (29 percent) sites. These two villages were the only ones to exhibit significant seasonal variation (at a 5 percent threshold) in prevalence values. Infections were mainly caused by *Trypanosoma congolense* as recorded in 65 percent to 67 percent of cases, followed by *T. vivax* found in 29 percent to 35 percent of infections, and rarely *Trypanosoma brucei*, with 1 percent of cases. The average haematocrit value in the rainy season was the highest (26 percent) with

significant seasonal variation. In entomological terms, two riparian glossine species have been identified in all seasons with flies trap⁻¹ day⁻¹ (FTD) from 3.45 to 5.29 for *Glossina palpalis* species and varying between 0.98 and 2.80 for *Glossina tachinoides*. In the rainy season, *Glossina palpalis* exhibited a significant correlation ($\alpha = 0.05$) between the density of glossines and the infection rate of cattle. Treatment failures (resistance) to diminazene aceturate were suspected in several sites.

15497. **Waiswa, C. & Kabasa, J. D.**, 2010. Experiences with an in-training community service model in the control of zoonotic sleeping sickness in Uganda. *Journal of Veterinary Medical Education*, **37** (3): 276-281.

Department of Veterinary Medicine, Makerere University, Kampala, Uganda.
[cwaiswa@vetmed.mak.ac.ug].

By 2006, the acute and zoonotic *Trypanosoma brucei rhodesiense* sleeping sickness in Uganda was spreading northward, leading to fear of a merger with the chronic *Trypanosoma brucei gambiense* type that affects people in the northwest of the country. Eliminating infection in cattle was urgent because they had been confirmed to be spreading the zoonotic type, and eliminating infection would reduce the animal reservoir and subsequently reduce transmission of sleeping sickness. In this article, we describe how the staff and students of the Faculty of Veterinary Medicine, Makerere University, adjusted their approach to training veterinary students who could provide the urgently needed manpower to enable the community to halt spread of the disease. Because it was not usual for university staff and students to implement disease control activities, the Government of Uganda had to delegate this responsibility to Makerere University. In turn, the university had to explore available opportunities in its training and outreach mandates. A model was developed that proved to be an effective hands-on training strategy while helping to control a disease that was threatening the health of people in a community that was just recovering from an armed rebellion. In total, 66 students and supervisors participated in the 10-week-long mass treatment activities in the target area and treated more than 190 000 out of 220 000 targeted (>86 percent) cattle with diminazene aceturate and deltamethrin. Also, the graduates' performance improved, as indicated by 43.5 percent of graduates securing employment within less than a month after completing the course.

7. EXPERIMENTAL TRYPANOSOMOSIS

(a) DIAGNOSTICS

15498. **Abdo, J., Kristersson, T., Seitzer, U., Renneker, S., Merza, M. & Ahmed, J.**, 2010. Development and laboratory evaluation of a lateral flow device (LFD) for the serodiagnosis of *Theileria annulata* infection. *Parasitology Research*, **107** (5): 1241-1248.

Division of Veterinary Infection Biology and Immunology, Research Center Borstel, Parkallee 22, 23845 Borstel, Germany. [jahmed@fz-borstel.de].

Several DNA-based and serological tests have been established for the detection of *Theileria annulata* infection, including the polymerase chain reaction, reverse line blot and

loop-mediated isothermal amplification, indirect enzyme-linked immunosorbent assay (ELISA), and competitive ELISA. In this study, we have applied knowledge from the development and application of a recombinant protein-based indirect ELISA and competitive ELISA to establish a rapid test for point-of-care diagnosis of *T. annulata* infection in the field to be used by the veterinarian. For the development of a lateral flow test, the recombinantly expressed *T. annulata* surface protein (TaSP) was applied as the test antigen and an anti-TaSP antiserum as the control line. TaSP antigen conjugated to colloidal gold particles was used as the detection system for visualization at the test line for the binding of anti-TaSP antibody present in the serum of infected animals. The developed test specifically detected antibodies in the serum of animals experimentally infected with *T. annulata* and showed no cross-reactivity with serum from animals infected with other tested bovine pathogens (*Trypanosoma brucei*, *Anaplasma marginale*, *Babesia bigemina*, *Babesia bovis*, and *Theileria parva*). Testing of field samples was compared with results obtained by other serological tests, resulting in a sensitivity and specificity of 96.3 percent and 87.5 percent compared with the indirect fluorescence antibody test, 98.7 percent and 81.8 percent compared with the indirect ELISA, and 100 percent and 47.6 percent compared with the competitive ELISA. In conclusion, a rapid test for the detection of *T. annulata* infection (*T. annulata* lateral flow device, Ta-LFD) has been developed, which is easy to perform, delivers results to be read by the naked eye within 10 min., and is suitable for the detection of infection in field samples.

15499. **Bossard, G., Boulange, A., Holzmüller, P., Thevenon, S., Patrel, D. & Authie, E.,** 2010. Serodiagnosis of bovine trypanosomosis based on HSP70/BiP inhibition ELISA. *Veterinary Parasitology*, **173** (1-2): 39-47.

UMR17 Trypanosomes, IRD-CIRAD, Campus International de Baillarguet, F-34398 Montpellier, France. [bossard@cirad.fr].

Animal trypanosomosis is a serious constraint to livestock productivity in tropical and sub-tropical countries. The pathogenic trypanosomes in bovidae are *Trypanosoma congolense*, *T. vivax*, *T. brucei* and *T. evansi*. Current serological tests to detect trypanosome infections are based on the use of whole trypanosome lysates; their potential is limited by antigen instability, lack of reproducibility and lack of test specificity due to the antibody's long persistence after treatment. The development of new tests based on recombinant technology that could be standardized and applied on a large scale at low cost would be very helpful. The major invariant antigen recognized by *T. congolense* infected cattle belongs to the heat shock protein (HSP) 70 family and is closely related to mammalian immunoglobulin binding protein (BiP). To improve the initial ELISA based on a recombinant fragment of HSP70/BiP, we developed an inhibition ELISA using an anti-BiP monoclonal antibody and a full-length fusion protein expressed in *E. coli*. Here we report on the development of the test and provide an initial assessment of its performance using sets of sera from experimental infections and from naturally infected cattle maintained in tsetse infested areas of Africa. The HSP70/BiP-based inhibition ELISA shows a good sensitivity in cattle experimentally infected with *T. congolense*, with an improved sensitivity in secondary infections. One major advantage, particularly for its further application in national laboratories, is that one single set of reagents and one single procedure are sufficient to apply on different mammalian host species infected with different trypanosome species.

15500. Pruvot, M., Kamyngkird, K., Desquesnes, M., Sarataphan, N. & Jittapalpong, S., 2010. A comparison of six primer sets for detection of *Trypanosoma evansi* by polymerase chain reaction in rodents and Thai livestock. *Veterinary Parasitology*, **171** (3-4): 185-193.

Centre de Coopération internationale en Recherche Agronomique pour le Développement (CIRAD)-Département Systèmes Biologiques (Bios), UMR177-Trypanosomes, Montpellier, F-34000 France. [mpruvot@ucalgary.ca].

To face the worldwide threat of Surra caused by *Trypanosoma evansi*, international organizations have stressed the need to evaluate and standardize diagnostic tools. PCR detection of *T. evansi* has undergone considerable expansion during the last 20 years, but primer sets are often insufficiently assessed and compared. In this work, we compared the performances of six primer pairs-TBR1/2, ESAG6/7, TEPAN1/2, pMUTEC F/R, TRYP1 R/S, and TRYP4 R/S tested using purified *T. evansi* DNA serial dilutions, *T. evansi*-infected rat blood serial dilutions and Thai dairy cattle samples. The TBR1/2 primer set was able to detect 0.01 pg of purified DNA, and a parasitaemia below one parasite/ml in rat blood. It presented the highest sensitivity in cattle samples as well as a high specificity, without non-specific products or false positive reactions out of 84 negative cattle samples tested. ESAG6/7 showed equivalent results with purified DNA and rat samples but presented non-specific products with Thai dairy cattle samples, leading to results that were not interpretable. TEPAN1/2 was not able to detect less than 0.1 pg of purified DNA or 50 trypanosomes/ml in rat blood. In cattle, TEPAN1/2 primers detected only 36 percent of the positives detected by TBR1/2. Given the parasitaemic level detected, pMUTEC F/R, TRYP1 R/S and TRYP4 R/S were not more sensitive than classical microscopic examination using the buffy coat. TBR1/2, TEPAN1/2, pMUTEC F/R and TRYP4 R/S did not cross-react with *Babesia* sp., *Trypanosoma theileri* and *Anaplasma marginale*. TBR1/2 was the most sensitive primer set to detect *T. evansi* in purified DNA, rodent blood and cattle blood, and did not show cross reaction with the other pathogens tested. It should therefore be preferred for epidemiological surveys. These results confirmed that TBR1/2 primers remain the reference for the detection of Trypanozoon DNA and should therefore be included in subsequent evaluations of new diagnostic tools based on DNA detection.

(b) PATHOLOGY AND IMMUNOLOGY

[See also 33: 15562, 15569, 15582, 15589, 15596, 15604, 15612]

15501. Ali, A., Al-Sobayil, F. A., Tharwat, M. & Hassanein, K. M., 2010. Ovarian hydrobursitis in female camels (*Camelus dromedaries*): 1. Biochemical, bacterial and protozoal evaluation. *Theriogenology*. **Published online, December 9.**

Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, 51452 Buraydah, Qassim, Saudi Arabia. [ahmedali77@hotmail.com].

The aim of this study was to evaluate female camels affected with ovarian hydrobursitis (n = 31) for haematological and biochemical findings and for bacterial and protozoal

infections. Blood samples were obtained and surgical ablation of the affected bursa was performed. Bursal fluid, follicular fluid, and serum were subjected to hormonal and biochemical analyses. Bursal fluids were cultured and colonies were identified using the BioMerieux Vitek two compact system. The passive haemagglutination test was used for detection of *Trypanosoma evansi*, while the indirect ELISA technique was carried out for detection of anti-hydatid cyst antibodies. Neutrophilia was found in the affected animals ($p = 0.01$) with tendencies for monocytosis ($p = 0.06$) and eosinophilia ($p = 0.05$). Bursal fluid had a tendency for high estradiol-17 beta concentration compared with blood serum ($p = 0.07$). Progesterone and cholesterol concentrations were similar in bursal fluid, follicular fluid and serum. Total protein, phosphorus, and magnesium concentrations were greater ($p < 0.05$) in the bursal fluid than in serum. *Oligella urethralis*, *Alloiococcus otitis*, *Granulicatella adicens*, *Escherichia coli*, *Sphingobacterium thalpophilum*, *Streptococcus sanguinis*, *Aeromonas salmonicida*, *Pseudomonas stutzeri*, *Staphylococcus warneri*, *Staphylococcus hominis*, and *Rhizobium radiobacter* were isolated from 46.7 percent of bursal fluids. 9.7 percent of cases were positive for *T. evansi*. None was positive for hydatid cysts. Accordingly, we suggest that the ovarian hydrobursitis syndrome is initially an inflammatory process and the accumulated bursal fluid partially originates from follicular fluid.

15502. **Amin, D. N., Ngoyi, D. M., Nkwachi, G. M., Palomba, M., Rottenberg, M., Buscher, P., Kristensson, K. & Masocha, W.,** 2010. Identification of stage biomarkers for human African trypanosomiasis. *American Journal of Tropical Medicine & Hygiene*, **82** (6): 983-990.

Department of Neuroscience, Karolinska Institutet, Stockholm, SE-171 77 Sweden. [ndemamin@yahoo.co.uk].

Human African trypanosomiasis (HAT), caused by infection with sub-species of *Trypanosoma brucei* manifests as a haemolymphatic stage followed by an encephalitic stage. The distinction of the two stages needs improvement as drugs used for the late stage are highly toxic. Transcripts encoding 16 secreted proteins differentially expressed in the brains of mice at late stage *T. b. brucei* infection when the early stage drug suramin is no longer effective and different to immunoglobulins, chemokines, and cytokines, were selected by microarray analysis. Lipocalin 2 and secretory leukocyte peptidase inhibitor (SLPI) mRNA showed the highest differential expression in mice. These transcripts were also upregulated in brains from infected rats. Lipocalin 2 was increased in cerebrospinal fluid (CSF) from rats during late stage *T. b. brucei* infection. Protein levels of lipocalin 2, SLPI, and the chemokine CXCL10 were found increased in CSF from *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* late stage HAT compared with early stage.

15503. **Barnwell, E. M., van Deursen, F. J., Jeacock, L., Smith, K. A., Maizels, R. M., Acosta-Serrano, A. & Matthews, K.,** 2010. Developmental regulation and extracellular release of a VSG expression-site-associated gene product from *Trypanosoma brucei* bloodstream forms. *Journal of Cell Science*, **123** (19): 3401-3411.

Centre for Immunity, Infection and Evolution, Institute of Immunology and Infection Research, School of Biological Sciences, Kings' Buildings, University

of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK.
[keith.matthews@ed.ac.uk].

Trypanosomes evade host immunity by exchanging variant surface glycoprotein (VSG) coats. VSG genes are transcribed from telomeric expression sites, which contain a diverse family of expression-site-associated genes (ESAGs). We have discovered that the mRNAs for one ESAG family, ESAG9, are strongly developmentally regulated, being enriched in stumpy forms, a life-cycle stage in the mammalian bloodstream that is important for the maintenance of chronic parasite infections and for tsetse transmission. ESAG9 gene sequences are highly diverse in the genome and encode proteins with weak similarity to the massively diverse MASP proteins in *Trypanosoma cruzi*. We demonstrate that ESAG9 proteins are modified by N-glycosylation and can be shed to the external milieu, this being dependent upon coexpression with at least one other family member. The expression profile and extracellular release of ESAG9 proteins represent a novel and unexpected aspect of the transmission biology of trypanosomes in their mammalian host. We suggest that these molecules might interact with the external environment, with possible implications for infection chronicity or parasite transmission.

15504. **Black, S. J., Guirnalda, P., Frenkel, D., Haynes, C. & Bockstal, V.,** 2010. Induction and regulation of *Trypanosoma brucei* VSG-specific antibody responses. *Parasitology*, **137**(14): 2041-2049.

Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts, USA; Laboratory for Cellular and Molecular Immunology, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium; and Department of Molecular and Cellular Interactions, VIB, Brussels, Belgium. [sblack@vasci.umass.edu].

The review addresses how infection with *Trypanosoma brucei* affects the development, survival and functions of B lymphocytes in mice. It discusses (1) the contributions of antibodies to trypanosome clearance from the bloodstream, (2) how B lymphocytes, the precursors of antibody producing plasma cells, interact with membrane form variable surface glycoprotein (VSG), i.e. with monovalent antigen that is free to diffuse within the lipid bilayer of the trypanosome plasma membrane and consequently can cross-link B cell antigen specific receptors by indirect processes only and (3) the extent and underlying causes of dysregulation of humoral immune responses in infected mice, focusing on the impact of wild type and GPI-PLC^{-/-} trypanosomes on bone marrow and extramedullary B lymphopoiesis, B cell maturation and survival.

15505. **Blom-Potar, M. C., Chamond, N., Cosson, A., Jouvion, G., Droin-Bergere, S., Huerre, M. & Minoprio, P.,** 2010. *Trypanosoma vivax* infections: pushing ahead with mouse models for the study of Nagana. II. Immunobiological dysfunctions. *PLoS Neglected Tropical Diseases*, **4** (8): e792.

Laboratoire d'Immunobiologie des Infections à *Trypanosoma*, Département d'Immunologie, Institut Pasteur, Paris, France. [pmm@pasteur.fr].

Trypanosoma vivax is the main species involved in trypanosomosis, but very little is known about the immunobiology of the infective process caused by this parasite. Recently we

undertook to further characterize the main parasitological, haematological and pathological characteristics of mouse models of *T. vivax* infection and noted severe anaemia and thrombocytopenia coincident with rising parasitaemia. To gain more insight into the organism's immunobiology, we studied lymphocyte populations in central (bone marrow) and peripheral (spleen and blood) tissues following mouse infection with *T. vivax* and showed that the immune system apparatus is affected both quantitatively and qualitatively. More precisely, after an initial increase that primarily involves CD4⁺ T cells and macrophages, the number of splenic B cells decreases in a stepwise manner. Our results show that while infection triggers the activation and proliferation of haematopoietic stem cells, granulocyte-monocyte, common myeloid and megacaryocyte erythrocyte progenitors decrease in number in the course of the infection. An in-depth analysis of B-cell progenitors also indicated that maturation of pro-B into pre-B precursors seems to be compromised. This interferes with the mature B cell dynamics and renewal in the periphery. Altogether, our results show that *T. vivax* induces profound immunological alterations in myeloid and lymphoid progenitors which may prevent adequate control of *T. vivax* trypanosomosis.

15506. **Boulangé, A. F., Khamadi, S. A., Pillay, D., Coetzer, T. H. & Authie, E., 2010.** 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.

UMR 17 IRD-CIRAD Trypanosomes, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France. [boulange@cirad.fr, abk32a@gmail.com].

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/L 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 monomerizing at acidic pH <5. This attribute is of utmost importance in the context of an anti-disease vaccine, given that the epitopes recognized by the sera of trypanosome-infected trypanotolerant cattle appear dimer-specific.

15507. **Da Silva, A. S., Belle, L. P., Bitencourt, P. E., Perez, H. A., Thome, G. R., Costa, M. M., Oliveira, C. B., Teixeira, M. M., Moretto, M. B., Mazzanti, C. M., Lopes, S. T. & Monteiro, S. G.,** 2010. *Trypanosoma evansi*: adenosine deaminase activity in the brain of infected rats. *Experimental Parasitology*, **127** (1): 173-177.

Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Brazil. [aleksandro_ss@yahoo.com.br].

The study was undertaken to evaluate changes in the activity of adenosine deaminase (ADA) in brains of rats infected by *Trypanosoma evansi*. Each rat was intraperitoneally infected with 10^6 trypomastigotes either suspended in fresh (group A; n = 13) or cryopreserved blood (group B; n = 13). Thirteen animals were used as control (group C). ADA activity was estimated in the cerebellum, cerebral cortex, striatum, and hippocampus. No differences ($p > 0.05$) in ADA activity were observed in the cerebellum between infected and non-infected animals. Significant ($p < 0.05$) reductions in ADA activity occurred in cerebral cortex in acutely (day four post-infection; PI) and chronically (day 20 PI) infected rats. ADA activity was significantly ($p < 0.05$) decreased in the hippocampus in acutely infected rats, but significantly ($p < 0.05$) increased in the chronically infected rats. Significant ($p < 0.05$) reductions in ADA activity occurred in the striatum of chronically infected rats. Parasites could be found in peripheral blood and brain tissue through microscopic examination and PCR assay, respectively, in acutely and chronically infected rats. The reduction of ADA activity in the brain was associated with high levels of parasitaemia and anaemia in acute infections. Alterations in ADA activity of the brain in *T. evansi*-infected rats may have implications for the pathogenesis of the disease.

15508. **Da Silva, A. S., Monteiro, S. G., Goncalves, J. F., Spanevello, R., Schmatz, R., Oliveira, C. B., Costa, M. M., Franca, R. T., Jaques, J. A., Schetinger, M. R., Mazzanti, C. M. & Lopes, S. T.,** 2010. *Trypanosoma evansi*: immune response and acetylcholinesterase activity in lymphocytes from infected rats. *Experimental Parasitology*, **127** (2): 475-480.

Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Brazil. [aleksandro_ss@yahoo.com.br].

The existence of cholinergic receptors in the immune system cells is well documented. This study aimed to evaluate the acetylcholinesterase activity (AChE) in lymphocytes from rats infected with *Trypanosoma evansi* in acute and chronic phase disease. Twenty animals were infected with 10^6 trypomastigotes forms each and 10 were used as negative controls. The two groups of inoculated rats were formed according to the degree of parasitaemia and the period post-infection (PI). Group A: rats with four days PI and between 24 and 45 parasites/field (1 000x); group B: rats with 30 days PI and parasitaemia with jagged peaks between 0 and 1 parasite/field; group C: not infected animals. At four days PI (acute phase) and 30 days PI (chronic phase) the rats were anaesthetized to collect blood for haemogram and separation of lymphocytes. After separation, the AChE activity was measured in lymphocytes. It was observed that the number of lymphocytes increased significantly in group A compared with group C. The activity of AChE in lymphocytes significantly increased in acute phase and decreased in chronic phase in the infected rats when compared with not infected animals ($p < 0.05$). Statistical analysis showed a positive correlation between

the number of lymphocytes and AChE activity in lymphocytes in four days PI ($r^2 = 0.59$). Therefore, the infection by *T. evansi* influences AChE activity in lymphocytes of rats indicating changes in the responses of the cholinergic system in the acute phase, possibly due to the immune functions performed by these enzymes.

15509. **Ezeokonkwo, R. C., Ezeh, I. O., Onunkwo, J. I., Obi, P. O., Onyenwe, I. W. & Agu, W. E.,** 2010. Comparative haematological study of single and mixed infections of mongrel dogs with *Trypanosoma congolense* and *Trypanosoma brucei brucei*. *Veterinary Parasitology*, **173** (1-2): 48-54.

Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria. [romanusezeokonkwo2002@yahoo.com].

The haematological effects of single and mixed infections of *Trypanosoma congolense* and *Trypanosoma brucei brucei* were compared in experimentally infected mongrel dogs. Twenty mongrel dogs of both sexes aged between three and six months, and weighing between 2.5 and 5.9 kg were used for the study. The dogs were kept in clean metal cages in a fly-proof house and were adequately fed and given water *ad libitum*. The twenty dogs were divided into four groups of five dogs each. Group I dogs were uninfected controls, group II dogs were infected with *T. congolense*, group III dogs were infected with *T. brucei brucei* and group IV dogs were infected with both *T. congolense* and *T. brucei brucei*. Parasitaemia occurred in the infected dogs in groups II, III, and IV 10-13 days post-infection (PI) with mean pre-patent periods (PPP) of 12, 10, and 11 days respectively. Mixed infection persisted throughout the duration of the experiment. *T. brucei* predominated *T. congolense* in the mixed infection constituting about 70 percent of the trypanosomes. The significant ($p < 0.05$) decrease in the mean haemoglobin concentration (Hb) and packed cell volume (PCV) caused by the infection did not differ significantly ($p > 0.05$) between the infected groups. Also the significant ($p < 0.05$) reduction in the total white blood cell count (TWBC) caused by the infection did not differ significantly ($p > 0.05$) between the infected groups. The decline in the total WBC count was due primarily to significant ($p < 0.05$) reductions in the lymphocyte counts of the infected dogs. It was thus concluded that single or mixed infection of mongrel dogs with *T. congolense* and *T. brucei brucei* resulted in anaemia and leucopenia which did not differ significantly ($p > 0.05$) among the infected groups.

15510. **Gjini, E., Haydon, D. T., Barry, J. D. & Cobbold, C. A.,** 2010. Critical interplay between parasite differentiation, host immunity, and antigenic variation in trypanosome infections. *American Naturalist*, **176** (4): 424-439.

Department of Mathematics, University of Glasgow, University Gardens, UK. [egjini@maths.gla.ac.uk].

