interpretations. Larger studies are needed to continue the evaluation of this drug combination in the treatment of *T. b. gambiense* sleeping sickness.

14075. **Robays, J., Lefevre, P., Lutumba, P., Lubanza, S., Kande Betu Ku Mesu, V., Van der Stuyft, P. & Boelaert, M., 2007**. Drug toxicity and cost as barriers to community participation in HAT control in the Democratic Republic of Congo. *Tropical Medicine and International Health*, **12** (2): 290-298.

Institute of Tropical Medicine, Epidemiology and Disease Control Unit, Antwerp, Belgium. [jrobays@itg.be].

Active case-finding programmes by mobile teams are the cornerstone of West African Human African Trypanosomiasis (HAT) control. Low attendance rates of screening and low uptake of treatment after diagnosis are major problems. The objectives of this survey were to explore community perception of HAT, to assess acceptability of control activities and to identify barriers amenable to intervention. In September 2004, we conducted 33 focus group discussions with beneficiaries of the HAT control programme among various ethnic groups in two ecological settings (savannah and fluvial) of the Democratic Republic of Congo. The population had a very detailed knowledge and understanding of HAT transmission, utility of screening, symptoms and treatment. Melarsoprol treatment was feared for its side effects. The sudden death of previously asymptomatic people during treatment was attributed to witchcraft, to which one becomes more vulnerable when the diagnosis is disclosed in public. Lack of confidentiality was also a problem because HAT carries a stigma as a mental disease. Lumbar punctures, especially when performed in public, were disliked but less feared. Financial barriers were a major obstacle for many patients. In conclusion, less toxic drugs, lowering financial barriers and improving confidentiality would have considerable impact on the participation in population screening for HAT.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

[See also **30**: 14053]

14076. **Kaare, M. T., Picozzi, K., Mlengeya, T., Fevre, E. M., Mellau, L. S., Mtambo, M. M., Cleaveland, S. & Welburn, S. C., 2007.** Sleeping sickness-a re-emerging disease in the Serengeti? *Travel Medicine and Infectious Disease*, **5** (2): 117-124.

Department of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture, P.O. Box 3021, Morogoro, Tanzania.

Sleeping sickness is a re-emerging disease in the Serengeti ecosystem affecting both local people and tourists. Here we report the results of a survey to assess the prevalence of

trypanosomiasis in both domestic and wild animals from this area. Five hundred and eighteen cattle samples were collected from 12 villages that bordered the Serengeti National Park and 220 samples from 15 different wild animal species were collected from within the park. PCR analysis, directed against the human serum resistance associated gene SRA, identified human infective *Trypanosoma brucei rhodesiense* parasites in both cattle and warthogs.

14077. **Maharjan, M. & Mishra, D. R., 2006**. Trypanosomiasis in domestic animals of Makwanpur district, Nepal. *Annals of the New York Academy of Sciences*, **1081**: 320-321.

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Trypanosomiasis is an infectious emerging haemoprotozoan parasitic disease in domestical animals of Nepal. The disease was found to be in 16 of 240 (6.67 percent) domestic animals of Makawanpur district, out of which 9 of 105 were (8.57 percent) cattle; 5 of 75 (6.67 percent) buffalos, and 2 of 15 (13.3 percent) dogs, while none of the goats and pigs acquired infection. The disease was found to be most prevalent during the rainy season when 9 of 82 (10.98 percent) were infected and its prevalence was higher among cross breeds than in local breeds.

14078. **Racloz, V., Griot, C. & Stark, K. D., 2006.** Sentinel surveillance systems with special focus on vector-borne diseases. *Animal Health Research Reviews*, **7** (1-2): 71-79.

Swiss Federal Veterinary Office, Schwarzenburgstrasse 155, 3003 Bern, Switzerland and Institute of Virology and Immunoprophylaxis, Mittelhäusern, Switzerland.

In the past few decades, vector-borne diseases have been spreading into countries previously free of these agents. It is necessary for a surveillance method to be tailored to the biology of these agents in order to detect their incursion. Using a sentinel herd system, it is possible to target high-risk areas where occurrence is most probably due to vector presence. Since the 1970s, diseases such as Akabane, vesicular stomatitis and Bluetongue disease have successfully been monitored using cattle herds as sentinels in many countries such as Saudi Arabia, Australia, China, Indonesia, Sultanate of Oman and most recently in countries in Western Europe. This paper reviews the strengths and weaknesses of sentinel herd surveillance systems in general. In order to determine their efficacy, the following criteria were found to be essential: the choice of sentinel locations, sentinel animal, seasonality of sampling and diagnostic testing methods. We conclude that due to its ability to focus on a specific disease, sentinel herd systems have been successful in the early detection of the spread of a targeted agent. This review is used as a basis for recommendations for the development of future sentinel herd systems.