Increasing availability of pathogen genomic data offers new opportunities to understand the fundamental mechanisms of immune evasion and pathogen population dynamics during chronic infection. Motivated by the growing knowledge on the antigenic variation system of the sleeping sickness parasite, the African trypanosome, we introduce a mechanistic framework for modelling within-host infection dynamics. Our analysis focuses first on a single parasitaemia peak and then on the dynamics of multiple peaks that rely on stochastic switching between groups of parasite variants. A major feature of trypanosome

infections is the interaction between variant-specific host immunity and density-dependent parasite differentiation to transmission life stages. In this study, we investigate how the interplay between these two types of control depends on the modular structure of the parasite antigenic archive. Our model shows that the degree of synchronization in stochastic variant emergence determines the relative dominance of general over specific control within a single peak. A requirement for multiple-peak dynamics is a critical switch rate between blocks of antigenic variants, which implies constraints on variant surface glycoprotein (VSG) archive genetic diversification. Our study illustrates the importance of quantifying the links between parasite genetics and within-host dynamics and provides insights into the evolution of trypanosomes.

15511. **Hunig, T., Luhder, F., Elflein, K., Gogishvili, T., Frohlich, M., Guler, R., Cutler, A. & Brombacher, F.,** 2010. CD28 and IL-4: two heavyweights controlling the balance between immunity and inflammation. *Medical Microbiology & Immunology*, **199** (3): 239-246.

Institute for Virology and Immunobiology, University of Wurzburg, Versbacher Str. 7, Wurzburg, Germany. [huenig@vim.uni-wuerzburg.de].

The costimulatory receptor CD28 and IL-4Ralpha containing cytokine receptors play key roles in controlling the size and quality of pathogen-specific immune responses. Thus, CD28-mediated costimulation is needed for effective primary T-cell expansion and for the generation and activation of regulatory T-cells (Treg cells), which protect from immunopathology. Similarly, IL-4Ralpha signals are required for alternative activation of macrophages, which counteract inflammation by type 1 responses. Furthermore, immune modulation by CD28 and IL-4 is interconnected through the promotion of IL-4 producing T-helper 2 cells by CD28 signals. Using conditionally IL-4Ralpha and CD28 deleted mice, as well as monoclonal antibodies, which block or stimulate CD28, or mAb that deplete Treg cells, we have studied the roles of CD28 and IL-4Ralpha in experimental mouse models of virus (influenza), intracellular bacteria (*L. monocytogenes*, *M. tuberculosis*), and parasite infections (*T. congolense*, *L. major*). We observed that in some, but not all settings, Treg cells and type 2 immune deviation, including activation of alternative macrophages can be manipulated to protect the host either from infection or from immunopathology with an overall beneficial outcome. Furthermore, we provide direct evidence that secondary CD8 T-cell responses to i.c. bacteria are dependent on CD28-mediated costimulation.

15512. **Kim, H. S. & Cross, G. A.,** 2010. TOPO3alpha influences antigenic variation by monitoring expression-site-associated VSG switching in *Trypanosoma brucei*. *PLoS Pathogens*, **6** (7): e1000992.

Laboratory of Molecular Parasitology, The Rockefeller University, New York, USA.

Homologous recombination (HR) mediates one of the major mechanisms of trypanosome antigenic variation by placing a different variant surface glycoprotein (VSG) gene under the control of the active expression site (ES). It is believed that the majority of VSG switching events occur by duplicative gene conversion, but only a few DNA repair genes that are central to HR have been assigned a role in this process. Gene conversion

events that are associated with crossover are rarely seen in VSG switching, similar to mitotic HR. In other organisms, TOPO3alpha (Top3 in yeasts), a type IA topoisomerase, is part of a complex that is involved in the suppression of crossovers. We therefore asked whether a related mechanism might suppress VSG recombination. Using a set of reliable recombination and switching assays that could score individual switching mechanisms, we discovered that TOPO3alpha function is conserved in *Trypanosoma brucei* and that TOPO3alpha plays a critical role in antigenic switching. Switching frequency increased 10-40-fold in the absence of TOPO3alpha and this hyper-switching phenotype required RAD51. Moreover, the preference of 70-bp repeats for VSG recombination was mitigated, while homology regions elsewhere in ES were highly favoured, in the absence of TOPO3alpha. Our data suggest that TOPO3alpha may remove undesirable recombination intermediates constantly arising between active and silent ESSs, thereby balancing ES integrity against VSG recombination.

15513. **Kipper, M., Da Silva, A. S., Oliveira, C. B., Andretta, I., Paim, F. C., da Silva, C. B., Leon, R., Correa, K., Stainki, D. R., Lopes, S. T. & Monteiro, S. G., 2010.** Relationship between splenic sequestration and thrombocytopenia in *Trypanosoma evansi* infection in rats. *Research in Veterinary Science*. **In press, corrected proof.**

Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.
[sgmonteiro@uol.com.br].

Trypanosoma evansi infections in domestic animals are characterized by anaemia and thrombocytopenia. The cause of the platelets decrease is unknown, but researchers suggest that thrombocytopenia may result from damage of the bone marrow, reduced survival of platelets, auto-immune thrombocytopenia, disseminated intravascular coagulation and splenic sequestration. Some of these causes have already been tested by our research group and found to be unrelated. Therefore, this study has the objective of testing the hypothesis that splenic sequestration might be responsible for thrombocytopenia in *T. evansi*-infected rats. A total of 28 rats assigned to four groups were used in the experiment. Group A rats were splenectomized and infected with *T. evansi*, group B rats were infected with *T. evansi*, group C rats were splenectomized but not infected, and group D rats were normal controls. Five days post-infection all rats were anaesthetized and blood was collected in order to measure the number of circulating platelets, fibrinogen levels, prothrombin time (PT) and activated partial thromboplastin time (aPTT). The spleens of groups B and D were weighed at necropsy. The infected animals (groups A and B) showed a significant reduction in platelets and increased PT and aPTT when compared to negative control groups (groups C and D). Animals from group A showed increased levels of fibrinogen. The mean weight of spleen differed between group B (2.62g) and group D (0.55g). It was concluded that there is no relationship between thrombocytopenia and splenic sequestration in infection by *T. evansi*.

15514. **Magaz, S., Caljon, G., Tran, T., Stijlemans, B. & Radwanska, M., 2010.** Current status of vaccination against African trypanosomiasis. *Parasitology*, **137**(14): 2017-2027.

Unit of Cellular and Molecular Immunology, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium; Department of Molecular and Cellular Interactions, VIB, Rijvisschestraat 120, B-9052 Ghent, Belgium; Unit of Entomology, Institute of Tropical Medicine Antwerp (ITM), Nationalestraat

155, B-2000 Antwerp, Belgium; and COST Office, Avenue Louise 149, B-1050 Brussels, Belgium. [stemagez@vub.ac.be].

Anti-trypanosomiasis vaccination still remains the best theoretical option in the fight against a disease that is continuously hovering between its wildlife reservoir and its reservoir in man and livestock. While antigenic variation of the parasite surface coat has been considered the major obstacle in the development of a functional vaccine, recent research into the biology of B cells has indicated that the problems might go further than that. This paper reviews past and current attempts to design both anti-trypanosome vaccines and vaccines directed towards the inhibition of infection-associated pathology.

15515. **Masocha, W.**, 2010. CD200 receptors are differentially expressed and modulated by minocycline in the brain during *Trypanosoma brucei* infection. *Journal of Neuroimmunology*, **226** (1-2): 59-65.

Department of Applied Therapeutics, Faculty of Pharmacy, Kuwait University, Kuwait. [masocha@hsc.edu.kw].

Infection with *Trypanosoma brucei*, which causes African trypanosomiasis, activates microglia, which are constitutively maintained in a quiescent state through CD200-CD200 receptor interactions. C57BL/6 mice have one inhibitory receptor, CD200R and three activating members, CD200 receptor-like (RL)a-c. Infection increased MAC-1 (microglia marker), CD200RLa and CD200RLb, but not CD200, CD200R or CD200RLc transcript levels in the brains. Minocycline treatment inhibited the infection-induced elevation of MAC-1 and CD200RLa transcripts, but had no significant effect on CD200 or the other receptors. This suggests that CD200RLa might play a role in microglia/macrophage activation during trypanosome infection.

15516. **Mou, Z., Jia, P., Kuriakose, S., Khadem, F. & Uzonna, J. E.**, 2010. Interleukin-17-mediated control of parasitaemia in experimental *Trypanosoma congolense* infection in mice. *Infection & Immunity*, **78** (12): 5271-5279.

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

BALB/c mice are highly susceptible to experimental *Trypanosoma congolense* infections, whereas C57BL/6 mice are relatively resistant. Infected highly susceptible BALB/c mice die of systemic inflammatory response syndrome. Because interleukin-17 (IL-17) and Th17 cells regulate inflammatory responses, we investigated their role in the pathogenesis of experimental African trypanosomiasis in mice. We show that the production of IL-17 by spleen and liver cells and the serum IL-17 level increased after *T. congolense* infection in mice. Interestingly, infected highly susceptible BALB/c mice produced more IL-17 and had more Th17 cells than infected relatively resistant C57BL/6 mice. Paradoxically, neutralization of IL-17 with anti-IL-17 monoclonal antibody *in vivo* induced higher parasitaemias in both the susceptible and the relatively resistant mice. Interestingly, anti-IL-17 antibody-treated mice had higher serum levels of alanine aminotransferase and aspartate aminotransferase, and the production of IL-10 and nitric oxide by liver cells was markedly decreased. Moreover, recombinant IL-17-treated mice exhibited significantly faster parasite

control and lower peak parasitaemias compared with control mice. Collectively, these results suggest that the IL-17/Th17 axis plays a protective role in murine experimental African trypanosomiasis.

15517. **Nnadi, P. A., Ezeh, I. O., Kalu, K. C. & Ngene, A. A.,** 2010. The impact of dietary protein on the pathophysiology of porcine trypanosome infection. *Veterinary Parasitology*, **173** (3-4): 193-199.

Department of Animal Health and Production, University of Nigeria, Nsukka, Nigeria. [ajannadix@yahoo.com].

The influence of protein nutrition on porcine trypanosomosis was investigated in this study. Thirty six landrace/large white cross weanling pigs were used. Upon purchase, these were divided into two groups of 18 pigs each and these were housed separately to enable them adapt to our animal house management regimen. Post-adaptation, the pigs were divided into 6 groups A(1) and A(2), B(1), and B(2), and C(1) and C(2) (n=6). A(1) and A(2) were fed diet A, B(1) and B(2) diet B while C(1) and C(2) were fed diet C with 28 percent, 20 percent and 16 percent crude protein, respectively. Two-weeks after adaptation, groups A(1), B(1), and C(1) were infected with 3×10^6 *Trypanosoma brucei brucei* organisms intraperitoneally. Body weight, temperature and packed cell volume of all group members were determined a week prior to infection, on the day of infection and weekly thereafter until end of the study. Serum biochemistry was also concurrently determined. Three days post-infection, blood was collected from all the members of A(1), B(1) and C(1) and thoroughly screened microscopically for the presence of trypanosome organisms. This was repeated on subsequent days until all the infected animals developed patency by showing parasitaemia under wet mount. The result of this study showed that infection did not have any significant effect on the rate of weight gain except in group C ($p < 0.05$). Moreover, infections caused significant hyperthermia in all the infection groups ($p < 0.05$) with diet A showing the least response and C the most severe. Furthermore, diet did not have any effect on parasite establishment or parasitaemia as the prepatent period was similar in all the infection groups. There was also significant reduction in PCV whose severity also correlated with reduction in the protein dietary quality. Similar observation was also made on the total serum protein where significant hyperproteinaemia correlated with increasing dietary protein and the uninfected controls having higher serum protein relative to the infected. There was in addition parasite-induced hypoalbuminaemia whose severity was also graduated in favour of increasing protein level. The study demonstrated the protective influence of dietary protein on some of the pathophysiological features of porcine trypanosomosis.

15518. **Paulnock, D. M., Freeman, B. B. & Mansfield, J. M.,** 2010. Modulation of innate immunity by African trypanosomes. *Parasitology*, **37**(14): 2051-2063.

Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA; and Department of Medical Microbiology & Immunology, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA [mansfield@bact.wisc.edu].

The experimental studies of *brucei* group trypanosomes presented here demonstrate that the balance of host and parasite factors, especially IFN- γ GPI-s VSG respectively, and the

timing of cellular exposure to them, dictate the predominant MP and DC activation profiles present at any given time during infection and within specific tissues. The timing of changes in innate immune cell functions following infection consistently support the conclusion that the key events controlling host resistance occur within a short time following initial exposure to the parasite GPI substituents. Once the changes in MP and DC activities are initiated, there appears little that the host can do to reverse these changes and alter the final outcome of these regulatory events. Instead, despite the availability of multiple innate and adaptive immune mechanisms that can control parasites, there is an inability to control trypanosome numbers sufficiently to prevent the emergence and establishment of virulent trypanosomes that eventually kill the host. Overall it appears that trypanosomes have carefully orchestrated the host innate and adaptive immune response so that parasite survival and transmission, and alterations of host immunity, are to its ultimate benefit.

15519. **Schwede, A. & Carrington, M.**, 2010. Bloodstream form trypanosome plasma membrane proteins: antigenic variation and invariant antigens. *Parasitology*, **37**(14): 2029-2039.

Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, UK. [mc115@cam.ac.uk].

Trypanosoma brucei is exposed to the adaptive immune system and complement in the blood of its mammalian hosts. The aim of this review is to analyse the role and regulation of the proteins present on the external face of the plasma membrane in the long-term persistence of an infection and transmission. In particular, the following are addressed: (1) antigenic variation of the variant surface glycoprotein (VSG), (2) the formation of an effective VSG barrier shielding invariant surface proteins, and (3) the rapid uptake of VSG antibody complexes combined with degradation of the immunoglobulin and recycling of the VSG.

15520. **Stavropoulos, P. & Papavasiliou, F. N.**, 2010. Using *T. brucei* as a biological epitope-display platform to elicit specific antibody responses. *Journal of Immunological Methods*, **362** (1-2): 190-194.

Laboratory of Lymphocyte Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA. [stavrop@mail.rockefeller.edu].

The African trypanosome (*Trypanosoma brucei*) is transmitted by the bite of the tsetse vector to the mammalian bloodstream where it exists as a completely extracellular parasite. As a result of this exposure, the parasite elicits a robust immune response that is almost exclusively antibody mediated, and is extremely specific to the trypanosome coat displayed on the surface. This coat is comprised of ~11 million copies of a single gpi-linked molecule (the variable surface glycoprotein or VSG) and can therefore be used as a powerful platform for the immunogenic display of antigenic determinants. Here we describe a method to display repetitive, ordered arrays of linear epitopes on the surface of *T. brucei* and to then use the engineered organisms to generate specific anti-epitope antibody responses, upon injection into mice. This method offers an alternative approach to generating anti-peptide antibodies, and could be a useful option in cases where more traditional methods have failed.

15521. **Thompson, P. D., Tipney, H., Brass, A., Noyes, H., Kemp, S., Naessens, J. & Tassabehji, M.**, 2010. Claudin 13, a member of the claudin family regulated in

mouse stress induced erythropoiesis. *PLoS One*, **5** (9): e 12667.

Cancer Immunogenetics, University of Manchester, St Mary's Hospital, Manchester, UK. [m.tassabehji@manchester.ac.uk].

Mammals are able to rapidly produce red blood cells in response to stress. The molecular pathways used in this process are important in understanding responses to anaemia in multiple biological settings. Here we characterize the novel gene Claudin 13 (Cldn13), a member of the Claudin family of tight junction proteins using RNA expression, microarray and phylogenetic analysis. We present evidence that Cldn13 appears to be co-ordinately regulated as part of a stress induced erythropoiesis pathway and is a mouse-specific gene mainly expressed in tissues associated with haematopoietic function. CLDN13 phylogenetically groups with its genomic neighbour CLDN4, a conserved tight junction protein with a putative role in epithelial to mesenchymal transition, suggesting a recent duplication event. Mechanisms of mammalian stress erythropoiesis are of importance in anaemic responses and expression microarray analyses demonstrate that Cldn13 is the most abundant Claudin in spleen from mice infected with *Trypanosoma congolense*. In mice prone to anaemia (C57BL/6), its expression is reduced compared with strains which display a less severe anaemic response (A/J and BALB/c) and is differentially regulated in spleen during disease progression. Genes clustering with Cldn13 on microarrays are key regulators of erythropoiesis (Tal1, Trim10, E2f2), erythrocyte membrane proteins (Rhd and Gypa), associated with red cell volume (Tmcc2) and indirectly associated with erythropoietic pathways (Cdca8, Cdkn2d, Cenpk). Relationships between genes appearing co-ordinately regulated with Cldn13 post-infection provide new insights into the molecular regulation and pathways involved in stress induced erythropoiesis and suggest a novel, previously unreported role for claudins in correct cell polarization and protein partitioning prior to erythroblast enucleation.

15522. **Vankrunkelsven, A., De Ceulaer, K., Hsu, D., Liu, F. T., De Baetselier, P. & Stijlemans, B.**, 2010. Lack of galectin-3 alleviates trypanosomiasis-associated anaemia of inflammation. *Immunobiology*, **215** (9-10): 833-841.

Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium. [annvankrunkelsven@yahoo.com].

A typical pathological feature associated with experimental African trypanosomiasis (*Trypanosoma brucei* infection in mice) is anaemia of chronic disease (ACD), which is due to a sustained type 1 cytokine-mediated inflammation and hyperactivation of M1 macrophages. Galectin-3 (Gal-3) was amply documented to contribute to the onset and persistence of type 1 inflammatory responses and we herein document that this protein is strongly upregulated during *T. brucei* infection. We evaluated the involvement of Gal-3 in trypanosomiasis-associated anaemia using galectin-3 deficient (Gal3^{-/-}) mice. *T. brucei* infected Gal3^{-/-} mice manifested significant lower levels of anaemia during infection and survived twice as long as wild type mice. Moreover, such mice showed increased levels of serum IL-10 and reduced liver pathology (as evidenced by lower AST/ALT levels). In addition, there was also an increase in gene expression of iron export genes and a reduced expression of genes, which are associated with accumulation of cellular iron. Our data indicate that Gal-3 is involved in

the development of inflammation-associated anaemia during African trypanosomiasis, possibly due to a disturbed iron metabolism that in turn may also lead to liver malfunction.

15523. **Wheeler, R. J.**, 2010. The trypanolytic factor-mechanism, impacts and applications. *Trends in Parasitology*, **26** (9): 457-464.

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, UK. [richard.wheeler@path.ox.ac.uk].

The *Trypanosoma brucei* subspecies *T. brucei brucei* is non-human infective due to susceptibility to lysis by trypanolytic factor (TLF) in human serum. Reviewed here are the advances which have revealed apolipoprotein L1 (ApoL1), found in high density lipoprotein, as the lysis-inducing component of TLF, the means of uptake via haptoglobin-related protein receptor and the mechanism of resistance in *T. b. rhodesiense* via its serum resistance-associated (SRA) protein. The first practical steps to application of these discoveries are now in progress; transgenic animals expressing either baboon or minimally truncated human ApoL1 show resistance to both *T. b. brucei* and *T. b. rhodesiense*. This has major implications for treatment and prevention of human and animal African trypanosomiasis.

(c) CHEMOTHERAPEUTICS

[See also **33**: 15559, 15560, 15566, 15577, 15610, 15611]

15524. **Adeyemi, O. S. & Akanji, M. A.**, 2010. Biochemical changes in the kidney and liver of rats following administration of ethanolic extract of *Psidium guajava* leaves. *Human & Experimental Toxicology*. **e Publication ahead of print, November 5.**

Department of Chemical Sciences, College of Natural Sciences, Redeemer's University, Nigeria.

Further to a previous report on the anti-trypanosomal properties of *Psidium guajava* aqueous leaf extract in rats experimentally infected with *Trypanosoma brucei brucei*, we have evaluated the effects of the daily intraperitoneal administration of *P. guajava* leaf extract to rats on the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase (ACP) in the kidney, liver and serum. The results obtained revealed that the administration of the extract produced significant increases in the serum activities of AST, ALT, ALP and ACP when compared with the control ($p < 0.05$). Also AST, ALT, ALP and ACP activities in the tissues of animals administered the extract revealed inconsistent changes ($p < 0.05$) relative to controls. The increase in the serum activity of ALP may be an indicator that there was a likely compromise to the integrity of the plasma membrane as a result of the ethanolic extract administration. This could have caused leakages of the other enzymes investigated, which may explain the corresponding increases in the serum activities of AST, ALT and ACP observed.

15525. **Alloatti, A., Gupta, S., Gualdrón-Lopez, M., Igoillo-Esteve, M., Nguewa, P. A., Deumer, G., Wallemacq, P., Altabe, S. G., Michels, P. A. & Uttaro, A. D.**, 2010. Genetic and chemical evaluation of *Trypanosoma brucei* oleate desaturase as a candidate drug target. *PLoS One*, **5** (12): e14239.

Facultad de Ciencias Bioquímicas y Farmacéuticas, Instituto de Biología Molecular y Celular de Rosario, CONICET, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina. [toniuttaro@yahoo.com.ar].

Trypanosomes can synthesize polyunsaturated fatty acids. Previously, we have shown that they possess stearoyl-CoA desaturase (SCD) and oleate desaturase (OD) to convert stearate (C18) into oleate (C18:1) and linoleate (C18:2), respectively. Here we examine if OD is essential to these parasites. Cultured procyclic (insect-stage) form (PCF) and bloodstream-form (BSF) *Trypanosoma brucei* cells were treated with 12- and 13-thiastearic acid (12-TS and 13-TS), inhibitors of OD, and the expression of the enzyme was knocked down by RNA interference. The phenotype of these cells was studied. Growth of PCF *T. brucei* was totally inhibited by 100 μ M of 12-TS and 13-TS, with EC₅₀ values of 40+/-2 and 30+/-2 μ M, respectively. The BSF was more sensitive, with EC₅₀ values of 7+/-3 and 2+/-1 μ M, respectively. This growth phenotype was due to the inhibitory effect of thiastearates on OD and, to a lesser extent, on SCD. The enzyme inhibition caused a drop in total unsaturated fatty-acid level of the cells, with a slight increase in oleate but a drastic decrease in linoleate level, most probably affecting membrane fluidity. After knocking down OD expression in PCF, the linoleate content was notably reduced, whereas that of oleate drastically increased, maintaining the total unsaturated fatty-acid level unchanged. Interestingly, the growth phenotype of the RNAi-induced cells was similar to that found for thiastearate-treated trypanosomes, with the former cells growing twofold slower than the latter ones, indicating that the linoleate content itself and not only fluidity could be essential for normal membrane functionality. A similar deleterious effect was found after RNAi in BSF, even with a mere 8 percent reduction of OD activity, indicating that its full activity is essential. As OD is essential for trypanosomes and is not present in mammalian cells, it is a promising target for chemotherapy of African trypanosomiasis.

15526. **Bot, C., Hall, B. S., Bashir, N., Taylor, M. C., Helsby, N. A. & Wilkinson, S. R.,** 2010. Trypanocidal activity of aziridinyl nitrobenzamide prodrugs. *Antimicrobial Agents & Chemotherapy*, **54** (10): 4246-4252.

Queen Mary Pre-Clinical Drug Discovery Group, School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, UK. [s.r.wilkinson@qmul.ac.uk].

The trypanocidal agents nifurtimox and benznidazole both function as prodrugs and must undergo enzyme-mediated activation, a reaction catalyzed by type I nitroreductase (NTR). In the search for new parasitic therapies, we have utilized this finding to investigate whether aziridinyl nitrobenzamide derivatives have activity against bloodstream-form *Trypanosoma brucei* and *Trypanosoma cruzi* amastigotes, parasite stages that replicate in the mammalian host. For *T. cruzi* drug screening, we generated trypanosomes that expressed the luciferase reporter gene and optimized a mammalian infection model in a 96-well plate format. A subset of compounds having a 5-(aziridin-1-yl)-2,4-dinitrobenzyl structure was shown to be metabolized by purified *T. brucei* NTR and when screened against both parasite life cycle stages displayed significant growth-inhibitory properties: the most potent compounds generated 50 percent inhibitory concentrations of <1 μ M. The trypanocidal activity was shown to be NTR specific, since parasites overexpressing this enzyme were

hypersensitive to the aziridinyl dinitrobenzyl agents. We conclude that members of the aziridinyl nitrobenzamide class of nitroheterocycles provide new lead structures that have the potential to treat trypanosomal infections.

15527. **Bova, F., Ettari, R., Micale, N., Carnovale, C., Schirmeister, T., Gelhaus, C., Leippe, M., Grasso, S. & Zappala, M.**, 2010. Constrained peptidomimetics as antiplasmodial falcipain-2 inhibitors. *Bioorganic & Medicinal Chemistry*, **18** (14): 4928-4938.

Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, 98168 Messina, Italy. [rettari@pharma.unime.it].

Herein we report the synthesis of a series of novel constrained peptidomimetics 2-10 endowed with a dipeptide backbone (D-Ser-Gly) and a vinyl ester warhead, structurally related to a previously identified lead compound 1, an irreversible inhibitor of falcipain-2, the main haemoglobinase of the lethal malaria parasite *Plasmodium falciparum*. The new compounds were evaluated for their inhibition against falcipain-2, as well as against cultured *P. falciparum*. The inhibitory activity of the synthesized compounds was also evaluated against another protozoal cysteine protease, namely rhodesain of *Trypanosoma brucei rhodesiense*.

15528. **Canlas, J., Hudson, J. B., Sharma, M. & Nandan, D.**, 2010. *Echinacea* and trypanosomatid parasite interactions: growth-inhibitory and anti-inflammatory effects of *Echinacea*. *Pharmaceutical Biology*, **48** (9): 1047-1052.

Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada. [jbhudson@interchange.ubc.ca].

Herbal preparations derived from various species and parts of *Echinacea* (Asteraceae) have been advocated for various medical applications, as a result of the many antimicrobial and immunomodulatory activities attributed to them. In order to investigate their effects on parasites, four preparations of *Echinacea*, with distinct chemical compositions, were evaluated for growth inhibition of three species of trypanosomatids: *Leishmania donovani*, *Leishmania major*, and *Trypanosoma brucei*. In addition, one *Echinacea* preparation was tested for anti-inflammatory activity in cell culture models designed to measure pro-inflammatory cytokines induced by *L. donovani*. All preparations inhibited growth of the organisms, although with different relative potencies, and in some cases morphological changes were observed. However, there was no obvious correlation with the composition of the marker compounds, alkylamides, caffeic acid derivatives, and polysaccharides. *L. donovani* stimulated the production of the pro-inflammatory cytokines IL-6 and IL-8 in human bronchial epithelial cells and in human skin fibroblasts, but in both cases the standardized ethanol extract of *E. purpurea* (L.) Moench (Echinaforce) abolished the stimulation, indicating anti-inflammatory activity of this extract. Thus various *Echinacea* extracts can inhibit the proliferation of these parasites and at least one can reverse the pro-inflammatory activity of *Leishmania donovani*.

15529. **Chan, K. Y., Matthews, K. R. & Ersfeld, K.,** 2010. Functional characterisation and drug target validation of a mitotic kinesin-13 in *Trypanosoma brucei*. *PLoS Pathogens*, **6** (8).

Department of Biological Sciences, University of Hull, Hull, UK.
[k.ersfeld@hull.ac.uk].

Mitotic kinesins are essential for faithful chromosome segregation and cell proliferation. Therefore, in humans, kinesin motor proteins have been identified as anti-cancer drug targets and small molecule inhibitors are now tested in clinical studies. Phylogenetic analyses have assigned five of the approximately fifty kinesin motor proteins coded by the *Trypanosoma brucei* genome to the kinesin-13 family. Kinesins of this family have unusual biochemical properties because they do not transport cargo along microtubules but are able to depolymerise microtubules at their ends, therefore contributing to the regulation of microtubule length. In other eukaryotic genomes sequenced to date, only between one and three Kinesin-13s are present. We have used immunolocalization, RNAi-mediated protein depletion, biochemical *in vitro* assays and a mouse model of infection to study the single mitotic kinesin-13 in *T. brucei*. Subcellular localization of all five *T. brucei* kinesin-13s revealed distinct distributions, indicating that the expansion of this kinesin family in kinetoplastids is accompanied by functional diversification. Only a single kinesin (TbKif13-1) has a nuclear localization. Using active, recombinant TbKif13-1 in *in vitro* assays we experimentally confirm the depolymerizing properties of this kinesin. We analyse the biological function of TbKif13-1 by RNAi-mediated protein depletion and show its central role in regulating spindle assembly during mitosis. Absence of the protein leads to abnormally long and bent mitotic spindles, causing chromosome mis-segregation and cell death. RNAi-depletion in a mouse model of infection completely prevents infection with the parasite. Given its essential role in mitosis, proliferation, and survival of the parasite and the availability of a simple *in vitro* activity assay, TbKif13-1 has been identified as an excellent potential drug target.

15530. **Dawson, A., Tulloch, L. B., Barrack, K. L. & Hunter, W. N.,** 2010. High-resolution structures of *Trypanosoma brucei* pteridine reductase ligand complexes inform on the placement of new molecular entities in the active site of a potential drug target. *Acta Crystallographica D: Biological Crystallography*, **66** (Pt 12): 1334-1340.

Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee DD15EH, Scotland, UK.

Pteridine reductase (PTR1) is a potential target for drug development against parasitic *Trypanosoma* and *Leishmania* species. These protozoa cause serious diseases for which current therapies are inadequate. High-resolution structures have been determined, using data between 1.6 and 1.1 Å resolution, of *T. brucei* PTR1 in complex with pemetrexed, trimetrexate, cyromazine and a 2,4-diaminopyrimidine derivative. The structures provide insight into the interactions formed by new molecular entities in the enzyme active site with ligands that represent lead compounds for structure-based inhibitor development and to support early-stage drug discovery.

15531. **Delespaux, V., Vitouley, H. S., Marcotty, T., Speybroeck, N., Berkvens, D., Roy, K., Geerts, S. & Van den Bossche, P.,** 2010. Chemosensitization of *Trypanosoma congolense* strains resistant to isometamidium chloride by tetracyclines and enrofloxacin. *PLoS Neglected Tropical Diseases*, **4** (9): e828.

Animal Health Department, Institute of Tropical Medicine Antwerp, Antwerp, Belgium. [vdelespaux@itg.be].

Because of the development of resistance in trypanosomes to trypanocidal drugs, the livelihood of millions of livestock keepers in sub-Saharan Africa is threatened now more than ever. The existing compounds have become virtually useless and pharmaceutical companies are not keen on investing in the development of new trypanocides. We may have found a breakthrough in the treatment of resistant trypanosomal infections, through the combination of the trypanocide isometamidium chloride (ISM) with two affordable veterinary antibiotics. In a first experiment, groups of mice were inoculated with *Trypanosoma congolense* strains resistant to ISM and either left untreated or treated with (i) tetracycline, (ii) ISM or (iii) the combination of the antibiotic and the trypanocide. Survival analysis showed that there was a significant effect of treatment and resistance to treatment on the survival time. The groups treated with ISM (with or without antibiotic) survived significantly longer than the groups that were not treated with ISM ($p < 0.01$). The group treated with the combination trypanocide/antibiotic survived significantly longer than the group treated with ISM ($p < 0.01$). In a second experiment, groups of cattle were inoculated with the same resistant trypanosome strain and treated with (i) ISM, (ii) ISM associated with oxytetracycline, or (iii) ISM associated with enrofloxacin. All animals treated with ISM became parasitaemic. In the groups treated with ISM-oxytetracycline and ISM-enrofloxacin, 50 percent of the animals were cured. Animals from the groups treated with a combination trypanocide/antibiotic presented a significantly longer prepatent period than animals treated with ISM ($p < 0.001$). The impact of the disease on the haematocrit was low in all ISM treated groups. Yet, it was lower in the groups treated with the combination trypanocide/antibiotic ($p < 0.01$). It is concluded that after optimization of the administration protocol, this new therapeutic combination could constitute a promising treatment for livestock infected with drug-resistant *T. congolense*.

15532. **Durrant, J. D., Hall, L., Swift, R. V., Landon, M., Schnauffer, A. & Amaro, R. E.,** 2010. Novel naphthalene-based inhibitors of *Trypanosoma brucei* RNA editing ligase 1. *PLoS Neglected Tropical Diseases*, **4** (8): e803.

Biomedical Sciences Program, University of California San Diego, La Jolla, California, USA. [ramaro@uci.edu].

Neglected tropical diseases, including diseases caused by trypanosomatid parasites such as *Trypanosoma brucei*, cost tens of millions of disability-adjusted life-years annually. As the current treatments for African trypanosomiasis and other similar infections are limited, new therapeutics are urgently needed. RNA editing ligase 1 (REL1), a protein unique to trypanosomes and other kinetoplastids, was identified recently as a potential drug target. Motivated by the urgent need for novel trypanocidal therapeutics, we use an ensemble-based virtual-screening approach to discover new naphthalene-based TbREL1 inhibitors. The

predicted binding modes of the active compounds are evaluated within the context of the flexible receptor model and combined with computational fragment mapping to determine the most likely binding mechanisms. Ultimately, four new low- μ molar inhibitors are presented. Three of the four compounds may bind to a newly revealed cleft that represents a putative druggable site not evident in any crystal structure. Pending additional optimization, the compounds presented here may serve as precursors for future novel therapies useful in the fight against several trypanosomatid pathogens, including human African trypanosomiasis, a devastating disease that afflicts the vulnerable patient populations of sub-Saharan Africa.

15533. **Gigante, F., Kaiser, M., Brun, R. & Gilbert, I. H.**, 2010. Design and preparation of sterol mimetics as potential antiparasitics. *Bioorganic & Medicinal Chemistry*, **18** (20): 7291-7301.

Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Sir James Black Centre, Dundee, DD1 5EH, UK.

We have previously shown that azasterols have activity against *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* species, which are the causative agents of various neglected tropical diseases. In this paper, we discuss the replacement of the sterol core of the azasterols with sterol mimics. Various mimics were designed, and the structures were minimised to see if they could adopt a similar conformation to that of the azasterols. From this, two series of mimics were synthesised and then evaluated against the parasites. Compounds showed moderate activity.

15534. **Habila, N., Agbaji, A. S., Ladan, Z., Bello, I. A., Haruna, E., Dakare, M. A. & Atolagbe, T. O.**, 2010. Evaluation of *in vitro* activity of essential oils against *Trypanosoma brucei brucei* and *Trypanosoma evansi*. *Journal of Parasitology Research*, **2010**.

Biochemistry Division, National Research Institute for Chemical Technology, P.M.B 1052 Zaria, Nigeria.

Essential oils (EOs) from *Cymbopogon citratus* (CC), *Eucalyptus citriodora* (EC), *Eucalyptus camaldulensis* (ED), and *Citrus sinensis* (CS) were obtained by hydrodistillation process. The EOs were evaluated *in vitro* for activity against *Trypanosoma brucei brucei* (*Tbb*) and *Trypanosoma evansi* (*T. evansi*). The EOs were found to possess antitrypanosomal activity *in vitro* in a dose-dependent pattern in a short period of time. The drop in number of parasite over time was achieved with doses of 0.4 g/ml, 0.2 g/mL, and 0.1 g/mL for all the EOs. The concentration of 0.4 g/mL CC was more potent at 3 minutes and 2 minutes for *Tbb* and *T. evansi*, respectively. The GC-MS analysis of the EOs revealed presence of cyclobutane (96.09 percent) in CS, 6-octenal (77.11 percent) in EC, eucalyptol (75 percent) in ED, and citral (38.32 percent) in CC among several other organic compounds. The results are discussed in relation to trypanosome chemotherapy.

15535. **Haddad, M., Sauvain, M. & Deharo, E.**, 2010. *Curcuma* as a parasitocidal agent: a review. *Planta Medica*. **E Publication ahead of print, November 23.**

Laboratoire de pharmacochimie des substances naturelles et pharmacophores redox, UMR 152, UPS, Université de Toulouse, Toulouse, France.

Members of the *Curcuma* plant species (Zingiberaceae) have been used for centuries in cooking, cosmetics, staining and in traditional medicine as "omnipotent" remedies. Herbal preparations made with, and molecules extracted from, *Curcuma* have been shown to possess a wide variety of pharmacological properties against malignant proliferation, hormonal disorders, inflammation, and parasitosis among other conditions. This review evaluates *Curcuma* and its associated bioactive compounds, particularly focusing on studies examining the parasiticidal activity of these components against the tropical parasites *Plasmodium*, *Leishmania*, *Trypanosoma*, *Schistosoma* and more generally against other cosmopolitan parasites.

15536. **Huang, H. W., Mohan, S. K. & Yu, C.,** 2010. The NMR solution structure of human epidermal growth factor (hEGF) at physiological pH and its interactions with suramin. *Biochemical & Biophysical Research Communications*, **402** (4): 705-710.

Department of Chemistry, National Tsing Hua University, Taiwan, ROC. [d9623541@oz.nthu.edu.tw]. [d9623541@oz.nthu.edu.tw].

Human epidermal growth factor (hEGF) induces the proliferation, differentiation and survival of various cell types including tumour-derived cells. Generally, hEGF performs its biological function by binding to a specific receptor (hEGFR) on the cell surface, thereby inducing signal transduction. Suramin, a polysulphonated naphthylurea that acts as a growth factor blocker, exhibits antiproliferative activity against non-small cell lung cancer (NSCLC) cells that overexpress EGFR on the cell surface. We determined the solution structure of hEGF under physiological conditions and investigated the interaction of suramin with hEGF using isothermal titration calorimetry and NMR spectroscopy techniques. The solution structure of hEGF presented in this paper is different from the bound form of hEGF present in the crystal structure of the 2:2 EGF-EGFR complex because its C-tail contains a hydrophobic core. This conformational difference supports the hypothesis that hEGF undergoes a conformational change when it binds to hEGFR and subsequently induces signal transduction. Based on the docking structure of the hEGF-suramin complex, we demonstrated how suramin blocks hEGF by binding to its receptor binding site (the C-terminal region around Arg45) and inhibits the crucial conformational change.

15537. **Jeganathan, S., Sanderson, L., Dogruel, M., Rodgers, J., Croft, S. & Thomas, S. A.,** 2010. The distribution of nifurtimox across the healthy and trypanosome-infected murine blood-brain and blood-CSF barriers. *Journal of Pharmacology & Experimental Therapeutics*. **Published online before print, November 5.**

King's College London, Pharmaceutical Sciences Research Division, Hodgkin Building, Guy's Campus, London, SE1 1UL, UK. [sarah.thomas@kcl.ac.uk].

Nifurtimox, an anti-parasitic drug, is used to treat American trypanosomiasis (Chagas disease) and has shown promise in treating CNS-stage human African trypanosomiasis (HAT; sleeping sickness). In combination with other anti-parasitic 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-CSF barriers (K_{in} brain parenchyma was $50.8 \pm 9.0 \mu\text{L}/\text{min}^{-1} \cdot \text{g}^{-1}$). In fact the loss of barrier integrity associated with trypanosome infection failed to change the distribution of [^3H] nifurtimox to any significant extent suggesting there is not an effective paracellular barrier for [^3H] nifurtimox entry into the CNS. Our studies also indicate that [^3H] nifurtimox is not a substrate for P-glycoprotein, an efflux transporter expressed on the luminal membrane of the BBB. However, there was evidence of [^3H] nifurtimox interaction with transporters at both the blood-brain and blood-CSF barriers as demonstrated by cross-competition studies with the other anti-trypanosomal 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.

15538. Jones, D. C., Hallyburton, I., Stojanovski, L., Read, K. D., Frearson, J. A. & Fairlamb, A. H., 2010. Identification of a kappa-opioid agonist as a potent and selective lead for drug development against human African trypanosomiasis. *Biochemical Pharmacology*, **80** (10): 1478-1486.

Division of Biological Chemistry & Drug Discovery, College of Life Sciences,
University of Dundee, Dow Street, Dundee DD1 5EH, UK.
[a.h.fairlamb@dundee.ac.uk]

A resazurin-based cell viability assay was developed for phenotypic screening of the LOPAC 1280 "library of pharmacologically active compounds" against bloodstream forms of *Trypanosoma brucei* *in vitro* identifying 33 compounds with EC_{50} values $< 1 \mu\text{M}$. Counter-screening vs. normal diploid human fibroblasts (MRC5 cells) was used to rank these hits for selectivity, with the most potent ($< 70 \text{ nM}$) and selective (> 700 -fold) compounds being suramin and pentamidine. These are well-known antitrypanosomal drugs which demonstrate the robustness of the resazurin cell viability assay. The most selective novel inhibitor was (+)-trans-(1R,2R)-U50,488 having an EC_{50} value of 60 nM against *T. brucei* and 270-fold selectivity over human fibroblasts. Interestingly, (-)-U50,488, a known CNS-active kappa-opioid receptor agonist and other structurally related compounds were > 70 -fold less active or inactive, as were several mu- and kappa-opioid antagonists. Although (+)-U50,488 was well tolerated by the oral route and displayed good pharmaceutical properties, including high brain penetration, the compound was not curative in the mouse model of infection. Nonetheless, the divergence of antinociceptive and antitrypanosomal activity represents a promising start point for further exploratory chemistry. Bioinformatic studies did not reveal any obvious candidate opioid receptors and the target of this cytostatic compound is unknown. Among the other potent, but less selective screening hits were compound classes with activity against protein kinases, topoisomerases, tubulin, as well as DNA and energy metabolism.

15539. Kroubi, M., Daulouede, S., Karembe, H., Jallouli, Y., Howsam, M., Mossalayi, D., Vincendeau, P. & Betbeder, D., 2010. Development of a nanoparticulate formulation of diminazene to treat African trypanosomiasis. *Nanotechnology*, **21** (50): 505102.

EA 4483, IFR 114 IMPRT, Faculté de Médecine, Pôle recherche, Département de Physiologie, 1 place de Verdun, 59045 Lille Cedex, France. [dbetbeder@aol.com].

There is a real need to develop new therapeutic strategies for African trypanosomiasis infections. In our study, we developed a new drug delivery system of diminazene (DMZ), a trypanocidal drug registered for veterinary use. This drug candidate presents a limited efficacy, a poor affinity for brain tissue and instability. The development of colloidal formulations based on a porous cationic nanoparticle with an oily core (${}_{70}\text{DGNP}^+$), has potentially two advantages: stabilization of the drug and potential targeting of the parasite. We analysed two processes of drug loading: in process (DMZ was added during the preparation of ${}_{70}\text{DGNP}^+$) at 80 °C) and post-loading (DMZ was mixed with a ${}_{70}\text{DGNP}^+$ solution at room temperature). Poor stability of the drug was observed using the in process technique. When using the post-loading technique over 80 percent drug entrapment efficiency was obtained at a ratio of DMZ:phospholipids (wt:wt) < 5 percent. Moreover, DMZ loaded into ${}_{70}\text{DGNP}^+$ was found to be protected against oxidation and was stable for at least six months at 4 °C. Finally, *in vitro* tests on *T. b. brucei* showed an increased efficacy of DMZ loaded in ${}_{70}\text{DGNP}^+$.

15540. **Mallari, J. P., Zhu, F., Lemoff, A., Kaiser, M., Lu, M., Brun, R. & Guy, R. K.,** 2010. Optimization of purine-nitrile TbcatB inhibitors for use *in vivo* and evaluation of efficacy in murine models. *Bioorganic & Medicinal Chemistry*, **18** (23): 8302-8309.

University of California, San Francisco, 94143-2280, USA.
[kip.guy@stjude.org].

There are currently only four clinical drugs available for treating human African trypanosomiasis (HAT), three of which were developed over 60 years ago. Despite years of effort, there has been relatively little progress towards identifying orally available chemotypes active against the parasite *in vivo*. Here, we report the lead optimization of a purine-nitrile scaffold that inhibits the essential TbcatB protease and its evaluation in murine models. A lead inhibitor that had potent activity against the trypanosomal protease TbcatB *in vitro* and cultured parasites *ex vivo* was optimized by rationally driven medicinal chemistry to an inhibitor that is orally available, penetrates the CNS, has a promising pharmacokinetic profile, and is non-toxic at 200mg/kg in a repeat dosage study. Efficacy models using oral administration of this lead inhibitor showed a significantly increased survival time in *Trypanosoma brucei brucei* infected mice but little effect on *Trypanosoma brucei rhodesiense* infected mice.

15541. **Mulyaningsih, S., Youns, M., El-Readi, M. Z., Ashour, M. L., Nibret, E., Sporer, F., Herrmann, F., Reichling, J. & Wink, M.,** 2010. Biological activity of the essential oil of *Kadsura longipedunculata* (Schisandraceae) and its major components. *Journal of Pharmacy & Pharmacology*, **62** (8): 1037-1044.

Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Heidelberg, Germany.

The aim was to determine the chemical composition of the essential oil of *Kadsura longipedunculata* and the biological activity of the oil and its major components. The essential oil from stem bark of *Kadsura longipedunculata* was analysed by capillary gas chromatography (GLC/FID) and gas chromatography-mass spectrometry (GLC/MS). The ability of the oil to reduce diphenylpicrylhydrazine (DPPH) was used to evaluate the antioxidant activity. Inhibition of both lipoxygenase and prostaglandin E₂ was used to assess the anti-inflammatory activity. Antimicrobial activity was studied *in vitro* against a range of bacteria and fungi using diffusion and microdilution methods. Inhibition of trypanosome proliferation was assessed using resazurin as vital stain. The *in vitro* cytotoxicity of the essential oil on six human cancer cell lines (HepG2, MIA PaCa-2, HeLa, HL-60, MDA-MB-231 and SW-480) was examined using the MTT assay. Fifty compounds, representing 97.63 percent of total oil, were identified. Delta-cadinene (21.79 percent), camphene (7.27 percent), borneol (6.05 percent), cubenol (5.12 percent) and delta-cadinol (5.11 percent) were found to be the major components of the oil. The oil exerted a good antimicrobial activity against all gram-positive bacteria tested, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis*. *Streptococcus pyogenes* and *S. agalactiae* were the most sensitive bacteria with a minimal inhibitory concentration (MIC) of 60 µg/ml oil. The essential oil showed a moderate fungicidal activity against yeasts, but it did not show any activity against Gram-negative bacteria. The essential oil showed a good trypanocidal activity in *Trypanosoma b. brucei* with an IC₅₀ value of 50.52 +/- 0.029 µg/ml. Radical scavenging activity had an IC₅₀ value of 3.06 +/- 0.79 mg/ml. 5-lipoxygenase inhibition (IC₅₀ = 38.58 µg/ml) and prostaglandin E₂ production inhibition (28.82 percent at 25 µg/ml) accounted for anti-inflammatory activity of the oil. The oil exhibited some degree of cytotoxic activity against MIA PaCa-2, HepG-2 and SW-480 cell lines with IC₅₀ values of 133.53, 136.96 and 136.62 µg/ml, respectively. The oil increased caspase 3/7 activity (an indicator of apoptosis) 2.5-4 fold in MIA Paca-2 cells. Camphene and borneol did not show antioxidant activity. However, both compounds exhibited some degree of antimicrobial, trypanocidal, anti-inflammatory and cytotoxic activity. This investigation provided evidence for, and confirmed the efficacy of, *K. longipedunculata*, a traditionally used Chinese medicinal plant for the treatment of inflammation and infection.