14079. **Sehgal, R. N., Valkiunas, G., Iezhova, T. A. & Smith, T. B., 2006.** Blood parasites of chickens in Uganda and Cameroon with molecular descriptions of *Leucocytozoon schoutedeni* and *Trypanosoma gallinarum*. *Journal of*

Parasitology, **92 (**6): 1336-1343.

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

Using microscopy and PCR, we determined the prevalence of blood parasites in village chickens in Uganda and Cameroon. Of 148 individuals tested, 18.3 percent were infected with *Leucocytozoon schoutedeni* (Haemosporida, *Leucocytozoidae*) and 4.1 percent were infected with *Trypanosoma gallinarum* (Kinetoplastida, *Trypanosomatidae*). No other blood parasites were detected. Subsequent phylogenetic analysis of the cytochrome b gene of *L. schoutedeni* identified 2 distinct lineages that were found at all 3 sampling locations in Uganda. The sequence divergence between these 2 lineages is 1.5 percent. One of these lineages was also found in chickens in Cameroon, nearly 2,000 km distant. There are no morphological differences between blood stages of the parasites represented by the 2 different lineages, suggesting that cytochrome b gene sequence divergence can be as high as 1.5 percent within a single well-defined morphospecies of *Leucocytozoon*. We sequenced a portion of the small subunit ribosomal RNA gene (SSU rRNA) of *T. gallinarum*, and redescribe *T. gallinarum* for the first time since its discovery in 1911. These are the first assignments of DNA sequence data to these morphospecies of *Leucocytozoon* and *Trypanosoma* and may represent an example of intraspecific sequence divergence.

14080. **Ul Hasan, M., Muhammad, G., Gutierrez, C., Iqbal, Z., Shakoor, A. & Jabbar, A., 2006.** Prevalence of *Trypanosoma evansi* infection in equines and camels in the Punjab region, Pakistan. *Annals of the New York Academy of Sciences*, **1081**: 322-324.

Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan.

A cross-sectional study has been carried out in order to determine the prevalence of *Trypanosoma evansi* infection in susceptible hosts in the Punjab region (Pakistan). A total of 170 equines and 150 dromedary camels were examined. Five (3.3 percent) and 6 (4 percent) camels were positive at parasitological and serological examination, respectively. None of the equines tested positive at any method. These results seem to indicate that *T. evansi* infection has a relatively low prevalence in the Punjab region. However, efforts must be done in order to establish control measures in affected herds and avoid dissemination of the disease.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **30**: 14100, 14105]

14081**. Dia, M. L., 2006.** Parasites of the camel in Burkina Faso. *Tropical Animal Health and Production,* **38** (1): 17-21.

Laboratoire de Parasitologie, BP 167 Nouakchott, Mauritania. [mldsb@hotmail.com].

No abstract available.

14082. **Gonzales, J. L., Chacon, E., Miranda, M., Loza, A. & Siles, L. M., 2007.** Bovine trypanosomosis in the Bolivian Pantanal. *Veterinary Parasitology*, **146** (1-2): 9-16.

Laboratorio de Investigación y Diagnóstico Veterinario "LIDIVET", Av. Ejercito Nacional 153, P.O. Box 29, Santa Cruz, Bolivia.

Trypanosomosis caused by *Trypanosoma vivax* has been a constraint for cattle production in the Bolivian lowlands, since it was introduced in 1996. Flooded areas like the Bolivian Pantanal have a suitable environment for the presence and transmission of salivarian trypanosomes and farmers from that region often report trypanosomosis-like problems on their farms. The objective of the present study, therefore, was to characterize the epidemiology of bovine trypanosomosis in the Bolivian Pantanal. In order to achieve this objective, 202 cattle from the province of Angel Sandoval and 209 cattle from the province of German Busch were randomly sampled (the Pantanal is located in both provinces). Twenty-nine farms in both provinces were visited, the farmers interviewed, and biologic samples collected from their cattle. Samples were submitted for parasitological and PCR evaluation and the prevalence of bovine trypanosomosis was estimated for each province. Laboratory results were correlated with the sampled animals packed cell volume (PCV) and body condition (BC) scores and the observed *T. vivax* parasites measured for morphometry analysis. Results from this study show differences in morphometric measures between *T. vivax* parasites from each province. Differences between provinces were also observed in the *T. vivax*-related disease situation. While in Angel Sandoval the PCV and BC of *T. vivax*-affected animals were significantly lower than those of the *T. vivax*-negative animals, in German Busch no differences were observed in the PCV and BC of *T. vivax*-positive or negative animals. Animal prevalence of *T. vivax* in Angel Sandoval was 27.79 percent (95 percent CI: 14.52-44.28) and in German Busch was 19.03 percent (95 percent CI: 9.19-30.75). The *T. evansi* animal prevalence in each province was 0.99 percent (95 percent CI: 0.27-2.99) and 5.71 percent (95 percent CI: 2.43-12.19), respectively. Based on questionnaire and laboratory results, it was concluded that trypanosomosis is a primary constraint for cattle production in the Bolivian Pantanal.