15542. Nakamura, K., Fujioka, S., Fukumoto, S., Inoue, N., Sakamoto, K., Hirata, H., Kido, Y., Yabu, Y., Suzuki, T., Watanabe, Y., Saimoto, H., Akiyama, H. & Kita, K., 2010. Trypanosome alternative oxidase, a potential therapeutic target for sleeping sickness, is conserved among *Trypanosoma brucei* subspecies. *Parasitology International*, **59** (4): 560-564.

Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Tokyo 113-0033, Japan.
[kosnakamura@nihs.go.jp]

Trypanosoma brucei rhodesiense and *T. b. gambiense* are known causes of human African trypanosomiasis (HAT), or "sleeping sickness", which is deadly if untreated. We previously reported that a specific inhibitor of trypanosome alternative oxidase (TAO), ascofuranone, quickly kills African trypanosomes *in vitro* and cures mice infected with another subspecies, non-human infective *T. b. brucei*, in *in vivo* trials. As an essential factor for trypanosome survival, TAO is a promising drug target due to the absence of alternative

oxidases in the mammalian host. This study found TAO expression in HAT-causing trypanosomes; its amino acid sequence was identical to that in non-human infective *T. b. brucei*. The biochemical understanding of the TAO including its three-dimensional structure and inhibitory compounds against TAO could therefore be applied to all three *T. brucei* subspecies in search of a cure for HAT. Our *in vitro* study using *T. b. rhodesiense* confirmed the effectiveness of ascofuranone (IC₅₀ value: 1 nM) to eliminate trypanosomes in human infective strain cultures.

15543. Nare, B., Wring, S., Bacchi, C., Beaudet, B., Bowling, T., Brun, R., Chen, D., Ding, C., Freund, Y., Gaukel, E., Hussain, A., Jarnagin, K., Jenks, M., Kaiser, M., Mercer, L., Mejia, E., Noe, A., Orr, M., Parham, R., Plattner, J., Randolph, R., Rattendi, D., Rewerts, C., Sligar, J., Yarlett, N., Don, R. & Jacobs, R., 2010. Discovery of novel orally bioavailable oxaborole 6-carboxamides that demonstrate cure in a murine model of late-stage central nervous system African trypanosomiasis. *Antimicrobial Agents & Chemotherapy*, **54** (10): 4379-4388.

Scynexis, Inc, Research Triangle Park, North Carolina 27713, USA.
[bakela.nare@scynexis.com].

We report the discovery of novel boron-containing molecules, exemplified by N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-2-trifluoromethylbenzamide (AN3520) and 4-fluoro-N-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-6-yl)-2-trifluoromethylbenzamide (SCYX-6759), as potent compounds against *Trypanosoma brucei in vitro*, including the two subspecies responsible for human disease *T. b. rhodesiense* and *T. b. gambiense*. These oxaborole carboxamides cured stage 1 (haemolympathic) trypanosomiasis infection in mice when administered orally at 2.5 to 10 mg/kg of body weight for four consecutive days. In stage 2 disease (central nervous system [CNS] involvement), mice infected with *T. b. brucei* were cured when AN3520 or SCYX-6759 were administered intraperitoneally or orally (50 mg/kg) twice daily for seven days. Oxaborole-treated animals did not exhibit gross signs of compound-related acute or subchronic toxicity. Metabolism and pharmacokinetic studies in several species, including nonhuman primates, demonstrate that both SCYX-6759 and AN3520 are low-clearance compounds. Both compounds were well absorbed following oral dosing in multiple species and also demonstrated the ability to cross the blood-brain barrier with no evidence of interaction with the P-glycoprotein transporter. Overall, SCYX-6759 demonstrated superior pharmacokinetics, and this was reflected in better efficacy against stage 2 disease in the mouse model. On the whole, oxaboroles demonstrate potent activity against all *T. brucei* subspecies, excellent physicochemical profiles, *in vitro* metabolic stability, a low potential for CYP450 inhibition, a lack of active efflux by the P-glycoprotein transporter, and high permeability. These properties strongly suggest that these novel chemical entities are suitable leads for the development of new and effective orally administered treatments for human African trypanosomiasis.

15544. Ohashi-Suzuki, M., Yabu, Y., Ohshima, S., Nakamura, K., Kido, Y., Sakamoto, K., Kita, K., Ohta, N. & Suzuki, T., 2010. Differential kinetic activities of glycerol kinase among African trypanosome species: phylogenetic and therapeutic implications. *Journal of Veterinary Medical Science*. e Publication ahead of print, December 22.

Department of International Health Development, Division of Public Health, Graduate School of Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan.

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.

15545. **Pallavi, R., Roy, N., Nageshan, R. K., Talukdar, P., Pavithra, S. R., Reddy, R., Venketesh, S., Kumar, R., Gupta, A. K., Singh, R. K., Yadav, S. C. & Tatu, U.,** 2010. Heat shock protein 90 as a drug target against protozoan infections: biochemical characterization of HSP90 from *Plasmodium falciparum* and *Trypanosoma evansi* and evaluation of its inhibitor as a candidate drug. *Journal of Biological Chemistry*, **285** (49): 37964-37975.

Department of Biochemistry, Indian Institute of Science, Bangalore, Karnataka 560012, India. [tatu@biochem.iisc.ernet.in].

Using a pharmacological inhibitor of Hsp90 in cultured malarial parasite, we have previously implicated *Plasmodium falciparum* Hsp90 (PfHsp90) as a drug target against malaria. In this study, we have biochemically characterized PfHsp90 in terms of its ATPase activity and interaction with its inhibitor geldanamycin (GA) and evaluated its potential as a drug target in a preclinical mouse model of malaria. In addition, we have explored the potential of Hsp90 inhibitors as drugs for the treatment of *Trypanosoma* infection in animals. Our studies with full-length PfHsp90 showed it to have the highest ATPase activity of all known Hsp90s; its ATPase activity was six times higher than that of human Hsp90. Also, GA brought about more robust inhibition of PfHsp90 ATPase activity as compared with human Hsp90. Mass spectrometric analysis of PfHsp90 expressed in *P. falciparum* identified a site of acetylation that overlapped with Aha1 and p23 binding domain, suggesting its role in modulating Hsp90 multichaperone complex assembly. Indeed, treatment of *P. falciparum* cultures with a histone deacetylase inhibitor resulted in a partial dissociation of PfHsp90 complex. Furthermore, we found a well known, semisynthetic Hsp90 inhibitor, namely 17-(allylamino)-17-demethoxygeldanamycin, to be effective in attenuating parasite growth and prolonging survival in a mouse model of malaria. We also characterized GA binding to Hsp90 from another protozoan parasite, namely *Trypanosoma evansi*. We found 17-(allylamino)-17-demethoxygeldanamycin to potently inhibit *T. evansi* growth in a mouse model of trypanosomiasis. In all, our biochemical characterization, drug interaction, and

animal studies supported Hsp90 as a drug target and its inhibitor as a potential drug against protozoan diseases.

15546. **Ruda, G. F., Wong, P. E., Alibu, V. P., Norval, S., Read, K. D., Barrett, M. P. & Gilbert, I. H.**, 2010. Aryl phosphoramidates of 5-phospho erythronohydroxamic acid, a new class of potent trypanocidal compounds. *Journal of Medicinal Chemistry*, **53** (16): 6071-6078.

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

RNAi and enzymatic studies have shown the importance of 6-phosphogluconate dehydrogenase (6-PGDH) in *Trypanosoma brucei* for the parasite survival and make it an attractive drug target for the development of new treatments against human African trypanosomiasis. 2,3-O-Isopropylidene-4-erythrono hydroxamate is a potent inhibitor of parasite *Trypanosoma brucei* 6-phosphogluconate dehydrogenase (6-PGDH), the third enzyme of the pentose phosphate pathway. However, this compound does not have trypanocidal activity due to its poor membrane permeability. Consequently, we have previously reported a prodrug approach to improve the antiparasitic activity of this inhibitor by converting the phosphate group into a less charged phosphate prodrug. The activity of prodrugs appeared to be dependent on their stability in phosphate buffer. Here we have successfully further extended the development of the aryl phosphoramidate prodrugs of 2,3-O-isopropylidene-4-erythrono hydroxamate by synthesizing a small library of phosphoramidates and evaluating their biological activity and stability in a variety of assays. Some of the compounds showed high trypanocidal activity and good correlation of activity with their stability in fresh mouse blood.

15547. **Samant, B. S. & Sukhthankar, M. G.**, 2010. Compounds containing 2-substituted imidazole ring for treatment against human African trypanosomiasis. *Bioorganic & Medicinal Chemistry Letters*, **21**(3): 1015-1018.

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

A series of compounds containing 2-substituted imidazoles has been synthesized from imidazole and tested for its biological activity against human African trypanosomiasis (HAT). The 2-substituted 5-nitroimidazoles such as fexinidazole (7a) and 1-[4-(1-methyl-5-nitro-1H-imidazol-2-ylmethoxy)-pyridin-2-yl]-piperazine (9e) exhibited potent activity against *T. brucei* *in vitro* with low cytotoxicity and good solubility. The presence of the NO₂ group at the 5-position of the imidazole ring in 2-substituted imidazoles is the crucial factor to inhibit *T. brucei*.

15548. **Scala, F., Fattorusso, E., Menna, M., Tagliatalata-Scafati, O., Tierney, M., Kaiser, M. & Tasdemir, D.**, 2010. Bromopyrrole alkaloids as lead compounds against protozoan parasites. *Marine Drugs*, **8** (7): 2162-2174.

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II, Via D. Montesano, 49, I-80131, Napoli, Italy. [fernando.scale@unina.it].

In the present study, 13 bromopyrrole alkaloids, including the oroidin analogues hymenidin (2), dispacamide B (3) and dispacamide D (4), stevensine (5) and spongiacidin B (6), their derivatives lacking the imidazole ring bromoaldisin (7), longamide B (8) and longamide A (9), the dimeric oroidin derivatives sceptorin (10) and dibromopalau'amine (11), and the non-oroidin bromopyrrolohomarginin (12), manzacidin A (13), and agelongine (14), obtained from marine sponges belonging to *Axinella* and *Agelas* genera have been screened *in vitro* against four parasitic protozoa, i.e., two *Trypanosoma* species (*T. brucei rhodesiense* and *T. cruzi*), *Leishmania donovani* and *Plasmodium falciparum* (K1 strain, a chloroquine resistant strain), responsible of human diseases with high morbidity and, in the case of malaria, high mortality. Our results indicate longamide B (8) and dibromopalau'amine (11) to be promising trypanocidal and antileishmanial agents, while dispacamide B (3) and spongiacidin B (6) emerge as antimalarial lead compounds. In addition, evaluation of the activity of the test alkaloids (2-14) against three different enzymes (PfFabI, PfFabG, PfFabZ) involved in the *de novo* fatty acid biosynthesis pathway of *P. falciparum* (PFAS-II) identified bromopyrrolohomarginin (12) as a potent inhibitor of PfFabZ. The structural similarity within the series of tested molecules allowed us to draw some preliminary structure-activity relationships. Tests against the mammalian L6 cells revealed important clues on the therapeutic index of the metabolites. This is the first detailed study on the antiprotozoal potential of marine bromopyrrole alkaloids.

15549. **Schmidt, T. J., Kaiser, M. & Brun, R.,** 2010. Complete structural assignment of serratol, a cembrane-type diterpene from *Boswellia serrata*, and evaluation of its antiprotozoal activity. *Planta Medica*. **e Publication ahead of print, December 14.**

Institut für Pharmazeutische Biologie und Phytochemie (IPBP), Westfälische Wilhelms-Universität Münster, Münster, Germany.

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 *T. brucei* and *P. falciparum*. These activities were 10- to 15-fold higher than its cytotoxicity against rat skeletal myoblasts. While some reports exist on potential anti-inflammatory activity of *Boswellia* diterpenes, this is the first report on antiprotozoal activity of such a compound.

15550. **Tantangmo, F., Lenta, B. N., Boyom, F. F., Ngouela, S., Kaiser, M., Tsamo, E., Weniger, B., Rosenthal, P. J. & Vonthron-Senecheau, C.**, 2010. Antiprotozoal activities of some constituents of *Markhamia tomentosa* (Bignoniaceae). *Annals of Tropical Medicine & Parasitology*, **104** (5): 391-398.

Department of Organic Chemistry, Faculty of Science, TWAS Research Unit of University of Yaoundé I, PO Box 812, Yaoundé, Cameroon.

Phytochemical investigation of an ethyl-acetate extract of the stem bark of *Markhamia tomentosa* (Bignoniaceae), which had good antimalarial activity *in vitro*, resulted in the isolation of eight known compounds: 2-acetylnaphtho[2,3-b]furan-4,9-dione (1), 2-acetyl-6-methoxynaphtho[2,3-b]furan-4,9-dione (2), oleanolic acid (3), pomolic acid (4), 3-acetylpomolic acid (5), tormentic acid (6), beta-sitosterol (7) and beta-sitosterol-3-O-beta-D-glucopyranoside (8). The structures of these compounds were established by spectroscopic methods. Each of compounds 1, 2, 4 and 5 was evaluated *in vitro* for its antiprotozoal activities against the ring stages of two chloroquine-resistant strains of *Plasmodium falciparum* (K1 and W2), the amastigotes of *Leishmania donovani*, and the bloodstream trypomastigotes of *Trypanosoma brucei rhodesiense* (the species responsible for human malaria, visceral leishmaniasis and African trypanosomiasis, respectively). Although compounds 1 and 2 exhibited potent antiprotozoal activities, they also showed high toxicity against a mammalian (L-6) cell line.

15551. **Waag, T., Gelhaus, C., Rath, J., Stich, A., Leippe, M. & Schirmeister, T.**, 2010. Allicin and derivatives are cysteine protease inhibitors with antiparasitic activity. *Bioorganic & Medicinal Chemistry Letters*, **20** (18): 5541-5543.

Institute of Pharmacy and Food Chemistry, University of Wurzburg, Am Hubland, 97074 Wurzburg, Germany. [schirmei@pzc.uni-wuerzburg.de].

Allicin and derivatives thereof inhibit the CAC1 cysteine proteases falcipain 2, rhodesain, cathepsin B and L in the low μ molar range. The structure-activity relationship revealed that only derivatives with primary carbon atom in vicinity to the thiosulfinate sulphur atom attacked by the active-site Cys residue are active against the target enzymes. Some compounds also show potent antiparasitic activity against *Plasmodium falciparum* and *Trypanosoma brucei brucei*.

15552. **Ward, C. P., Burgess, K. E., Burchmore, R. J., Barrett, M. P. & de Koning, H. P.**, 2010. A fluorescence-based assay for the uptake of CPD0801 (DB829) by African trypanosomes. *Molecular & Biochemical Parasitology*, **174** (2): 145-149.

Division of Infection and Immunity, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK. [H.de-Koning@bio.gla.ac.uk].

Drug therapies currently used for second stage human African trypanosomiasis (HAT) exhibit problems with toxicity, difficulty of administration, and resistance linked to the loss of transporter function. Key to the development of new drugs for HAT is a better understanding of the transport properties of candidate compounds. Standard methods for studying transport utilize radio-labelled permeant or HPLC-MS, however the natural

fluorescence of many trypanocidal compounds can be exploited. Here we present a fluorescence-based assay for measuring uptake by trypanosomes of CPD0801, a drug candidate for second stage HAT. Sample fluorescence is measured in a 96-well format using a bench top fluorimeter. Our method is directly applicable to the study of other diamidines with similar fluorescent properties and readily adapted for use with other cell types or fluorescent molecules as we demonstrate for the veterinary trypanocide ethidium.

15553. **Worthen, C., Jensen, B. C. & Parsons, M.**, 2010. Diverse effects on mitochondrial and nuclear functions elicited by drugs and genetic knockdowns in bloodstream stage *Trypanosoma brucei*. *PLoS Neglected Tropical Diseases*, **4** (5): e678.

Seattle Biomedical Research Institute, Seattle, Washington, USA.
[marilyn.parsons@sabri.org].

The options for treating the fatal disease human African trypanosomiasis are limited to a few drugs that are toxic or facing increasing resistance. New drugs that kill the causative agents, subspecies of *Trypanosoma brucei*, are therefore urgently needed. Little is known about the cellular mechanisms that lead to death of the pathogenic bloodstream stage. We therefore conducted the first side by side comparison of the cellular effects of multiple death inducers that target different systems in bloodstream form parasites, including six drugs (pentamidine, prostaglandin D₂, quercetin, etoposide, camptothecin, and a tetrahydroquinoline) and six RNAi knockdowns that target distinct cellular functions. All compounds tested were static at low concentrations and killed at high concentrations. Dead parasites were rapidly quantified by forward and side scatter during flow cytometry, as confirmed by ethidium homodimer and esterase staining, making these assays convenient for quantitating parasite death. The various treatments yielded different combinations of defects in mitochondrial potential, reactive oxygen species, cell cycle, and genome segregation. No evidence was seen for phosphatidylserine exposure, a marker of apoptosis. Reduction in ATP levels lagged behind decreases in live cell number. Even when the impact on growth was similar at 24 hours, drug-treated cells showed dramatic differences in their ability to further proliferate, demonstrating differences in the reversibility of effects induced by the diverse compounds. Parasites showed different phenotypes depending on the treatment, but none of them were clear predictors of whether apparently live cells could go on to proliferate after drugs were removed. We therefore suggest that clonal proliferation assays may be a useful step in selecting anti-trypanosomal compounds for further development. Elucidating the genetic or biochemical events initiated by the compounds with the most profound effects on subsequent proliferation may identify new means to activate death pathways.

15554. **Wube, A. A., Bucar, F., Gibbons, S., Asres, K., Rattray, L. & Croft, S. L.**, 2010. Antiprotozoal activity of drimane and coloratane sesquiterpenes towards *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum* in vitro. *Phytotherapy Research*, **24** (10): 1468-1472.

Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Karl-Franzens University Graz, Universitaetsplatz 4/1, A-8010 Graz, Austria.
[franz.bucar@uni-graz.at].

The extracts and 12 sesquiterpenes obtained from the East African medicinal plant *Warburgia ugandensis* Sprague (Canellaceae) were assessed for their antiplasmodial activity against the chloroquine-sensitive (3D7) and chloroquine-resistant (K1) strains of *Plasmodium falciparum* and antitrypanosomal activity against *Trypanosoma brucei rhodesiense*. The dichloromethane extract displayed strong antiplasmodial and antitrypanosomal activities with IC₅₀ values of 8.10 and 1.10 µg/mL against the K1 strain of the malaria parasite and the STIB900 strain of *T. b. rhodesiense*, respectively. Among the compounds evaluated for inhibition of trypomastigotes, both drimane and coloratane sesquiterpenes possessing aldehyde groups at positions 8 and 9 were found to show most antitrypanosomal activity with IC₅₀ values in the range 0.56-6.4 µM. The antiplasmodial assays also revealed that the six coloratane and six drimane sesquiterpenes isolated from this extract exhibited significant antitrypanosomal activity with IC₅₀ values ranging from 0.45 to 114 µM. Among the compounds tested against the malarial parasite *P. falciparum* 11?-hydroxymuzigadiolide (3) was most active with an IC₅₀ value of 6.40 µM.

15555. **Zoidis, G., Kolocouris, N., Kelly, J. M., Prathalingam, S. R., Naesens, L. & De Clercq, E.**, 2010. Design and synthesis of bioactive adamantanaminoalcohols and adamantanamines. *European Journal of Medicinal Chemistry*, **45** (11): 5022-5030.

Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Athens, Panepistimioupoli-Zografou, GR-15771 Athens, Greece. [zoidis@pharm.uoa.gr].

Adamantanamines 16, 18, 21, 24, 27, 28, 30, 32, 35, 36, 37, 40, 46 and 48 were synthesized and tested for anti-influenza A virus and trypanocidal activity. The stereoelectronic requirements for optimal antiviral and trypanocidal potency were investigated. The effect of introducing a hydroxyl group close to the amino group on this class of compounds was examined for the first time. Aminoalcohol 24 proved to be the most active of the compounds tested against influenza A virus, being 6-fold more active than amantadine, equipotent to rimantadine and 26-fold more potent than ribavirin. Aminoalcohols 36 and 37 were found to have considerable activity against bloodstream forms of the African trypanosome, *Trypanosoma brucei*, being almost 10 times more potent than rimantadine.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

15556. **Liao, D. & Shen, J.**, 2010. Studies of quinapyramine-resistance of *Trypanosoma brucei evansi* in China. *Acta Tropica*, **116** (3): 173-177.

Sichuan Academy of Animal Science, Lane 7 Niu Sha Road, Chengdu, Sichuan, PR China. [liaodjin@yahoo.com.cn].

In the present article, we summarize our studies of antitrypanocidal resistance of *Trypanosoma brucei evansi* in recent years, the analysis of quinapyramine sensitive *T. b. evansi* in China, the biological characteristics of quinapyramine resistant *T. b. evansi* populations, and

biological materials associated with quinapyramine resistance in *T. b. evansi* population. Firstly, the correlative assays of effective dosage of quinapyramine on *T. b. evansi* disease between *in vivo* and *in vitro* methods showed that their relationship was parabolic with positive correlation. On the other hand, the IC₅₀ and CD₁₀₀ values of 12 *T. b. evansi* isolates, AHB, GDB1, GDB2, HNB, JSB1, JSB2, YNB, ZJB, GDH, GXM, HBM and XJCA, collected from buffaloes, horses, mules and camels across nine provinces of China were examined using the two methods. Among them, nine isolates, AHB, GDB1, GDB2, HNB, JSB1, JSB2, YNB, ZJB and GDH, became quinapyramine sensitive *T. b. evansi*. Secondly, *T. evansi* populations could rapidly become antrycide resistant when they were passed through immunosuppressed mice treated with low doses of the drug. But, the replication rate of trypanosomes with antrycide resistance decreased as the level of drug resistance increased. Thirdly, the analysis of the HK, G6PDH, ALAT and ASAT isoenzymes showed that they were not involved in the quinapyramine resistance of *T. b. evansi*. But the protein bands of 15.79kDa and 19.76kDa might be involved in the antrycide resistance of *T. b. evansi* populations. At the genetic level, the gene, TbTA1, could be amplified from the *T. b. evansi* isolate sensitive to quinapyramine as could the *T. b. evansi* isolate with quinapyramine resistance using not only the RT-PCR technique, but also PCR technique. We used the SSH (suppression subtractive hybridization) to clone highly or low expressed cDNA fragments caused by the production of antrycide resistance in *T. b. evansi*. The five low and nine high expressed new cDNA fragments were amplified. Among them, the three low expressed cDNA fragments had the same sequence of 65 amino acids and the three high expressed cDNA fragments were located in chromosome VI, like *T. brucei*. Lastly, more work needs to be done in order to elucidate the mechanism of quinapyramine resistance of *T. b. evansi*.

15557. **Van den Bossche, P., Chitanga, S., Masumu, J., Marcotty, T. & Delespau, V.,** 2010. Virulence in *Trypanosoma congolense* savannah subgroup. A comparison between strains and transmission cycles. *Parasite Immunology*. **e Publication ahead of print, December 30.**

Institute of Tropical Medicine, Animal Health Department, Nationalestraat 155, B-2000 Antwerp, Belgium; Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, South Africa; and Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, Great East Road Campus, P.O. Box 32379, Lusaka, Zambia. [vdelespau@itg.be].

Trypanosoma congolense strains have been shown to differ in their virulence both between subgroups and within the savannah subgroup between strains. This review revisits these findings and complements them with information on the virulence of *T. congolense* savannah subgroup strains isolated from cattle (domestic transmission cycle) in different geographical areas and of strains isolated in protected areas where trypanotolerant wildlife species are the reservoir of the trypanosomes (sylvatic transmission cycle). The virulence of a total of 62 *T. congolense* savannah subgroup strains (50 domestic and 12 sylvatic), determined using a standard protocol in mice, was compared. Virulence varied substantially between strains with, depending on the strain, the median survival time of infected mice varying from five to more than sixty days. The proportion of highly virulent strains (median survival time < 10 days) was significantly ($p = 0.005$) higher in strains from the sylvatic transmission cycle. The analysis highlights repercussions of the domestication of the

trypanosomiasis transmission cycle that may have to be taken in consideration in the development of trypanosomiasis control strategies.

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

15558. **Ammerman, M. L., Presnyak, V., Fisk, J. C., Foda, B. M. & Read, L. K.,** 2010. TbRGG2 facilitates kinetoplastid RNA editing initiation and progression past intrinsic pause sites. *Rna*, **16** (11): 2239-2251.

Department of Microbiology and Immunology, University at Buffalo School of Medicine, Buffalo, New York 14214, USA. [lread@buffalo.edu].

TbRGG2 is an essential kinetoplastid RNA editing accessory factor that acts specifically on pan-edited RNAs. To understand the mechanism of TbRGG2 action, we undertook an in-depth analysis of edited RNA populations in TbRGG2 knockdown cells and an *in vitro* examination of the biochemical activities of the protein. We demonstrate that TbRGG2 down-regulation more severely impacts editing at the 5' ends of pan-edited RNAs than at their 3' ends. The initiation of editing is reduced to some extent in TbRGG2 knockdown cells. In addition, TbRGG2 plays a post-initiation role as editing becomes stalled in TbRGG2-depleted cells, resulting in an overall decrease in the 3' to 5' progression of editing. Detailed analyses of edited RNAs from wild-type and TbRGG2-depleted cells reveal that TbRGG2 facilitates progression of editing past intrinsic pause sites that often correspond to the 3' ends of cognate guide RNAs (gRNAs). In addition, noncanonically edited junction regions are either absent or significantly shortened in TbRGG2-depleted cells, consistent with impaired gRNA transitions. Sequence analysis further suggests that TbRGG2 facilitates complete utilization of certain gRNAs. *In vitro* RNA annealing and *in vivo* RNA unwinding assays demonstrate that TbRGG2 can modulate RNA-RNA interactions. Collectively, these data are consistent with a model in which TbRGG2 facilitates initiation and 3' to 5' progression of editing through its ability to affect gRNA utilization, both during the transition between specific gRNAs and during usage of certain gRNAs.

15559. **Baker, N., Alsford, S. & Horn, D.,** Genome-wide RNAi screens in African trypanosomes identify the nifurtimox activator NTR and the eflornithine transporter AAT6. *Molecular & Biochemical Parasitology*, **176**(1): 55-57.

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

To be effective, therapeutic compounds must typically enter target cells and, in some cases, must be concentrated or modified. Thus, uptake and activation mechanisms often form the basis of selectivity against infectious agents. Loss-of-function screens can be used to identify proteins involved in drug uptake and metabolism and may also identify clinically relevant potential resistance mechanisms. We used a genome-scale RNA interference (RNAi) library to identify loss-of-function resistance mechanisms in bloodstream form *Trypanosoma brucei*. Nifurtimox-eflornithine combination therapy (NECT) was recently introduced for human African trypanosomiasis and we focus on these drugs here. Screens for resistance to nifurtimox and a related drug, benznidazole, identified loss of nitroreductase (NTR) pro-drug

activator function. A screen for resistance to the amino-acid analogue, eflornithine, identified loss of amino-acid transporter (AAT6) function. Our results confirm recent findings and suggest that NTR or AAT6 loss-of-function represents major potential mechanisms of resistance to these drugs. Thus, bloodstream-form *T. brucei* RNAi libraries present a versatile tool for selective genetic screening and for the rapid identification of drug-activation, uptake and potential resistance mechanisms.

15560. **Bakker, B. M., Krauth-Siegel, R. L., Clayton, C., Matthews, K., Girolami, M., Westerhoff, H. V., Michels, P. A., Breiting, R. & Barrett, M. P.**, 2010. The silicon trypanosome. *Parasitology*, **137** (9): 1333-1341.

Department of Pediatrics, Center for Liver, Digestive and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. [b.m.bakker@med.umcg.nl].

African trypanosomes have emerged as promising unicellular model organisms for the next generation of systems biology. They offer unique advantages, due to their relative simplicity, the availability of all standard genomics techniques and a long history of quantitative research. Reproducible cultivation methods exist for morphologically and physiologically distinct life-cycle stages. The genome has been sequenced, and microarrays, RNA-interference and high-accuracy metabolomics are available. Furthermore, the availability of extensive kinetic data on all glycolytic enzymes has led to the early development of a complete, experiment-based dynamic model of an important biochemical pathway. Here we describe the achievements of trypanosome systems biology so far and outline the necessary steps towards the ambitious aim of creating a “silicon trypanosome”, a comprehensive, experiment-based, multi-scale mathematical model of trypanosome physiology. We expect that, in the long run, the quantitative modelling enabled by the silicon trypanosome will play a key role in selecting the most suitable targets for developing new anti-parasite drugs.

15561. **Bakker, B. M., van Eunen, K., Jeneson, J. A., van Riel, N. A., Bruggeman, F. J. & Teusink, B.**, 2010. Systems biology from micro-organisms to human metabolic diseases: the role of detailed kinetic models. *Biochemical Society Transactions*, **38** (5): 1294-1301.

Department of Liver, Digestive and Metabolic Diseases, University Medical Centre Groningen, University of Groningen, The Netherlands. [b.m.bakker@med.umcg.nl].

Human metabolic diseases are typically network diseases. This holds not only for multifactorial diseases, such as metabolic syndrome or Type 2 diabetes, but even when a single gene defect is the primary cause, where the adaptive response of the entire network determines the severity of disease. The latter may differ between individuals carrying the same mutation. Understanding the adaptive responses of human metabolism naturally requires a systems biology approach. Modelling of metabolic pathways in micro-organisms and some mammalian tissues has yielded many insights, qualitative as well as quantitative, into their control and regulation. Yet, even for a well-known pathway such as glycolysis, precise predictions of metabolite dynamics from experimentally determined enzyme kinetics

have been only moderately successful. In the present review, we compare kinetic models of glycolysis in three cell types (African trypanosomes, yeast and skeletal muscle), evaluate their predictive power and identify limitations in our understanding. Although each of these models has its own merits and shortcomings, they also share common features. For example, in each case independently measured enzyme kinetic parameters were used as input. Based on these “lessons from glycolysis”, we will discuss how to make best use of kinetic computer models to advance our understanding of human metabolic diseases.

15562. **Bosschaerts, T., Guilliams, M., Stijlemans, B., Morias, Y., Engel, D., Tacke, F., Herin, M., De Baetselier, P. & Beschin, A.**, 2010. Tip-DC development during parasitic infection is regulated by IL-10 and requires CCL2/CCR2, IFN-gamma and MyD88 signalling. *PLoS Pathogens*, **6** (8): e1001045. doi:10.1371/journal.ppat.1001045.

Department of Molecular and Cellular Interactions, VIB, Brussels, Belgium.
[abeschin@vub.ac.be].

The development of classically activated monocytic cells (M1) is a prerequisite for effective elimination of parasites, including African trypanosomes. However, persistent activation of M1 that produce pathogenic molecules such as TNF and NO contributes to the development of trypanosome infection-associated tissue injury including liver cell necrosis in experimental mouse models. Aiming to identify mechanisms involved in regulation of M1 activity, we have recently documented that during *Trypanosoma brucei* infection, CD11b⁺Ly6C⁺CD11c⁺ TNF and iNOS producing DCs (Tip-DCs) represent the major pathogenic M1 liver subpopulation. By using gene expression analyses, KO mice and cytokine neutralizing antibodies, we show here that the conversion of CD11b⁺Ly6C⁺ monocytic cells to pathogenic Tip-DCs in the liver of *T. brucei* infected mice consists of a three-step process including (i) a CCR2-dependent but CCR5- and Mif-independent step crucial for emigration of CD11b⁺Ly6C⁺ monocytic cells from the bone marrow but dispensable for their blood to liver migration; (ii) a differentiation step of liver CD11b⁺Ly6C⁺ monocytic cells to immature inflammatory DCs (CD11c⁺ but CD80/CD86/MHC-II(low)) which is IFN-gamma and MyD88 signalling independent; and (iii) a maturation step of inflammatory DCs to functional (CD80/CD86/MHC-II(high)) TNF and NO producing Tip-DCs which is IFN-gamma and MyD88 signalling dependent. Moreover, IL-10 could limit CCR2-mediated egression of CD11b⁺Ly6C⁺ monocytic cells from the bone marrow by limiting Ccl2 expression by liver monocytic cells, as well as their differentiation and maturation to Tip-DCs in the liver, showing that IL-10 works at multiple levels to dampen Tip-DC mediated pathogenicity during *T. brucei* infection. A wide spectrum of liver diseases associates with alteration of monocyte recruitment, phenotype or function, which could be modulated by IL-10. Therefore, investigating the contribution of recruited monocytes to African trypanosome induced liver injury could potentially identify new targets to treat hepatic inflammation in general, and during parasite infection in particular.

15563. **Cai, S., Fang, J., Li, Q. S., Borchardt, R. T., Kuczera, K., Middaugh, C. R. & Schowen, R. L.**, 2010. Comparative kinetics of cofactor association and dissociation for the human and trypanosomal S-adenosylhomocysteine hydrolases. 3. Role of lysyl and tyrosyl residues of the C-terminal extension. *Biochemistry*, **49** (38): 8434-8441.

Department of Molecular Biosciences, The University of Kansas, Lawrence, Kansas 66047, USA.

On the basis of the available X-ray structures of S-adenosylhomocysteine hydrolases (SAHs), free energy simulations employing the MM-GBSA approach were applied to predict residues important to the differential cofactor binding properties of human and trypanosomal SAHs (Hs-SAHH and Tc-SAHH), within 5 Å of the cofactor NAD⁺/NADH binding site. Among the 38 residues in this region, only four are different between the two enzymes. Surprisingly, the four nonidentical residues make no major contribution to differential cofactor binding between Hs-SAHH and Tc-SAHH. On the other hand, four pairs of identical residues are shown by free energy simulations to differentiate cofactor binding between Hs-SAHH and Tc-SAHH. Experimental mutagenesis was performed to test these predictions for a lysine residue and a tyrosine residue of the C-terminal extension that penetrates a partner subunit to form part of the cofactor binding site. The K431A mutant of Tc-SAHH (TcK431A) loses its cofactor binding affinity but retains the wild type's tetrameric structure, while the corresponding mutant of Hs-SAHH (HsK426A) loses both cofactor affinity and tetrameric structure. The tyrosine mutants HsY430A and TcY435A alter the NAD(+) association and dissociation kinetics, with HsY430A increasing the cofactor equilibrium dissociation constant from approximately 10 nM (Hs-SAHH) to approximately 800 nM and TcY435A increasing the cofactor equilibrium dissociation constant from approximately 100 nM (Tc-SAHH) to approximately 1 mM. Both changes result from larger increases in the off rate combined with smaller decreases in the on rate. These investigations demonstrate that computational free energy decomposition may be used to guide experimental studies by suggesting sensitive sites for mutagenesis. Our finding that identical residues in two orthologous proteins may give significantly different binding free energy contributions strongly suggests that comparative studies of homologous proteins should investigate not only different residues but also identical residues in these proteins.

15564. Ceylan, S., Seidel, V., Ziebart, N., Berndt, C., Dirdjaja, N. & Krauth-Siegel, R. L., 2010. The dithiol glutaredoxins of African trypanosomes have distinct roles and are closely linked to the unique trypanothione metabolism. *Journal of Biological Chemistry*, **285** (45): 35224-35237.

Biochemie-Zentrum der Universität Heidelberg, Im Neuenheimer Feld 328, D-69120 Heidelberg, Germany. [luise.krauth-siegel@bzh.uni-heidelberg.de].

Trypanosoma brucei, the causative agent of African sleeping sickness, possesses two dithiol glutaredoxins (Grx1 and Grx2). Grx1 occurs in the cytosol and catalyzes protein deglutathionylations with k_{cat}/K_m -values of up to $2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. It accelerates the reduction of ribonucleotide reductase by trypanothione although less efficiently than the parasite trypanredoxin and has low insulin disulfide reductase activity. Despite its classical CPYC active site, Grx1 forms dimeric iron-sulphur complexes with GSH, glutathionylspermidine, or trypanothione as non-protein ligands. Thus, contrary to the generally accepted assumption, replacement of the Pro is not a prerequisite for cluster formation. *T. brucei* Grx2 shows an unusual CQFC active site, and orthologues occur exclusively in trypanosomatids. Grx2 is enriched in mitochondria, and fractionated digitonin lysis resulted in a co-elution with cytochrome c, suggesting localization in the mitochondrial intermembrane space. Grx2 catalyzes the reduction of insulin disulfide but not of ribonucleotide reductase and exerts

deglutathionylation activity 10-fold lower than that of Grx1. RNA interference against Grx2 caused a growth retardation of procyclic cells consistent with an essential role. Grx1 and Grx2 are constitutively expressed with cellular concentrations of about 2 μM and 200 nM respectively, in both the mammalian bloodstream and insect procyclic forms. Trypanothione reduces the disulphide form of both proteins with apparent rate constants that are three orders of magnitude higher than those with glutathione. Grx1 and, less efficiently, also Grx2 catalyze the reduction of GSSG by trypanothione. Thus, the Grxs play exclusive roles in the trypanothione-based thiol redox metabolism of African trypanosomes.

15565. **Chan, K. Y. & Ersfeld, K.,** 2010. The role of the kinesin-13 family protein TbKif13-2 in flagellar length control of *Trypanosoma brucei*. *Molecular & Biochemical Parasitology*, **174** (2): 137-140.

Department of Biological Sciences, University of Hull, Hull, UK.
[k.ersfeld@hull.ac.uk].

TbKif13-2, a member of the microtubule-depolymerising Kinesin-13 family was localized at the tip of the flagellum in *Trypanosoma brucei*. Its predicted activity suggested a role in the regulation of axonemal length. However, using gene deletion and overexpression of TbKif13-2 we show that in procyclic *T. brucei*, this kinesin has only a very limited effect on flagellar length. Gene deletion resulted in no significant elongation of the flagellum and overexpression only slightly decreased flagellar length and the rate of growth of a new flagellum during cell division. This is in contrast to studies in *Leishmania major*, where overexpression of the TbKif13-2 homologue resulted in a significant length reduction of the flagellum. Knockout of TbKif13-2 has, however, an effect on the initial growth of the emerging new flagellum. In conclusion, we show that TbKif13-2 has only a marginal impact on flagellar length in *T. brucei*.

15566. **Cristodero, M., Seebeck, T. & Schneider, A.,** 2010. Mitochondrial translation is essential in bloodstream forms of *Trypanosoma brucei*. *Molecular Microbiology*, **78** (3): 757-769.

Department of Chemistry and Biochemistry, University of Bern, Freiestr. 3, CH-3012 Bern, Switzerland. [andre.schneider@ibc.unibe.ch].

The parasitic protozoa *Trypanosoma brucei* has a complex life cycle. Oxidative phosphorylation is highly active in the procyclic form but absent from bloodstream cells. The mitochondrial genome encodes several gene products that are required for oxidative phosphorylation, but it completely lacks tRNA genes. For mitochondrial translation to occur, the import of cytosolic tRNAs is therefore essential for procyclic *T. brucei*. Whether the same is true for the bloodstream form has not been studied so far. Here we show that the steady-state levels of mitochondrial tRNAs are essentially the same in both life stages. Editing of the imported tRNA(Tip) also occurs in both forms as well as in mitochondria of *Trypanosoma evansi*, which lacks a genome and a translation system. These results show that mitochondrial tRNA import is a constitutive process that must be mediated by proteins that are expressed in both forms of the life cycle and that are not encoded in the mitochondrial genome. Moreover, bloodstream cells lacking either mitochondria-specific translation elongation factor Tu or mitochondrial tryptophanyl-tRNA synthetase are not viable indicating that mitochondrial

translation is also essential in this stage. Both of these proteins show trypanosomatid-specific features and may therefore be excellent novel drug targets.

15567. **de Jesus, T. C., Tonelli, R. R., Nardelli, S. C., da Silva Augusto, L., Motta, M. C., Girard-Dias, W., Miranda, K., Ulrich, P., Jimenez, V., Barquilla, A., Navarro, M., Docampo, R. & Schenkman, S.**, 2010. Target of rapamycin (TOR)-like 1 kinase is involved in the control of polyphosphate levels and acidocalcisome maintenance in *Trypanosoma brucei*. *Journal of Biological Chemistry*, **285** (31): 24131-24140.

Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Sao Paulo, Sao Paulo 04023-062, Brazil. [sschenkman@unifesp.br].

The targets of rapamycin (TOR) kinases are highly conserved protein kinases that integrate signals from nutrients and growth factors to coordinate cell growth and cell cycle progression. It has been previously described that two TOR kinases control cell growth in the protozoan parasite *Trypanosoma brucei*, the causative agent of African trypanosomiasis. Here we studied an unusual TOR-like protein named TbTOR-like 1 containing a PDZ domain and found exclusively in kinetoplastids. TbTOR-like 1 localizes to unique cytosolic granules. After hyperosmotic stress, the localization of the protein shifts to the cell periphery, different from other organelle markers. Ablation of TbTOR-like 1 causes a progressive inhibition of cell proliferation, producing parasites accumulating in the S/G₂ phase of the cell cycle. TbTOR-like 1 knocked down cells have an increased area occupied by acidic vacuoles, known as acidocalcisomes, and are enriched in polyphosphate and pyrophosphate. These results suggest that TbTOR-like 1 might be involved in the control of acidocalcisome and polyphosphate metabolism in *T. brucei*.

15568. **Degrasse, J. A. & Devos, D.**, 2010. A functional proteomic study of the *Trypanosoma brucei* nuclear pore complex: an informatic strategy. *Methods in Molecular Biology*, **673**: 231-238.

US Food and Drug Administration, College Park, MD, USA.

The nuclear pore complex (NPC) is the sole mediator of transport between the nucleus and the cytoplasm. The NPC is composed of about 30 distinct proteins, termed nucleoporins or nups. The yeast and mammalian NPCs have been extensively studied. However, the two species are relatively closely related. Thus, to reveal details about NPC evolution, we chose to characterize the NPC of a distantly related organism, *Trypanosoma brucei*. We took a subcellular proteomic approach and used several complementary strategies to identify 865 proteins associated with the nuclear envelope. Over 50 percent of approximately 8 100 open reading frames of *T. brucei* have little or no known function because *T. brucei* is distantly related to model metazoa and fungi (Berriman et al., *Science* 309:416-422, 2005). By sequence similarity alone, we could identify only five nucleoporins. This chapter outlines our strategy to identify 17 additional nucleoporins as well as contribute functional annotation data to the *T. brucei* genome database.

15569. **Denninger, V., Fullbrook, A., Bessat, M., Ersfeld, K. & Rudenko, G.**, 2010. The FACT subunit TbSpt16 is involved in cell cycle specific control of VSG expression sites in *Trypanosoma brucei*. *Molecular Microbiology*, **78** (2): 459-474.

Division of Cell and Molecular Biology, Sir Alexander Fleming Building,
Imperial College, South Kensington, London SW72AZ, UK.
[gloria.rudenko@imperial.ac.uk].

The African trypanosome *Trypanosoma brucei* monoallelically expresses one of more than 1 000 variant surface glycoprotein (VSG) genes. The active VSG is transcribed from one of about 15 telomeric VSG expression sites (ESs). It is unclear how monoallelic expression of VSG is controlled, and how inactive VSG ESs are silenced. Here, we show that blocking synthesis of the *T. brucei* FACT subunit TbSpt16 triggers a G2/early M phase cell cycle arrest in both bloodstream and insect form *T. brucei*. Segregation of *T. brucei* minichromosomes in these stalled cells is impaired, implicating FACT in maintenance of centromeres. Strikingly, knock-down of TbSpt16 results in 20- to 23-fold derepression of silent VSG ES promoters in bloodstream form *T. brucei*, with derepression specific to the G2/M cell cycle stage. In insect form *T. brucei* TbSpt16 knock-down results in 16- to 25-fold VSG ES derepression. Using chromatin immunoprecipitation (ChIP), TbSpt16 was found to be particularly enriched at the promoter region of silent but not active VSG ESs in bloodstream form *T. brucei*. The chromatin remodeler FACT is therefore implicated in maintenance of repressed chromatin present at silent VSG ES promoters, but is also essential for chromosome segregation presumably through maintenance of functional centromeres.

15570. **Diskar, M., Zenn, H. M., Kaupisch, A., Kaufholz, M., Brockmeyer, S., Sohmen, D., Berrera, M., Zaccolo, M., Boshart, M., Herberg, F. W. & Prinz, A., 2010.** Regulation of cAMP-dependent protein kinases: the human protein kinase X (PrKX) reveals the role of the catalytic subunit alphaH-alpha loop. *Journal of Biological Chemistry*, **285** (46): 35910-35918.

Department of Biochemistry, University of Kassel, Heinrich-Plett-Strasse 40,
34132 Kassel, Germany. [ankeprinz@uni-kassel.de].

cAMP-dependent protein kinases are reversibly complexed with any of the four isoforms of regulatory (R) subunits, which contain either a substrate or a pseudosubstrate autoinhibitory domain. The human protein kinase X (PrKX) is an exemption as it is inhibited only by pseudosubstrate inhibitors, i.e. RIalpha or RIIbeta but not by substrate inhibitors RIIalpha or RIIbeta. Detailed examination of the capacity of five PrKX-like kinases ranging from human to protozoa (*Trypanosoma brucei*) to form holoenzymes with human R subunits in living cells shows that this preference for pseudosubstrate inhibitors is evolutionarily conserved. To elucidate the molecular basis of this inhibitory pattern, we applied bioluminescence resonance energy transfer and surface plasmon resonance in combination with site-directed mutagenesis. We observed that the conserved alphaH-alpha loop residue Arg-283 in PrKX is crucial for its RI over RII preference, as a R283L mutant was able to form a holoenzyme complex with wild type RII subunits. Changing the corresponding alphaH-alpha loop residue in PKA Calpha (L277R) significantly destabilized holoenzyme complexes *in vitro*, as cAMP-mediated holoenzyme activation was facilitated by a factor of 2-4, and lead to a decreased affinity of the mutant C subunit for R subunits, significantly affecting RII containing holoenzymes.

15571. **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., 2010. Global analysis of protein palmitoylation in African trypanosomes. *Eukaryotic Cell*. **Published online ahead of print, December 30.**

Departments of Pathology and Microbiology-Immunology, and Department of Pediatrics, Northwestern University, Chicago, Illinois, USA; The Border Biomedical Research Center, Department of Biological Sciences, University of Texas at El Paso, El Paso, Texas 79968, USA; Department of Pathology and Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan 48109, USA; and Laboratory of Genetics and Molecular Cardiology, Heart Institute (InCor), University of Sao Paulo, Sao Paulo, SP 05403-000, Brazil. [icalmeida@utep.edu].

Many eukaryotic proteins are posttranslationally modified by the thioesterification of cysteine thiols to long chain fatty acids. This modification, protein palmitoylation, is catalyzed 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 catalyzed 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, 2D LC-MS/MS 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.

15572. **Flaspohler, J. A., Jensen, B. C., Saveria, T., Kifer, C. T. & Parsons, M., 2010.** A novel protein kinase localized to lipid droplets is required for droplet biogenesis in trypanosomes. *Eukaryotic Cell*, **9** (11): 1702-1710.

Seattle Biomedical Research Institute, WA 98109, USA.
[marilyn.parsons@seattlebiomed.org].