14083. **Gutierrez, C., Corbera, J. A., Morales, M. & Buscher, P., 2006**. Trypanosomosis in goats: current status. *Annals of the New York Academy of Sciences*, **1081**: 300-310.

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Trypanosomosis is a major constraint on ruminant livestock production in Africa, Asia, and South America. The principal host species affected varies geographically, but buffalo, cattle, camels, and horses are particularly sensitive. Natural infections with *Trypanosoma congolense, T. vivax, T. brucei,* and *T. evansi* have been described in goats. Trypanosomosis in goats produces acute, subacute, chronic, or subclinical forms, *T. vivax, T. congolense* and *T. evansi* being the most invasive trypanosomes for goats. However, the role of goats in the epidemiology of trypanosomosis is largely discussed and not well understood.

Thus, it has commonly been assumed that trypanosomosis presents a subclinical course and that goats do not play an important role in the epidemiology of the disease. This can partially be due to parasitemia caused by trypanosomes which has been considered low in goats. However, this assumption is currently undergoing a critical reappraisal because of goats may also serve as a reservoir of trypanosome infection for other species, including the human beings in the case of *T. brucei rhodesiense*. The present article describes the current status of trypanosomosis in goats in Africa, Asia, and South America. Pathogenesis, clinical features, diagnosis, and treatment of the different trypanosomes are also described. The possible role of goats in the epidemiology of the disease in the different areas is also discussed.

14084. **Mochabo, M. O., Kitala, P. M., Gathura, P. B., Ogara, W. O., Eregae, E. M., Kaitho, T. D. & Catley, A., 2006**. The socio-economic impact of important camel diseases as perceived by a pastoralist community in Kenya. *Onderstepoort Journal of Veterinary Research*, **73** (4): 269-274.

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This paper presents the results of a study conducted in a pastoral community in Kenya using participatory appraisal approaches. The objective of the study was to assess the socio-economic impact of camel trypanosomosis (surra) according to the perceptions of the pastoralists. Four livestock grazing units were conveniently selected and in each of them, three groups of key informants comprising five to eight persons were selected for the participatory exercises. Five camel diseases were listed in order of importance according to their severity and frequency of occurrence including trypanosomosis, mange, non-specific diarrhoea, tick infestations and haemorrhagic septicaemia. The losses listed as incurred due to the five diseases were: losses in milk, meat, blood, fats and hides, dowry payments, and depreciation in sale of animals, losses due to infertility and abortions, and losses due to the cost of treatment. There was good agreement ($P < 0.05$) between the informant groups on the losses incurred as a result of the diseases for all the selected loss indicators. Surra and mange were given high median scores on all the indicators while non-specific diarrhoea, tick infestations, and haemorrhagic septicaemia received moderate median scores. Based on the study findings it is concluded that the camel plays a central role in the lives of Turkana pastoralists and that surra has a devastating social and economic impact. There is a need for veterinary and policy decision-makers to focus more attention on the control of surra in this arid and semi-arid area of Kenya.

14085**. Muhammad, G., Saqib, M., Sajid, M. S. & Naureen, A., 2007.** *Trypanosoma evansi* infections in Himalayan black bears (*Selenarctos thibetanus*). *Journal of Zoo and Wildlife Medicine*, **38**(1): 97-100.

Department of Clinical Medicine and Surgery, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.