Ubiquitous among eukaryotes, lipid droplets are organelles that function to coordinate intracellular lipid homeostasis. Their morphology and abundance are affected by numerous genes, many of which are involved in lipid metabolism. In this report we identify a *Trypanosoma brucei* protein kinase, LDK, and demonstrate its localization to the periphery of lipid droplets. Association with lipid droplets was abrogated when the hydrophobic domain of LDK was deleted, supporting a model in which the hydrophobic domain is associated with

or inserted into the membrane monolayer of the organelle. RNA interference knockdown of LDK modestly affected the growth of mammalian bloodstream-stage parasites but did not affect the growth of insect (procyclic)-stage parasites. However, the abundance of lipid droplets dramatically decreased in both cases. This loss was dominant over treatment with myriocin or growth in delipidated serum, both of which induce lipid body biogenesis. Growth in delipidated serum also increased LDK autophosphorylation activity. Thus, LDK is required for the biogenesis or maintenance of lipid droplets and is one of the few protein kinases specifically and predominantly associated with an intracellular organelle.

15573. **Franklin, J. B. & Ullu, E.**, 2010. Biochemical analysis of PIFTC3, the *Trypanosoma brucei* orthologue of nematode DYF-13, reveals interactions with established and putative intraflagellar transport components. *Molecular Microbiology*, **78** (1): 173-186.

Department of Cell Biology, School of Medicine, Yale University, New Haven, CT 06536, USA. [elisabetta.ullu@yale.edu].

DYF-13, originally identified in *Caenorhabditis elegans* within a collection of dye-filling chemosensory mutants, is one of several proteins that have been classified as putatively involved in intraflagellar transport (IFT), the bidirectional movement of protein complexes along cilia and flagella and specifically in anterograde IFT. Although genetic studies have highlighted a fundamental role of DYF-13 in nematode sensory cilium and trypanosome flagellum biogenesis, biochemical studies on DYF-13 have lagged behind. Here, we show that in *Trypanosoma brucei* the orthologue to DYF-13, PIFTC3, participates in a macromolecular complex of approximately 660 kDa. Mass spectroscopy of affinity-purified PIFTC3 revealed several components of IFT complex B as well as orthologues of putative IFT factors DYF-1, DYF-3, DYF-11/Elipsa and IFTA-2. DYF-11 was further analysed and shown to be concentrated near the basal bodies and in the flagellum, and to be required for flagellum elongation. In addition, by coimmunoprecipitation we detected an interaction between DYF-13 and IFT122, a component of IFT complex A, which is required for retrograde transport. Thus, our biochemical analysis supports the model, proposed by genetic analysis in *C. elegans*, that the trypanosome orthologue of DYF-13 plays a central role in the IFT mechanism.

15574. **Frederiks, F., van Welsem, T., Oudgenoeg, G., Heck, A. J., Janzen, C. J. & van Leeuwen, F.**, 2010. Heterologous expression reveals distinct enzymatic activities of two DOT1 histone methyltransferases of *Trypanosoma brucei*. *Journal of Cell Science*, **123** (23): 4019-4023.

Division of Gene Regulation, The Netherlands Cancer Institute, 1066 CX, Amsterdam, The Netherlands. [cjanzen@lmu.de].

Dot1 is a highly conserved methyltransferase that modifies histone H3 on the nucleosome core surface. In contrast to yeast, flies, and humans where a single Dot1 enzyme is responsible for all methylation of H3 lysine 79 (H3K79), African trypanosomes express two DOT1 proteins that methylate histone H3K76 (corresponding to H3K79 in other organisms) in a cell-cycle-regulated manner. Whereas DOT1A is essential for normal cell cycle progression, DOT1B is involved in differentiation and control of antigenic variation of

this protozoan parasite. Analysis of DOT1A and DOT1B in trypanosomes or *in vitro*, to understand how H3K76 methylation is controlled during the cell cycle, is complicated by the lack of genetic tools and biochemical assays. To eliminate these problems, we developed a heterologous expression system in yeast. Whereas *Trypanosoma brucei* DOT1A predominantly dimethylated H3K79, DOT1B trimethylated H3K79 even in the absence of dimethylation by DOT1A. Furthermore, DOT1A activity was selectively reduced by eliminating ubiquitylation of H2B. The tail of histone H4 was not required for activity of DOT1A or DOT1B. These findings in yeast provide new insights into possible mechanisms of regulation of H3K76 methylation in *Trypanosoma brucei*.

15575. **Glover, L., Jun, J. & Horn, D.,** 2010. Microhomology-mediated deletion and gene conversion in African trypanosomes. *Nucleic Acids Research*. **Advanced access, published October 21.**

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

Antigenic variation in African trypanosomes is induced by DNA double-strand breaks (DSBs). In these protozoan parasites, DSB repair (DSBR) is dominated by homologous recombination (HR) and microhomology-mediated end joining (MMEJ), while non-homologous end joining (NHEJ) has not been reported. To facilitate the analysis of chromosomal end-joining, we established a system whereby inter-allelic repair by HR is lethal due to loss of an essential gene. Analysis of intrachromosomal end joining in individual DSBR survivors exclusively revealed MMEJ-based deletions but no NHEJ. A survey of microhomologies typically revealed sequences of between five and 20 bp in length with several mismatches tolerated in longer stretches. Mean deletions were of 54 bp on the side closest to the break and 284 bp in total. Break proximity, microhomology length and GC-content all favoured repair and the pattern of MMEJ described above was similar at several different loci across the genome. We also identified interchromosomal gene conversion involving HR and MMEJ at different ends of a duplicated sequence. While MMEJ-based deletions were RAD51-independent, one-sided MMEJ was RAD51 dependent. Thus, we describe the features of MMEJ in *Trypanosoma brucei*, which is analogous to micro single-strand annealing; and RAD51 dependent, one-sided MMEJ. We discuss the contribution of MMEJ pathways to genome evolution, subtelomere recombination and antigenic variation.

15576. **Goodhead, I., Archibald, A., Amwayi, P., Brass, A., Gibson, J., Hall, N., Hughes, M. A., Limo, M., Iraqi, F., Kemp, S. J. & Noyes, H. A.,** 2010. A comprehensive genetic analysis of candidate genes regulating response to *Trypanosoma congolense* infection in mice. *PLoS Neglected Tropical Diseases*, **4** (11): e880.

Centre for Genomic Research, School of Biological Sciences, University of Liverpool, Liverpool, UK. [harry@liv.ac.uk].

African trypanosomes are protozoan parasites that cause "sleeping sickness" in humans and a similar disease in livestock. Trypanosomes also infect laboratory mice and three major quantitative trait loci (QTL) that regulate survival time after infection with *T. congolense* have been identified in two independent crosses between susceptible A/J and BALB/c mice, and the resistant C57BL/6. These were designated *Tir1*, *Tir2* and *Tir3* for *Trypanosoma*

infection response, and range in size from 0.9-12 cM. Mapping loci regulating survival time after *T. congolense* infection in an additional cross revealed that susceptible C3H/HeJ mice have alleles that reduce survival time after infection at *Tir1* and *Tir3* QTL, but not at *Tir2*. Next-generation resequencing of a 6.2 Mbp region of mouse chromosome 17, which includes *Tir1*, identified 1 632 common single nucleotide polymorphisms (SNP) including a probably damaging non-synonymous SNP in *Pram1* (PML-RAR alpha-regulated adaptor molecule 1), which was the most plausible candidate QTL gene in *Tir1*. Genome-wide comparative genomic hybridisation identified 12 loci with copy number variants (CNV) that correlate with differential gene expression, including *Cd244* (natural killer cell receptor 2B4), which lies close to the peak of *Tir3c* and has gene expression that correlates with CNV and phenotype, making it a strong candidate QTL gene at this locus. By systematically combining next-generation DNA capture and sequencing, array-based comparative genomic hybridisation (aCGH), gene expression data and SNP annotation we have developed a strategy that can generate a short list of polymorphisms in candidate QTL genes that can be functionally tested.

15577. **Gupta, S., Cordeiro, A. T. & Michels, P. A.,** 2010. Glucose-6-phosphate dehydrogenase is the target for the trypanocidal action of human steroids. *Molecular & Biochemical Parasitology*. **In press, corrected proof.**

Research Unit for Tropical Diseases, de Duve Institute, TROP 74.39, Avenue Hippocrate 74, B-1200 Brussels, Belgium. [shree.chemistry@gmail.com].

Steroids such as dehydroepiandrosterone (DHEA) and epiandrosterone (EA) exert multiple effects in mammals including the inhibition of glucose-6-phosphate dehydrogenase (G6PDH). Initially, the inhibition was considered specific for the mammalian enzyme. The beneficial effect of these steroids on infections by protists and nematodes was attributed to stimulation of the immune system. However, we showed previously that DHEA and EA also inhibit *Trypanosoma brucei* and *T. cruzi* G6PDH, with low μM K_i values, but not the enzyme from *Leishmania* species, and kill *in vitro* cultured trypanosomes. We report here that, contrary to wild-type trypanosomes, mutant bloodstream-form *T. brucei* cells expressing *L. mexicana* G6PDH are not susceptible to the steroids, proving that G6PDH is the *in situ* target. Moreover, bromo-derivatives of the steroids show 50-100 fold lower K_i values for the enzyme and display an increased potency to kill the parasites. Therefore, the compounds offer promise for use in development of parasite-selective drugs.

15578. **Hoog, J. L., Gluenz, E., Vaughan, S. & Gull, K.,** 2010. Ultrastructural investigation methods for *Trypanosoma brucei*. *Methods in Cell Biology*, **96**: 175-196.

Sir William Dunn School of Pathology, University of Oxford, Oxford OX13RE, UK.

Trypanosoma brucei is a unicellular parasite causing African sleeping sickness in cattle and humans. Due to the ease with which these cells can be cultured and genetically manipulated, it has emerged as a model organism for the kinetoplastids. In this chapter we describe the preparation of *T. brucei* for transmission electron microscopy. A thorough explanation of conventional sample preparation through chemical fixation of whole cells and detergent extracted cytoskeletons followed by dehydration and Epon embedding is given. We

also introduce a novel high-pressure freezing protocol, which followed by rapid freeze substitution and HM20 embedding generates *T. brucei* samples displaying good cell morphology, which are suitable for immunocytochemistry.

15579. **Igoillo-Esteve, M., Mazet, M., Deumer, G., Wallemacq, P. & Michels, P. A.,** 2010. Glycosomal ABC transporters of *Trypanosoma brucei*: Characterization of their expression, topology and substrate specificity. *International Journal of Parasitology*. **In press, corrected proof.**

Research Unit for Tropical Diseases, de Duve Institute and Laboratory of Biochemistry, Université catholique de Louvain, TROP 74.39, Avenue Hippocrate 74, B-1200 Brussels, Belgium. [paul.michels@uclouvain.be].

Metabolism in trypanosomatids is compartmentalized with major pathways, notably glycolysis, present in peroxisome-like organelles called glycosomes. To date, little information is available about the transport of metabolites through the glycosomal membrane. Previously, three ATP-binding cassette (ABC) transporters, called GAT1-3 for Glycosomal ABC Transporters 1 to 3, have been identified in the glycosomal membrane of *Trypanosoma brucei*. Here we report that GAT1 and GAT3 are expressed both in bloodstream and procyclic form trypanosomes, whereas GAT2 is mainly or exclusively expressed in bloodstream-form cells. Protease protection experiments showed that the nucleotide-binding domain of GAT1 and GAT3 is exposed to the cytosol, indicating that these transporters mediate the ATP-dependent uptake of solutes from the cytosol into the glycosomal lumen. Depletion of GAT1 and GAT3 by RNA interference in procyclic cells grown in glucose-containing medium did not affect growth. Surprisingly, GAT1 depletion enhanced the expression of the very different GAT3 protein. Expression knockdown of GAT1, but not GAT3, in procyclic cells cultured in glucose-free medium was lethal. Depletion of GAT1 in glucose-grown procyclic cells caused a modification of the total cellular fatty-acid composition. No or only minor changes were observed in the levels of most fatty acids, including oleate (C18:1), nevertheless the linoleate (C18:2) abundance was significantly increased upon GAT1 silencing. Furthermore, glycosomes purified from procyclic wild-type cells incorporate oleoyl-CoA in a concentration- and ATP-dependent manner, whilst this incorporation was severely reduced in glycosomes from cells in which GAT1 levels had been decreased. Together, these results strongly suggest that GAT1 serves to transport primarily oleoyl-CoA, but possibly also other fatty acids, from the cytosol into the glycosomal lumen and that its depletion results in a cellular linoleate accumulation, probably due to the presence of an active oleate desaturase. The role of intraglycosomal oleoyl-CoA and its essentiality when the trypanosomes are grown in the absence of glucose, are discussed.

15580. **Inoue, M., Yasuda, K., Uemura, H., Yasaka, N., Inoue, H., Sei, Y., Horikoshi, N. & Fukuma, T.,** 2010. Phosphorylation-dependent protein interaction with *Trypanosoma brucei* 14-3-3 proteins that display atypical target recognition. *PLoS One*, **5** (12): e15566.

Division of Eukaryotic Microbiology, Department of Infectious Medicine, Kurume University School of Medicine, Kurume, Japan. [inouedna@med.kurume-u.ac.jp].

The 14-3-3 proteins are structurally conserved throughout eukaryotes and participate in protein kinase signalling. All 14-3-3 proteins are known to bind to evolutionally conserved phosphoserine-containing motifs (modes 1 and/or 2) with high affinity. In *Trypanosoma brucei*, 14-3-3I and II play pivotal roles in motility, cytokinesis and the cell cycle. However, none of the *T. brucei* 14-3-3 binding proteins have previously been documented. Initially we showed that *T. brucei* 14-3-3 proteins exhibit far lower affinity to those peptides containing RSxpSxP (mode 1) and RxY/FxpSxP (mode 2) (where x is any amino acid residue and pS is phosphoserine) than human 14-3-3 proteins, demonstrating the atypical target recognition by *T. brucei* 14-3-3 proteins. We found that the putative *T. brucei* protein phosphatase 2C (PP2c) binds to *T. brucei* 14-3-3 proteins utilizing its mode 3 motif (-pS/pTx(1-2)-COOH, where x is not Pro). We constructed eight chimeric PP2c proteins replacing its authentic mode 3 motif with potential mode 3 sequences found in the *Trypanosoma brucei* genome database, and tested their binding. As a result, *T. brucei* 14-3-3 proteins interacted with three out of eight chimeric proteins including two with high affinity. Importantly, *T. brucei* 14-3-3 proteins co-immunoprecipitated with an uncharacterized full-length protein containing identified high-affinity mode 3 motif, suggesting that both proteins form a complex *in vivo*. In addition, a synthetic peptide derived from this mode 3 motif binds to *T. brucei* 14-3-3 proteins with high affinity. Because of the atypical target recognition of *T. brucei* 14-3-3 proteins, no 14-3-3-binding proteins have been successfully identified in *T. brucei* until now whereas over 200 human 14-3-3-binding proteins have been identified. This report describes the first discovery of the *T. brucei* 14-3-3-binding proteins and their binding motifs. The high-affinity phosphopeptide will be a powerful tool to identify novel *T. brucei* 14-3-3-binding proteins.

15581. **Kabututu, Z. P., Thayer, M., Melehani, J. H. & Hill, K. L.,** 2010. CMF70 is a subunit of the dynein regulatory complex. *Journal of Cell Science*, **123** (20): 3587-3595.

Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, CA 90095, USA.

Flagellar motility drives propulsion of several important pathogens and is essential for human development and physiology. Motility of the eukaryotic flagellum requires coordinate regulation of thousands of dynein motors arrayed along the axoneme, but the proteins underlying dynein regulation are largely unknown. The dynein regulatory complex, DRC, is recognized as a focal point of axonemal dynein regulation, but only a single DRC subunit, trypanin/PF2, is currently known. The component of motile flagella 70 protein, CMF70, is broadly and uniquely conserved among organisms with motile flagella, suggesting a role in axonemal motility. Here we demonstrate that CMF70 is part of the DRC from *Trypanosoma brucei*. CMF70 is located along the flagellum, co-sediments with trypanin in sucrose gradients and co-immunoprecipitates with trypanin. RNAi knockdown of CMF70 causes motility defects in a wild-type background and suppresses flagellar paralysis in cells with central pair defects, thus meeting the functional definition of a DRC subunit. Trypanin and CMF70 are mutually conserved in at least five of six extant eukaryotic clades, indicating that the DRC was probably present in the last common eukaryotic ancestor. We have identified only the second known subunit of this ubiquitous dynein regulatory system, highlighting the utility of combined genomic and functional analyses for identifying novel subunits of axonemal sub-complexes.

15582. **Kieft, R., Capewell, P., Turner, C. M., Veitch, N. J., MacLeod, A. & Hajduk, S.,** 2010. Mechanism of *Trypanosoma brucei gambiense* (group 1) resistance to human trypanosome lytic factor. *Proceedings of the National Academy of Sciences USA*, **107** (37): 16137-16141.

Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA. [gvwa08@udcf.gla.ac.uk].

Human innate immunity against most African trypanosomes, including *Trypanosoma brucei*, is mediated by a minor subclass of toxic serum HDL, called trypanosome lytic factor-1 (TLF-1). This HDL contains two primate specific proteins, apolipoprotein L-1 and haptoglobin (Hp)-related protein, as well as apolipoprotein A-1. These assembled proteins provide a powerful defence against trypanosome infection. *Trypanosoma brucei rhodesiense* causes human African sleeping sickness because it has evolved an inhibitor of TLF-1, serum resistance-associated (SRA) protein. *Trypanosoma brucei gambiense* lacks the SRA gene, yet it infects humans. As transfection of *T. b. gambiense* (group 1) is not possible, we initially used *in vitro*-selected TLF-1-resistant *T. b. brucei* to examine SRA-independent mechanisms of TLF-1 resistance. Here we show that TLF-1 resistance in *T. b. brucei* is caused by reduced expression of the Hp/Hb receptor gene (TbbHpHbR). Importantly, *T. b. gambiense* (group 1) also showed a marked reduction in uptake of TLF-1 and a corresponding decrease in expression of *T. b. gambiense* Hp/Hb receptor (TbgHpHbR). Ectopic expression of TbbHpHbR in TLF-1-resistant *T. b. brucei* rescued TLF-1 uptake, demonstrating that decreased TbbHpHbR expression conferred TLF-1 resistance. Ectopic expression of TbgHpHbR in TLF-1-resistant *T. b. brucei* failed to rescue TLF-1 killing, suggesting that coding sequence changes altered Hp/Hb receptor binding affinity for TLF-1. We propose that the combination of coding sequence mutations and decreased expression of TbgHpHbR directly contribute to parasite evasion of human innate immunity and infectivity of group 1 *T. b. gambiense*.

15583. **Koestler, T., von Haeseler, A. & Ebersberger, I.,** 2010. FACT: functional annotation transfer between proteins with similar feature architectures. *BMC Bioinformatics*, **11**: 417.

Center for Integrative Bioinformatics Vienna, Max F Perutz Laboratories, Dr Bohrgasse 9, A-1030 Vienna, Austria.

The increasing number of sequenced genomes provides the basis for exploring the genetic and functional diversity within the tree of life. Only a tiny fraction of the encoded proteins undergoes a thorough experimental characterization. For the remainder, bioinformatics annotation tools are the only means to infer their function. Exploiting significant sequence similarities to already characterized proteins, commonly taken as evidence for homology, is the prevalent method to deduce functional equivalence. Such methods fail when homologues are too diverged, or when they have assumed a different function. Finally, due to convergent evolution, functional equivalence is not necessarily linked to common ancestry. Therefore complementary approaches are required to identify functional equivalents. We present the Feature Architecture Comparison Tool <http://www.cibiv.at/FACT> to search for functionally equivalent proteins. FACT uses the

similarity between feature architectures of two proteins, i.e., the arrangements of functional domains, secondary structure elements, and compositional properties, as a proxy for their functional equivalence. A scoring function measures feature architecture similarities, which enables searching for functional equivalents in entire proteomes. Our evaluation of 9 570 EC classified enzymes revealed that FACT, using the full feature set outperformed the existing architecture-based approaches by identifying significantly more functional equivalents as highest scoring proteins. We show that FACT can identify functional equivalents that share no significant sequence similarity. However, when the highest scoring protein of FACT is also the protein with the highest local sequence similarity, it is in 99 percent of the cases functionally equivalent to the query. We demonstrate the versatility of FACT by identifying a missing link in the yeast glutathione metabolism and also by searching for the human Golga5 equivalent in *Trypanosoma brucei*. In conclusion, FACT facilitates a quick and sensitive search for functionally equivalent proteins in entire proteomes. FACT is complementary to approaches using sequence similarity to identify proteins with the same function. Thus, FACT is particularly useful when functional equivalents need to be identified in evolutionarily distant species, or when functional equivalents are not homologous. The most reliable annotation transfers, however, are achieved when feature architecture similarity and sequence similarity are jointly taken into account.

15584. **Kolev, N. G., Franklin, J. B., Carmi, S., Shi, H., Michaeli, S. & Tschudi, C.,** 2010. The transcriptome of the human pathogen *Trypanosoma brucei* at single-nucleotide resolution. *PLoS Pathogens*, **6** (9): e1001090.

School of Public Health, Yale University, New Haven, Connecticut, USA.
[christian.tschudi@yale.edu].

The genome of *Trypanosoma brucei*, the causative agent of African trypanosomiasis, was published five years ago, yet identification of all genes and their transcripts remains to be accomplished. Annotation is challenged by the organization of genes transcribed by RNA polymerase II (Pol II) into long unidirectional gene clusters with no knowledge of how transcription is initiated. Here we report a single-nucleotide resolution genomic map of the *T. brucei* transcriptome, adding 1 114 new transcripts, including 103 non-coding RNAs, confirming and correcting many of the annotated features and revealing an extensive heterogeneity of 5' and 3' ends. Some of the new transcripts encode polypeptides that are either conserved in *T. cruzi* and *Leishmania major* or were previously detected in mass spectrometry analyses. High-throughput RNA sequencing (RNA-Seq) was sensitive enough to detect transcripts at putative Pol II transcription initiation sites. Our results, as well as recent data from the literature, indicate that transcription initiation is not solely restricted to regions at the beginning of gene clusters, but may occur at internal sites. We also provide evidence that transcription at all putative initiation sites in *T. brucei* is bidirectional, a recently recognized fundamental property of eukaryotic promoters. Our results have implications for gene expression patterns in other important human pathogens with similar genome organization (*Trypanosoma cruzi*, *Leishmania* sp.) and revealed heterogeneity in pre-mRNA processing that could potentially contribute to the survival and success of the parasite population in the insect vector and the mammalian host.

15585. **Kramer, S., Kimblin, N. C. & Carrington, M.**, 2010. Genome-wide *in silico* screen for CCCH-type zinc finger proteins of *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*. *BMC Genomics*, **11**: 283. doi:10.1186/1471-2164-11-283.

Department of Biochemistry, University of Cambridge, Cambridge, UK. [sk503@cam.ac.uk].

CCCH type zinc finger proteins are RNA binding proteins with regulatory functions at all stages of mRNA metabolism. The best-characterized member, tritetrarproline (TTP), binds to AU rich elements in 3' UTRs of unstable RNAs, mediating their degradation. In kinetoplastids, CCCH type zinc finger proteins have been identified as being involved in the regulation of the life cycle and possibly the cell cycle. To date, no systematic listing of CCCH proteins in kinetoplastids is available. We have identified the complete set of CCCH type zinc finger proteins in the available genomes of the kinetoplastid protozoa *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*. One fifth (20 percent) of all CCCH motifs fall into non-conventional classes and many had not been previously identified. One third of all CCCH proteins have more than one CCCH motif, suggesting multivalent RNA binding. One third have additional recognizable domains. The vast majority are unique to Kinetoplastida or to a subgroup within. Two exceptions are of interest: the putative orthologue of the mRNA nuclear export factor Mex67 and a 3'-5' exoribonuclease restricted to *Leishmania* species. CCCH motifs are absent from these proteins in other organisms and might be unique, novel features of the Kinetoplastida homologues. Of the others, several have a predicted, and in one case experimentally confirmed, connection to the ubiquitination pathways, for instance a HECT-type E3 ubiquitin ligase. The total number of kinetoplastid CCCH proteins is similar to the number in higher eukaryotes but lower than in yeast. A comparison of the genomic loci between the Trypanosomatidae homologues provides insight into both the evolution of the CCCH proteins as well as the CCCH motifs. This study provides the first systematic listing of the Kinetoplastida CCCH proteins. The number of CCCH proteins with more than one CCCH motif is larger than previously estimated, due to the identification of non-conventional CCCH motifs. Experimental approaches are now necessary to examine the functions of the many unique CCCH proteins as well as the function of the putative Mex67 and the *Leishmania* 3'-5' exoribonuclease.

15586. **Lacomble, S., Vaughan, S., Gadelha, C., Morphew, M. K., Shaw, M. K., McIntosh, J. R. & Gull, K.**, 2010. Basal body movements orchestrate membrane organelle division and cell morphogenesis in *Trypanosoma brucei*. *Journal of Cell Science*, **123** (17): 2884-2891.

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

The defined shape and single-copy organelles of *Trypanosoma brucei* mean that it provides an excellent model in which to study how duplication and segregation of organelles is interfaced with morphogenesis of overall cell shape and form. The centriole or basal body of eukaryotic cells is often seen to be at the centre of such processes. We have used a combination of electron microscopy and electron tomography techniques to provide a detailed three-dimensional view of duplication of the basal body in trypanosomes. We show that the basal body duplication and maturation cycle exerts an influence on the intimately

associated flagellar pocket membrane system that is the portal for secretion and uptake from this cell. At the start of the cell cycle, a probasal body is positioned anterior to the basal body of the existing flagellum. At the G1-S transition, the probasal body matures, elongates and invades the pre-existing flagellar pocket to form the new flagellar axoneme. The new basal body undergoes a spectacular anti-clockwise rotation around the old flagellum, while its short new axoneme is associated with the pre-existing flagellar pocket. This rotation and subsequent posterior movements result in division of the flagellar pocket and ultimately sets parameters for subsequent daughter cell morphogenesis.

15587. **Lee, J. H., Cai, G., Panigrahi, A. K., Dunham-Ems, S., Nguyen, T. N., Radolf, J. D., Asturias, F. J. & Gunzl, A.,** 2010. A TFIIF-associated mediator head is a basal factor of small nuclear spliced leader RNA gene transcription in early-diverged trypanosomes. *Molecular & Cellular Biology*, **30** (23): 5502-5513.

Department of Genetics and Developmental Biology, University of Connecticut Health Center, 400 Farmington Avenue, Farmington, CT 06030-6403, USA. [gunzl@uchc.edu].

Genome annotation suggested that early-diverged kinetoplastids possess a reduced set of basal transcription factors. More recent work, however, on the lethal parasite *Trypanosoma brucei* identified extremely divergent orthologues of TBP, TFIIA, TFIIB, and TFIIF which, together with the small nuclear RNA-activating protein complex, form a transcription preinitiation complex (PIC) at the spliced leader (SL) RNA gene (SLRNA) promoter. The SL RNA is a small nuclear RNA and a trans splicing substrate for the maturation of all pre-mRNAs which is metabolized continuously to sustain gene expression. Here, we identified and biochemically characterized a novel TFIIF-associated protein complex in *T. brucei* (Med-T) consisting of nine subunits whose amino acid sequences are conserved only among kinetoplastid organisms. Functional analyses *in vivo* and *in vitro* demonstrated that the complex is essential for cell viability, SLRNA transcription, and PIC integrity. Molecular structure analysis of purified Med-T and Med-T/TFIIF complexes by electron microscopy revealed that Med-T corresponds to the mediator head module of higher eukaryotes. These data therefore show that mediator is a basal factor for small nuclear SL RNA gene transcription in trypanosomes and that the basal transcription function of mediator head is a characteristic feature of eukaryotes which developed early in their evolution.

15588. **Liang, S. & Connell, G. J.,** 2010. Identification of specific inhibitors for a trypanosomatid RNA editing reaction. *Rna*, **16** (12): 2435-2441.

Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455, USA. [conne018@umn.edu].

Several mitochondrial mRNAs of the trypanosomatid protozoa are edited through the post-transcriptional insertion and deletion of uridylates. The reaction has provided insights into basic cellular biology and is also important as a potential therapeutic target for the diseases caused by trypanosomatid pathogens. Despite this importance, the field has been hindered by the lack of specific inhibitors that could be used as probes of the reaction mechanism or developed into novel therapeutics. In this study, an electrochemiluminescent aptamer-switch was utilized in a high-throughput screen for inhibitors of a trypanosomatid

RNA editing reaction. The screen identified GW5074, mitoxantrone, NF 023, protoporphyrin IX, and D-sphingosine as inhibitors of insertion editing, with IC_{50} values ranging from 1 to 3 μ M. GW5074 and protoporphyrin IX are demonstrated to inhibit at or before the endonuclease cleavage that initiates editing and will be valuable biochemical probes for the early events of the *in vitro* reaction. Since protoporphyrin IX and sphingosine are both naturally present within the trypanosomatids, their effectiveness as *in vitro* inhibitors is also suggestive of the potential for *in vivo* modulatory roles.

15589. **Luder, C. G., Campos-Salinas, J., Gonzalez-Rey, E. & van Zandbergen, G.,** 2010. Impact of protozoan cell death on parasite-host interactions and pathogenesis. *Parasites & Vectors*, **3**: 116. doi:10.1186/1756-3305-3-116.

Institute for Medical Microbiology, Georg-August-University, Kreuzbergerring 57, 37075 Gottingen, Germany. [clueder@gwdg.de].

PCD in protozoan parasites has emerged as a fascinating field of parasite biology. This not only relates to the underlying mechanisms and their evolutionary implications but also to the impact on the parasite-host interactions within mammalian hosts and arthropod vectors. During recent years, common functions of apoptosis and autophagy in protozoa and during parasitic infections have emerged. Here, we review how distinct cell death pathways in *Trypanosoma*, *Leishmania*, *Plasmodium* or *Toxoplasma* may contribute to regulation of parasite cell densities in vectors and mammalian hosts, to differentiation of parasites, to stress responses, and to modulation of the host immunity. The examples provided indicate crucial roles of PCD in parasite biology. The existence of PCD pathways in these organisms and the identification as being critical for parasite biology and parasite-host interactions could serve as a basis for developing new anti-parasitic drugs that take advantage of these pathways.

15590. **Luginbuehl, E., Ryter, D., Schranz-Zumkehr, J., Oberholzer, M., Kunz, S. & Seebeck, T.,** 2010. The N terminus of phosphodiesterase TbrPDEB1 of *Trypanosoma brucei* contains the signal for integration into the flagellar skeleton. *Eukaryotic Cell*, **9** (10): 1466-1475.

Institute of Cell Biology, University of Bern, Switzerland. [thomas.seebeck@izb.unibe.ch].

The precise subcellular localization of the components of the cyclic AMP (cAMP) signalling pathways is a crucial aspect of eukaryotic intracellular signalling. In the human pathogen *Trypanosoma brucei*, the strict control of cAMP levels by cAMP-specific phosphodiesterases is essential for parasite survival, both in cell culture and in the infected host. Among the five cyclic nucleotide phosphodiesterases identified in this organism, two closely related isoenzymes, *T. brucei* PDEB1 (TbrPDEB1) (PDEB1) and TbrPDEB2 (PDEB2) are predominantly responsible for the maintenance of cAMP levels. Despite their close sequence similarity, they are distinctly localized in the cell. PDEB1 is mostly located in the flagellum, where it forms an integral part of the flagellar skeleton. PDEB2 is mainly located in the cell body, and only a minor part of the protein localizes to the flagellum. The current study, using transfection of procyclic trypanosomes with green fluorescent protein (GFP) reporters, demonstrates that the N termini of the two enzymes are essential for determining their final subcellular localization. The first 70 amino acids of PDEB1 are

sufficient to specifically direct a GFP reporter to the flagellum and to lead to its detergent-resistant integration into the flagellar skeleton. In contrast, the analogous region of PDEB2 causes the GFP reporter to reside predominantly in the cell body. Mutagenesis of selected residues in the N-terminal region of PDEB2 demonstrated that single amino acid changes are sufficient to redirect the reporter from a cell body location to stable integration into the flagellar skeleton.

15591. **Maric, D., Epting, C. L. & Engman, D. M.**, Composition and sensory function of the trypanosome flagellar membrane. 2010. *Current Opinion in Microbiology*, **13** (4): 466-472.

Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA. [d-engman@northwestern.edu].

A cilium is an extension of the cell that contains an axonemal complex of microtubules and associated proteins bounded by a membrane which is contiguous with the cell body membrane. Cilia may be nonmotile or motile, the latter having additional specific roles in cell or fluid movement. The term flagellum refers to the motile cilium of free-living single cells (e.g. bacteria, archaea, spermatozoa, and protozoa). In eukaryotes, both nonmotile and motile cilia possess sensory functions. The ciliary interior (cilioplasm) is separated from the cytoplasm by a selective barrier that prevents passive diffusion of molecules between the two domains. The sensory functions of cilia reside largely in the membrane and signals generated in the cilium are transduced into a variety of cellular responses. In this review we discuss the structure and biogenesis of the cilium, with special attention to the trypanosome flagellar membrane, its lipid and protein composition and its proposed roles in sensing and signalling.

15592. **Marino, K., Guther, M. L., Wernimont, A. K., Amani, M., Hui, R. & Ferguson, M. A.**, 2010. Identification, subcellular localization, biochemical properties, and high-resolution crystal structure of *Trypanosoma brucei* UDP-glucose pyrophosphorylase. *Glycobiology*, **20** (12): 1619-1630.

Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK. [m.a.j.ferguson@dundee.ac.uk].

The protozoan parasite *Trypanosoma brucei* is the causative agent of the cattle disease Nagana and human African sleeping sickness. Glycoproteins play key roles in the parasite's survival and infectivity, and the *de novo* biosyntheses of the sugar nucleotides UDP-galactose (UDP-Gal), UDP-N-acetylglucosamine, and GDP-fucose have been shown to be essential for their growth. The only route to UDP-Gal in *T. brucei* is through the epimerization of UDP-glucose (UDP-Glc) by UDP-Glc 4'-epimerase. UDP-Glc is also the glucosyl donor for the unfolded glycoprotein glucosyltransferase (UGGT) involved in glycoprotein quality control in the endoplasmic reticulum and is the presumed donor for the synthesis of base J (beta-D-glucosylhydroxymethyluracil), a rare deoxynucleotide found in telomere-proximal DNA in the bloodstream form of *T. brucei*. Considering that UDP-Glc plays such a central role in carbohydrate metabolism, we decided to characterize UDP-Glc biosynthesis in *T. brucei*. We identified and characterized the parasite UDP-glucose pyrophosphorylase (TbUGP), responsible for the formation of UDP-Glc from glucose-1-phosphate and UTP, and localized the enzyme to the peroxisome-like glycosome organelles of the parasite. Recombinant

TbUGP was shown to be enzymatically active and specific for glucose-1-phosphate. The high-resolution crystal structure was also solved, providing a framework for the design of potential inhibitors against the parasite enzyme.

15593. **Martin, D. M., Nett, I. R., Vandermoere, F., Barber, J. D., Morrice, N. A. & Ferguson, M. A.**, 2010. Prophossi: automating expert validation of phosphopeptide-spectrum matches from tandem mass spectrometry. *Bioinformatics*, **26** (17): 2153-2159.

Division of Biological Chemistry and Drug Discovery, College of LifeSciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK. [d.m.a.martin@dundee.ac.uk].

Complex patterns of protein phosphorylation mediate many cellular processes. Tandem mass spectrometry (MS/MS) is a powerful tool for identifying these post-translational modifications. In high-throughput experiments, mass spectrometry database search engines, such as MASCOT provide a ranked list of peptide identifications based on hundreds of thousands of MS/MS spectra obtained in a mass spectrometry experiment. These search results are not in themselves sufficient for confident assignment of phosphorylation sites as identification of characteristic mass differences requires time-consuming manual assessment of the spectra by an experienced analyst. The time required for manual assessment has previously rendered high-throughput confident assignment of phosphorylation sites challenging. We have developed a knowledge base of criteria, which replicate expert assessment, allowing more than half of cases to be automatically validated and site assignments verified with a high degree of confidence. This was assessed by comparing automated spectral interpretation with careful manual examination of the assignments for 501 peptides above the 1 percent false discovery rate (FDR) threshold corresponding to 259 putative phosphorylation sites in 74 proteins of the *Trypanosoma brucei* proteome. Despite this stringent approach, we are able to validate 80 of the 91 phosphorylation sites (88 percent) positively identified by manual examination of the spectra used for the MASCOT searches with a FDR < 15 percent. High-throughput computational analysis can provide a viable second stage validation of primary mass spectrometry database search results. Such validation gives rapid access to a systems level overview of protein phosphorylation in the experiment under investigation. A GPL licensed software implementation in Perl for analysis and spectrum annotation is available in the supplementary material and a web server can be assessed online at <http://www.compbio.dundee.ac.uk/prophossi>.

15594. **Mijares, A., Concepcion, J. L., Vielma, J. R. & Portillo, R.**, 2010. Immune detection of acetylcholinesterase in subcellular compartments of *Trypanosoma evansi*. *Parasitology Research*, **108** (1): 1-5.

Centro de Biofísica y Bioquímica, Laboratorio de Fisiología de Parasitos, Instituto Venezolano de Investigaciones Científicas, A.P. 20632, Caracas 1020A, Venezuela.

Trypanosoma evansi is a worldwide distributed haemoparasite with a strong economic impact in veterinary activities. Despite widespread knowledge about the aetiology of the disease caused by *T. evansi*, there are few detailed studies about the metabolism of this

parasite. The aim of this study was to determine the presence of acetylcholinesterase (AChE) in *T. evansi* through a strategy of subcellular localization and confocal microscopy. The localization of the AChE by differential and isopycnic centrifugation strategy showed that this enzyme has a predominant localization in the glycosome, similar to hexokinase, and it is not present in either the cytosol or the plasma membrane. This study shows novel data that help to understand the non-neuronal role of AChE in the Trypanosomatidae family.

15595. **Moshiri, H. & Salavati, R.,** 2010. A fluorescence-based reporter substrate for monitoring RNA editing in trypanosomatid pathogens. *Nucleic Acids Research*, **38** (13): e138.

Institute of Parasitology, McGill University, 2111 Lakeshore Road, Ste. Anne de Bellevue, Montreal, Quebec H9X3V9, Canada. [reza.salavati@mcgill.ca].

RNA editing regulates mitochondrial gene expression in trypanosomatid pathogens by creating functional mRNAs. It is catalyzed by a multi-protein complex (the editosome), and is found to be essential in both insect stage and mammalian blood stream form of *Trypanosoma brucei*. This particular form of RNA editing is unique to trypanosomatids, and thus provides a suitable drug target in trypanosomatid pathogens. Here, we demonstrate the feasibility of a rapid and sensitive fluorescence-based reporter assay to monitor RNA editing based on ribozyme activity. We could validate our new assay using previously identified inhibitors against the essential RNA editing ligase. The principle advantages of this assay are: (i) the use of non-radioactively labelled materials, (ii) sensitivity afforded by fluorescence instrumentation applicable to high-throughput screening of chemical inhibitors against the essential editosome and (iii) a rapid and convenient 'mix and measure' type of assay in low volume with a high signal to noise ratio. This assay should enhance rapid identification and characterization of the editosome inhibitors primarily based on the overall composition of the editosomes from *T. brucei*. These inhibitors could also be tested against the editosomes from the closely related pathogens including *T. cruzi* and *Leishmania* species.

15596. **Narayanan, M. S., Kushwaha, M., Ersfeld, K., Fullbrook, A., Stanne, T. M. & Rudenko, G.,** 2010. NLP is a novel transcription regulator involved in VSG expression site control in *Trypanosoma brucei*. *Nucleic Acids Research*. **Published online, November 13.**

Division of Cell and Molecular Biology, Sir Alexander Fleming Building, Imperial College London, South Kensington, London SW7 2AZ, UK; Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK; and Department of Biological Sciences and Hull York Medical School, University of Hull, Cottingham Road, Hull HU6 7RX, UK. [gloria.rudenko@imperial.ac.uk].

Trypanosoma brucei mono-allelically expresses one of approximately 1500 variant surface glycoprotein (VSG) genes while multiplying in the mammalian bloodstream. The active VSG is transcribed by RNA polymerase I in one of approximately 15 telomeric VSG expression sites (ESs). *T. brucei* is unusual in controlling gene expression predominantly post-transcriptionally, and how ESs are mono-allelically controlled remains a mystery. Here we identify a novel transcription regulator, which resembles a nucleoplasm-like protein

(NLP) with an AT-hook motif. NLP is key for ES control in bloodstream form *T. brucei*, as NLP knockdown results in 45- to 65-fold derepression of the silent VSG221 ES. NLP is also involved in repression of transcription in the inactive VSG basic copy arrays, minichromosomes and procyclin loci. NLP is shown to be enriched on the 177- and 50-bp simple sequence repeats, the non-transcribed regions around rDNA and procyclin, and both active and silent ESs. Blocking NLP synthesis leads to downregulation of the active ES, indicating that NLP plays a role in regulating appropriate levels of transcription of ESs in both their active and silent state. Discovery of the unusual transcription regulator NLP provides new insight into the factors that are critical for ES control.

15597. **Nilsson, D., Gunasekera, K., Mani, J., Osteras, M., Farinelli, L., Baerlocher, L., Roditi, I. & Ochsenreiter, T.,** 2010. Spliced leader trapping reveals widespread alternative splicing patterns in the highly dynamic transcriptome of *Trypanosoma brucei*. *PLoS Pathogens*, **6** (8): e1001037.

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

Trans-splicing of leader sequences onto the 5'ends of mRNAs is a widespread phenomenon in protozoa, nematodes and some chordates. Using parallel sequencing we have developed a method to simultaneously map 5'splice sites and analyze the corresponding gene expression profile, that we term spliced leader trapping (SLT). The method can be applied to any organism with a sequenced genome and trans-splicing of a conserved leader sequence. We analyzed the expression profiles and splicing patterns of bloodstream and insect forms of the parasite *Trypanosoma brucei*. We detected the 5' splice sites of 85 percent of the annotated protein-coding genes and, contrary to previous reports, found up to 40 percent of transcripts to be differentially expressed. Furthermore, we discovered more than 2 500 alternative splicing events, many of which appear to be stage-regulated. Based on our findings we hypothesize that alternatively spliced transcripts present a new means of regulating gene expression and could potentially contribute to protein diversity in the parasite. The entire dataset can be accessed online at TriTrypDB or through: <http://splicer.unibe.ch/>.

15598. **Obado, S. O., Bot, C., Echeverry, M. C., Bayona, J. C., Alvarez, V. E., Taylor, M. C. & Kelly, J. M.,** 2010. Centromere-associated topoisomerase activity in bloodstream form *Trypanosoma brucei*. *Nucleic Acids Research*. **Published online, September 22.**

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK; Laboratorio de Parasitología - Facultad de Medicina, Universidad Nacional de Colombia-Sede, Bogotá, Colombia; and Instituto de Investigaciones Biotecnológicas, Universidad Nacional de General San Martín, Avenida General Paz 5445, INTI, Edificio 19, San Martín (1650), Buenos Aires, Argentina. [john.kelly@lshtm.ac.uk].

Topoisomerase-II accumulates at centromeres during prometaphase, where it resolves the DNA catenations that represent the last link between sister chromatids. Previously, using approaches including etoposide-mediated topoisomerase-II cleavage, we mapped centromeric

domains in trypanosomes, early branching eukaryotes in which chromosome segregation is poorly understood. Here, we show that in bloodstream form *Trypanosoma brucei*, RNAi-mediated depletion of topoisomerase-IIalpha, but not topoisomerase-IIbeta, results in the abolition of centromere-localized activity and is lethal. Both phenotypes can be rescued by expression of the corresponding enzyme from *T. cruzi*. Therefore, processes which govern centromere-specific topoisomerase-II accumulation/activation have been functionally conserved within trypanosomes, despite the long evolutionary separation of these species and differences in centromeric DNA organization. The variable carboxyl terminal region of topoisomerase-II has a major role in regulating biological function. We therefore generated *T. brucei* lines expressing *T. cruzi* topoisomerase-II truncated at the carboxyl terminus and examined activity at centromeres after the RNAi-mediated depletion of the endogenous enzyme. A region necessary for nuclear localization was delineated to six residues. In other organisms, sumoylation of topoisomerase-II has been shown to be necessary for regulated chromosome segregation. Evidence that we present here suggests that sumoylation of the *T. brucei* enzyme is not required for centromere-specific cleavage activity.

15599. **Ohshima, S., Ohashi-Suzuki, M., Miura, Y., Yabu, Y., Okada, N., Ohta, N. & Suzuki, T.**, 2010. TbUNC119 and its binding protein complex are essential for propagation, motility, and morphogenesis of *Trypanosoma brucei* procyclic form cells. *PLoS One*, **5** (12): e15577. [0897vip@tmd.ac.jp].

Department of Core Laboratory, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.

Flagellum-mediated motility of *Trypanosoma brucei* is considered to be essential for the parasite to complete stage development in the tsetse fly vector, while the mechanism by which flagellum-mediated motility is controlled is not fully understood. We thus compared *T. brucei* whole gene products (amino acid sequence) with *Caenorhabditis elegans* UNC (uncoordinated) proteins, in order to find uncharacterized motility-related *T. brucei* genes. Through *in silico* analysis, we found 88 gene products which were highly similar to *C. elegans* UNC proteins and categorized them as TbCEUN (*T. brucei* gene products which have high similarity to *C. elegans* UNC proteins). Approximately two thirds of the 88 TbCEUN gene products were kinesin-related molecules. A gene product highly similar to *C. elegans* UNC119 protein was designated as TbUNC119. RNAi-mediated depletion of TbUNC119 showed no apparent phenotype. However, knock-down analysis of both TbUNC119 and its binding protein (TbUNC119BP) which was found by yeast two-hybrid analysis showed characteristic phenotypes, including reduced motility, morphological change (extended cell shape), and cellular apoptosis. Based on the observed phenotypes, possible functions of the TbUNC119 and TbUNC119BP are discussed.

15600. **Paris, Z., Changmai, P., Rubio, M. A., Zikova, A., Stuart, K. D., Alfonzo, J. D. & Lukes, J.**, 2010. The Fe/S cluster assembly protein Isd11 is essential for tRNA thiolation in *Trypanosoma brucei*. *Journal of Biological Chemistry*, **285** (29): 22394-22402.

Biology Centre, Institute of Parasitology, Czech Academy of Sciences, and Faculty of Sciences, University of South Bohemia, 37005 Ceske Budejovice (Budweis), Czech Republic. [jula@paru.cas.cz].

Fe/S clusters are part of the active site of many enzymes and are essential for cell viability. In eukaryotes the cysteine desulphurase Nfs (IscS) donates the sulphur during Fe/S cluster assembly and was thought sufficient for this reaction. Moreover, Nfs is indispensable for tRNA thiolation, a modification generally required for tRNA function and protein synthesis. Recently, Isd11 was discovered as an integral part of the Nfs activity at an early step of Fe/S cluster assembly. Here we show, using a combination of genetic, molecular, and biochemical approaches, that Isd11, in line with its strong association with Nfs, is localized in the mitochondrion of *T. brucei*. In addition to its involvement in Fe/S assembly, Isd11 also takes part in both cytoplasmic and mitochondrial tRNA thiolation, whereas Mtu1, another protein proposed to collaborate with Nfs in tRNA thiolation, is required for this process solely within the mitochondrion. Taken together these data place Isd11 at the centre of these sulphur transactions and raise the possibility of a connection between Fe/S metabolism and protein synthesis, helping to integrate two seemingly unrelated pathways.

15601. **Price, H. P., Peltan, A., Stark, M. & Smith, D. F.,** 2010. The small GTPase ARL2 is required for cytokinesis in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology*, **173** (2): 123-131.

Centre for Immunology and Infection, Department of Biology/Hull York Medical School, University of York, York YO10 5YW, UK. [hp502@york.ac.uk].

The Arf-like (Arl) small GTPases have a diverse range of functions in the eukaryotic cell. Metazoan Arl2 acts as a regulator of microtubule biogenesis, binding to the tubulin-specific chaperone cofactor D. Arl2 also has a mitochondrial function through its interactions with BART and ANT-1, the only member of the Ras superfamily to be found in this organelle to date. In the present study, we describe characterization of the Arl2 orthologue in the protozoan parasite *Trypanosoma brucei*. Modulation of TbARL2 expression in bloodstream form parasites by RNA interference (RNAi) causes inhibition of cleavage furrow formation, resulting in a severe defect in cytokinesis and the accumulation of multinucleated cells. RNAi of TbARL2 also results in loss of acetylated alpha tubulin but not of total tubulin from cellular microtubules. While overexpression of TbARL2^{myc} also leads to a defect in cytokinesis, an excess of untagged protein has no effect on cell division, demonstrating the importance of the extreme C-terminus in correct function. TbARL2 overexpressing cells (either myc-tagged or untagged) have an increase in acetylated tubulin. Our data indicate that Arl2 has a fundamentally conserved role in trypanosome microtubule biogenesis that correlates with tubulin acetylation.

15602. **Reifur, L., Yu, L. E., Cruz-Reyes, J., Vanhartesvelt, M. & Koslowsky, D. J.,** 2010. The impact of mRNA structure on guide RNA targeting in kinetoplastid RNA editing. *PLoS One*, **5** (8): e12235.

Comparative Medicine and Integrative Biology Program, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, USA. [koslowsk@msu.edu].

Mitochondrial mRNA editing in *Trypanosoma brucei* requires the specific interaction of a guide RNA with its cognate mRNA. Hundreds of gRNAs are involved in the editing process, each needing to target their specific editing domain within the target message. We hypothesized that the structure surrounding the mRNA target may be a limiting factor and involved in the regulation process. In this study, we selected four mRNAs with distinct target structures and investigated how sequence and structure affected efficient gRNA targeting. Two of the mRNAs, including the ATPase subunit 6 and ND7-550 (5' end of NADH dehydrogenase subunit 7) that have open, accessible anchor binding sites show very efficient gRNA targeting. Electrophoretic mobility shift assays indicate that the cognate gRNA for ND7-550 had 10-fold higher affinity for its mRNA than the A6 pair. Surface plasmon resonance studies indicate that the difference in affinity was due to a four-fold faster association rate. As expected, mRNAs with considerable structure surrounding the anchor binding sites were less accessible and had very low affinity for their cognate gRNAs. *In vitro* editing assays indicate that efficient pairing is crucial for gRNA directed cleavage. However, only the A6 substrate showed gRNA-directed cleavage at the correct editing site. This suggests that different gRNA/mRNA pairs may require different "sets" of accessory factors for efficient editing. By characterizing a number of different gRNA/mRNA interactions, we may be able to define a "bank" of RNA editing substrates with different putative chaperone and other co-factor requirements. This will allow the more efficient identification and characterization of transcript specific RNA editing accessory proteins.

15603. **Ribeiro, C. M., Pontes, M. J., Bird, S., Chadzinska, M., Scheer, M., Verburg-van Kemenade, B. M., Savelkoul, H. F. & Wiegertjes, G. F., 2010.** Trypanosomiasis-induced Th17-like immune responses in carp. *PLoS One*, **5** (9): e13012.

Cell Biology and Immunology Group, Department of Animal Sciences,
Wageningen University, Wageningen, The Netherlands.
[geert.wiegertjes@wur.nl].

In mammalian vertebrates, the cytokine interleukin (IL)-12 consists of a heterodimer between p35 and p40 subunits whereas interleukin-23 is formed by a heterodimer between p19 and p40 subunits. During an immune response, the balance between IL-12 and IL-23 can depend on the nature of the pathogen associated molecular pattern (PAMP) recognized by, for example TLR2, leading to a preferential production of IL-23. IL-23 production promotes a Th17-mediated immune response characterized by the production of IL-17A/F and several chemokines, important for neutrophil recruitment and activation. For the cold blooded vertebrate common carp, only the IL-12 subunits have been described so far. Common carp is the natural host of two protozoan parasites: *Trypanoplasma borreli* and *Trypanosoma carassii*. We found that these parasites negatively affect p35 and p40a gene expression in carp. Transfection studies of HEK293 and carp macrophages show that *T. carassii*-derived PAMPs are agonists of carp TLR2, promoting p19 and p40c gene expression. The two protozoan parasites induce different immune responses as assessed by gene expression and histological studies. During *T. carassii* infections, in particular, we observed a propensity to induce p19 and p40c gene expression, suggestive of the formation of IL-23. Infections with *T. borreli* and *T. carassii* lead to an increase of IFN-gamma2 gene expression whereas IL-17A/F2 gene expression was only observed during *T. carassii* infections. The moderate increase in the number of splenic macrophages during *T. borreli* infection contrasts the marked increase in the number of splenic neutrophilic granulocytes during *T. carassii*

infection, along with an increased gene expression of metalloproteinase-9 and chemokines. This is the first study that provides evidence for a Th17-like immune response in fish in response to infection with a protozoan parasite.

15604. **Rudenko, G.**, 2010. Epigenetics and transcriptional control in African trypanosomes. *Essays in Biochemistry*, **48** (1): 201-219.

Division of Cell and Molecular Biology, Sir Alexander Fleming Building, Imperial College, South Kensington, London SW72AZ, UK. [gloria.rudenko@imperial.ac.uk].

The African trypanosome *Trypanosoma brucei* is a unicellular parasite which causes African sleeping sickness. Transcription in African trypanosomes displays some unusual features, as most of the trypanosome genome is transcribed as extensive polycistronic RNA Pol II (polymerase II) transcription units that are not transcriptionally regulated. In addition, RNA Pol I is used for transcription of a small subset of protein coding genes in addition to the rDNA (ribosomal DNA). These Pol I-transcribed protein coding genes include the VSG (variant surface glycoprotein) genes. Although a single trypanosome has many hundreds of VSG genes, the active VSG is transcribed in a strictly monoallelic fashion from one of approx. 15 telomeric VSG ESs (expression sites). Originally, it was thought that chromatin was not involved in the transcriptional control of ESs; however, this view is now being re-evaluated. It has since been shown that the active ES is depleted of nucleosomes compared with silent ESs. In addition, a number of proteins involved in chromatin remodelling or histone modification and which play a role in ES silencing (including TbISWI [*T. brucei* ISWI (imitation-switch protein)] and DOT1B) have recently been identified. Lastly, the telomere-binding protein TbRAP1 (*T. brucei* RAP1) has been shown to establish a repressive gradient extending from the ES telomere end up to the ES promoter. We still need to determine which epigenetic factors are involved in 'marking' the active ES as part of the counting mechanism of monoallelic exclusion. The challenge will come in determining how these multiple regulatory layers contribute to ES control.

15605. **Sharma, S., Singha, U. K. & Chaudhuri, M.**, Role of Tob55 on mitochondrial protein biogenesis in *Trypanosoma brucei*. 2010. *Molecular & Biochemical Parasitology*, **174** (2): 89-100.

Department of Microbiology and Immunology, School of Medicine, Meharry Medical College, Nashville, TN 37208, USA. [mchaudhuri@mmc.edu].

Mitochondrial outer membrane (MOM) proteins in parasitic protozoa like *Trypanosoma brucei* are poorly characterized. In fungi and higher eukaryotes, Tob55 is responsible for the assembly of beta-barrel proteins in the MOM. Here we show that *T. brucei* Tob55 (TbTob55) has considerable similarity in its primary and secondary structure to Tob55 from other species. TbTob55 is localized in *T. brucei* MOM and is essential for procyclic cell survival. Induction of Tob55 RNAi decreased the level of the voltage-dependent anion channel (VDAC) within 48 h. Although the primary effect is on VDAC, induction of TbTob55 RNAi for 96 h or more also decreased the levels of other nucleus encoded mitochondrial proteins. In addition, the mitochondrial membrane potential was reduced at this later time point possibly due to a reduction in the level of the proteins

involved in oxidative phosphorylation. However, mitochondrial structure was not altered due to depletion of Tob55. *In vitro* protein import of VDAC into mitochondria with a 50-60 percent reduction of TbTob55 was reduced about 40 percent in comparison to uninduced control. In addition, the import of presequence-containing proteins such as, cytochrome oxidase subunit 4 (COIV) and trypanosome alternative oxidase (TAO) was affected by about 20 percent under this condition. Depletion of VDAC levels by RNAi did not affect the import of either COIV or TAO. Furthermore, TbTob55 over expression increased the steady state level of VDAC as well as the level of the assembled protein complex of VDAC, suggesting that similar to other eukaryotes TbTob55 is involved in assembly of MOM beta-barrel proteins and plays an indirect role in the biogenesis of mitochondrial preproteins destined for the mitochondrial inner membrane.

15606. **Shateri Najafabadi, H. & Salavati, R.,** 2010. Functional genome annotation by combined analysis across microarray studies of *Trypanosoma brucei*. *PLoS Neglected Tropical Diseases*, **4** (8): e810.

Institute of Parasitology, McGill University, Ste Anne de Bellevue, Quebec, Canada. [reza.salavati@mcgill.ca].

Functional annotation of trypanosomatid genomes has been a daunting task due to the low similarity of their genes with annotated genes of other organisms. Three recent studies have provided gene expression profiles in several different conditions and life stages for one of the main disease-causing trypanosomatids, *Trypanosoma brucei*. These data can be used to study the gene functions and regulatory mechanisms in this organism. Combining the data from three different microarray studies of *T. brucei*, we show that functional linkages among *T. brucei* genes can be identified based on gene coexpression, leading to a powerful approach for gene function prediction. These predictions can be further improved by considering the expression profiles of orthologous genes from other trypanosomatids. Furthermore, gene expression profiles can be used to discover potential regulatory elements within 3' untranslated regions. These results suggest that although trypanosomatids do not regulate genes at transcription level, trypanosomatid genes with related functions are coregulated post-transcriptionally via modulation of mRNA stability, implying the presence of complex regulatory networks in these organisms. Our analysis highlights the demand for a thorough transcript profiling of *T. brucei* genome in parallel with other trypanosomatid genomes, which can provide a powerful means to improve their functional annotation.

15607. **Smirlis, D., Duszenko, M., Ruiz, A. J., Scoulica, E., Bastien, P., Fasel, N. & Soteriadou, K.,** 2010. Targeting essential pathways in trypanosomatids gives insights into protozoan mechanisms of cell death. *Parasites & Vectors*, **3**: 107.

Laboratory of Molecular Parasitology, Department of Microbiology, Hellenic Pasteur Institute, 127 Bas, Sofias Ave., 11521 Athens, Greece. [penny@pasteur.gr].

Apoptosis is a normal component of the development and health of multicellular organisms. However, apoptosis is now considered a prerogative of unicellular organisms, including the trypanosomatids of the genera *Trypanosoma* spp. and *Leishmania* spp., causative agents of some of the most important neglected human diseases. Trypanosomatids

show typical hallmarks of apoptosis, although they lack some of the key molecules contributing to this process in metazoans, like caspase genes, Bcl-2 family genes and the TNF-related family of receptors. Despite the lack of these molecules, trypanosomatids appear to have the basic machinery to commit suicide. The components of the apoptotic execution machinery of these parasites are slowly coming into light, by targeting essential processes and pathways with different apoptogenic agents and inhibitors. This review will be confined to the events known to drive trypanosomatid parasites to apoptosis.

15608. **Spitznagel, D., O'Rourke, J. F., Leddy, N., Hanrahan, O. & Nolan, D. P.,** 2010. Identification and characterization of an unusual class I myosin involved in vesicle traffic in *Trypanosoma brucei*. *PLoS One*, **5** (8): e12282.

Molecular Parasitology Group, School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland. [denolan@tcd.ie].

Myosins are a multimember family of motor proteins with diverse functions in eukaryotic cells. African trypanosomes possess only two candidate myosins and thus represent a useful system for functional analysis of these motors. One of these candidates is an unusual class I myosin (TbMyo1) that is expressed at similar levels but organized differently during the life cycle of *Trypanosoma brucei*. This myosin localizes to the polarized endocytic pathway in bloodstream forms of the parasite. This organization is actin dependent. Knock down of TbMyo1 results in a significant reduction in endocytic activity, a cessation in cell division and eventually cell death. A striking morphological feature in these cells is an enlargement of the flagellar pocket, which is consistent with an imbalance in traffic to and from the surface. In contrast, TbMyo1 is distributed throughout procyclic forms of the tsetse vector and a loss of approximately 90 percent of the protein has no obvious effects on growth or morphology. These results reveal a life cycle stage specific requirement for this myosin in essential endocytic traffic and represent the first description of the involvement of a motor protein in vesicle traffic in these parasites.

15609. **Tkacz, I. D., Gupta, S. K., Volkov, V., Romano, M., Haham, T., Tulinski, P., Lebenthal, I. & Michaeli, S.,** 2010. Analysis of spliceosomal proteins in trypanosomatids reveals novel functions in mRNA processing. *Journal of Biological Chemistry*, **285** (36): 27982-27999.

Mina and Everard Goodman Faculty of Life Sciences and Advanced Materials and Nanotechnology Institute, Bar-Ilan University, Ramat-Gan 52900, Israel. [michaes@mail.biu.ac.il].

In trypanosomatids, all mRNAs are processed via trans-splicing, although cis-splicing also occurs. In trans-splicing, a common small exon, the spliced leader (SL), which is derived from a small SL RNA species, is added to all mRNAs. Sm and Lsm proteins are core proteins that bind to U snRNAs and are essential for both these splicing processes. In this study, SmD3- and Lsm3-associated complexes were purified to homogeneity from *Leishmania tarentolae*. The purified complexes were analyzed by mass spectrometry, and 54 and 39 proteins were purified from SmD3 and Lsm complexes, respectively. Interestingly, among the proteins purified from Lsm3, no mRNA degradation factors were detected, as in Lsm complexes from other eukaryotes. The U1A complex was purified and identified by mass

spectrometry analysis, in addition to U1 small nuclear ribonucleoprotein (snRNP) proteins, additional co-purified proteins, including the polyadenylation factor CPSF73. Defects observed in cells silenced for U1 snRNP proteins suggest that the U1 snRNP functions exclusively in cis-splicing, although U1A also participates in polyadenylation and affects trans-splicing. The study characterized several trypanosome-specific nuclear factors involved in snRNP biogenesis, whose function was elucidated in *Trypanosoma brucei*. Conserved factors such as PRP19 which functions at the heart of every cis-spliceosome, also affect SL RNA modification; GEMIN2, a protein associated with SMN (survival of motor neurons) and implicated in selective association of U snRNA with core Sm proteins in trypanosomes, is a master regulator of snRNP assembly. This study demonstrates the existence of trypanosomatid-specific splicing factors but also that conserved snRNP proteins possess trypanosome-specific functions.

15610. **Vandemeulebroucke, A., Minici, C., Bruno, I., Muzzolini, L., Tornaghi, P., Parkin, D. W., Versees, W., Steyaert, J. & Degano, M., 2010.** Structure and mechanism of the 6-oxopurine nucleosidase from *Trypanosoma brucei brucei*. *Biochemistry*, **49** (41): 8999-9010.

Department of Molecular and Cellular Interactions and Structural Biology
Brussels, Vrije Universiteit Brussel, Brussels, Belgium.
[degano.massimo@hsr.it].

Trypanosomes are purine-auxotrophic parasites that depend upon nucleoside hydrolase (NH) activity to salvage nitrogenous bases necessary for nucleic acid and cofactor synthesis. Nonspecific and purine-specific NHs have been widely studied, yet little is known about the 6-oxopurine-specific isozymes, although they are thought to play a primary role in the catabolism of exogenously derived nucleosides. Here, we report the first functional and structural characterization of the inosine-guanosine-specific NH from *Trypanosoma brucei brucei*. The enzyme shows near diffusion-limited efficiency coupled with a clear specificity for 6-oxopurine nucleosides achieved through a catalytic selection of these substrates. Pre-steady-state kinetic analysis reveals ordered product release, and a rate-limiting structural rearrangement that is associated with the release of the product, ribose. The crystal structure of this trypanosomal NH determined to 2.5 Å resolution reveals distinctive features compared to those of both purine- and pyrimidine-specific isozymes in the framework of the conserved and versatile NH fold. Nanomolar iminoribitol-based inhibitors identified in this study represent important lead compounds for the development of novel therapeutic strategies against trypanosomal diseases.

15611. **Vincent, I. M., Creek, D., Watson, D. G., Kamleh, M. A., Woods, D. J., Wong, P. E., Burchmore, R. J. & Barrett, M. P., 2010.** A molecular mechanism for efloornithine resistance in African trypanosomes. *PLoS Pathogens*, **6** (11): e1001204.

University of Glasgow, Glasgow, UK. [m.barrett@bio.gla.ac.uk].

Human African trypanosomiasis, endemic to sub-Saharan Africa, is invariably fatal if untreated. Its causative agent is the protozoan parasite *Trypanosoma brucei*. Eflornithine is used as a first line treatment for human African trypanosomiasis, but there is a risk that resistance could thwart its use, even when used in combination therapy with nifurtimox.

Eflornithine resistant trypanosomes were selected *in vitro* and subjected to biochemical and genetic analysis. The resistance phenotype was verified *in vivo*. Here we report the molecular basis of resistance. While the drug's target, ornithine decarboxylase, was unaltered in resistant cells and changes to levels of metabolites in the targeted polyamine pathway were not apparent, the accumulation of eflornithine was shown to be diminished in resistant lines. An amino acid transporter gene, TbAAT6 (Tb927.8.5450), was found to be deleted in two lines independently selected for resistance. Ablating expression of this gene in wildtype cells using RNA interference led to acquisition of resistance while expression of an ectopic copy of the gene introduced into the resistant deletion lines restored sensitivity, confirming the role of TbAAT6 in eflornithine action. Eflornithine resistance is easy to select through loss of a putative amino acid transporter, TbAAT6. The loss of this transporter will be easily identified in the field using a simple PCR test, enabling more appropriate chemotherapy to be administered.

15612. **Wang, Q. P., Kawahara, T. & Horn, D.,** 2010. Histone deacetylases play distinct roles in telomeric VSG expression site silencing in African trypanosomes. *Molecular Microbiology*, **77** (5): 1237-1245.

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

African trypanosomes evade the host immune response through antigenic variation, which is achieved by periodically expressing different variant surface glycoproteins (VSGs). VSG expression is monoallelic such that only one of approximately 15 telomeric VSG expression sites (ESs) is transcribed at a time. Epigenetic regulation is involved in VSG control but our understanding of the mechanisms involved remains incomplete. Histone deacetylases are potential drug targets for diseases caused by protozoan parasites. Here, using recombinant expression we show that the essential *Trypanosoma brucei* deacetylases, DAC1 (class I) and DAC3 (class II) display histone deacetylase activity. Both DAC1 and DAC3 are nuclear proteins in the bloodstream stage parasite, while only DAC3 remains concentrated in the nucleus in insect-stage cells. Consistent with developmentally regulated localization, DAC1 antagonizes SIR2rp1-dependent telomeric silencing only in the bloodstream form, indicating a conserved role in the control of silent chromatin domains. In contrast, DAC3 is specifically required for silencing at VSG ES promoters in both bloodstream and insect-stage cells. We conclude that DAC1 and DAC3 play distinct roles in subtelomeric gene silencing and that DAC3 represents the first readily druggable target linked to VSG ES control in the African trypanosome.

15613. **Wickstead, B., Carrington, J. T., Gluenz, E. & Gull, K.,** 2010. The expanded kinesin-13 repertoire of trypanosomes contains only one mitotic kinesin indicating multiple extra-nuclear roles. *PLoS One*, **5** (11): e15020.

Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom. [bill.wickstead@path.ox.ac.uk].

Kinesin-13 proteins have a critical role in animal cell mitosis, during which they regulate spindle microtubule dynamics through their depolymerization activity. Much of what is known about kinesin-13 function emanates from a relatively small sub-family of proteins

containing MCAK and Kif2A/B. However, recent work on kinesins from the much more widely distributed, ancestral kinesin-13 family, which includes human Kif24, have identified a second function in flagellum length regulation that may exist either alongside or instead of the mitotic role. The African trypanosome *Trypanosoma brucei* encodes seven distinct kinesin-13 proteins, allowing scope for extensive specialization of roles. Here, we show that of all the trypanosomal kinesin-13 proteins, only one is nuclear. This protein, TbKIN13-1, is present in the nucleoplasm throughout the cell cycle, but associates with the spindle during mitosis, which in trypanosomes is closed. TbKIN13-1 is necessary for the segregation of both large and mini-chromosomes in this organism and reduction in TbKIN13-1 levels mediated by RNA interference causes defects in spindle disassembly with spindle-like structures persisting in non-mitotic cells. A second kinesin-13 is localised to the flagellum tip, but the majority of the kinesin-13 family members are in neither of these cellular locations. These data show that the expanded kinesin-13 repertoire of trypanosomes is not associated with diversification of spindle-associated roles. TbKIN13-1 is required for correct spindle function, but the extra-nuclear localization of the remaining paralogues suggests that the biological roles of the kinesin-13 family are wider than previously thought.

15614. **Zhang, X., Cui, J., Nilsson, D., Gunasekera, K., Chanfon, A., Song, X., Wang, H., Xu, Y. & Ochsenreiter, T.,** 2010. The *Trypanosoma brucei* MitoCarta and its regulation and splicing pattern during development. *Nucleic Acids Research*, **38** (21): 7378-7387.

Department of Biomedical Engineering, Nanjing University of Aeronautics and Astronautics, Nanjing, Jiangsu 210016 China. [xyn@bmb.uga.edu].

It has long been known that trypanosomes regulate mitochondrial biogenesis during the life cycle of the parasite; however, the mitochondrial protein inventory (MitoCarta) and its regulation remain unknown. We present a novel computational method for genome-wide prediction of mitochondrial proteins using a support vector machine-based classifier with approximately 90 percent prediction accuracy. Using this method, we predicted the mitochondrial localization of 468 proteins with high confidence and have experimentally verified the localization of a subset of these proteins. We then applied a recently developed parallel sequencing technology to determine the expression profiles and the splicing patterns of a total of 1065 predicted MitoCarta transcripts during the development of the parasite, and showed that 435 of the transcripts significantly changed their expressions while 630 remain unchanged in any of the three life stages analyzed. Furthermore, we identified 298 alternatively splicing events, a small subset of which could lead to dual localization of the corresponding proteins.

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