The Asiatic or Himalayan black bear (*Selenarctos thibetanus*) is an endangered species. In South Asian countries, captive tamed Himalayan bears are commonly used by roving bear-charmers to entertain the people in rural and urban areas. In captivity, this species confronts

several psychophysical traumas and communicable diseases, which are prevalent in other domestic species. The present report describes four cases of *Trypanosoma evansi* infection in live Himalayan charming bears, which originated from the Faisalabad and Jhang districts of Pakistan. The condition was characterized by pyrexia, accelerated pulse, tachypnea, depression, anaemic mucous membranes, and ataxia (n = 3). Microscopic examination of peripheral blood films revealed moderate (n = 2) or high (n = 2) numbers of *T. evansi*. All four bears were treated twice at 3-day intervals with suramin sodium by using almost twice the dosage recommended for common domestic animals (10 mg/kg). The treated bears were found aparasitaemic on repeat blood testing on days 5, 7, and 10 post-treatment. No adverse effects were noted and all four cases recovered in 3-7 days after completion of the second round of treatment. One bear died 8 days after the second treatment (day 11). This is the first report of *T. evansi* in bears.

(c) TRYPANOTOLERANCE

14086. **Berthier, D., Chantal, I., Thevenon, S., Marti, J., Piquemal, D. & Maillard, J. C., 2006**. Bovine transcriptome analysis by SAGE technology during an experimental *Trypanosoma congolense* infection. *Annals of the New York Academy of Sciences*, **1081**: 286-299.

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In central and sub-Saharan Africa, trypanosomosis is a tsetse fly-transmitted disease, which is considered as the most important impediment to livestock production in the region. However, several indigenous West African taurine breeds (*Bos taurus*) present remarkable tolerance to the infection. This genetic capability, named trypanotolerance, results from numerous biological mechanisms most probably under multigenic dependences, among which are control of the trypanosome infection by limitation of parasitemia and control of severe anaemia due to the pathogenic effects. Today, some postgenomic biotechnologies, such as transcriptome analyses, allow characterization of the full expressed genes involved in the majority of animal diseases under genetic control. One of them is serial analysis of gene expression (SAGE) technology, which consists of the construction of mRNA transcript libraries for qualitative and quantitative analysis of the entire genes expressed or inactivated at a particular step of cellular activation. We developed four different mRNA transcript libraries from white blood cells on a N'Dama trypanotolerant animal during an experimental *Trypanosoma congolense* (*T. congolense*) infection: one before experimental infection (ND0), one at the parasitaemia peak (NDm), one at the minimal packed cell volume (NDa), and the last one at the end of the experiment after normalization (NDf). Bioinformatic comparisons in bovine genomic databases allowed us to obtain more than 75,000 sequences, among which are several known genes, some others are already described as expressed sequence tags (ESTs), and the last are completely new, but probably functional in trypanotolerance. The knowledge of all identified named or unnamed genes involved in trypanotolerance characteristics will allow us to use them in a field marker-assisted selection strategy and in microarrays prediction sets for bovine trypanotolerance.

14087. **Bosso, N.A., Cissé, M.F., van der Waaij, E.H., Fall, A. & van Arendonk, J.A.M., 2007.** Genetic and phenotypic parameters of body weight in West African Dwarf goat and Djallonké sheep. *Small Ruminant Research*, **67** (2-3): 271-278.

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The International Trypanotolerance Centre's small ruminant breeding programme was initiated in 1995. The aim was to increase the efficiency of meat production and the trypanotolerance of the animals (sheep and goat). To achieve that goal, selection was based on estimated breeding values for daily weight gain from 4 to 12 months of age measured on trypanosome challenge. The purpose of this study was to estimate genetic parameters for growth traits and to evaluate genetic trends in West African Dwarf goat and Djallonké sheep resulting from the breeding programme under a low input production environment. Data for West African Dwarf goat and Djallonké sheep included birth weight (BW), weaning weight (W120), yearling weight (W360), pre-weaning (GR0–4) and post-weaning (GR4–12) growth rate. The data were analysed using an animal model that accounted for fixed effects of sex, year of birth, season of birth, parity of the dam, type of birth and the interaction year by season of birth. Estimates of heritability for BW, W120, W360, GR0–4 and GR4–12 were 0.5, 0.43, 0.30, 0.32 and 0.11 for goats and 0.39, 0.54, 0.21, 0.54 and 0.23 for sheep, respectively. The genetic correlation between BW and W120 was high for goats (0.74) and moderate for sheep (0.47). Genetic correlations between W120 and GR4–12 were high (0.92) for goats and moderate (0.49) for sheep. Between GR0–4 and BW the correlation was positive but low for sheep (0.26) and moderate for goats (0.60). Positive trends were found in mean estimated breeding values for animals born in the period 1995–2002 which demonstrated the effectiveness of the implemented breeding programmes.

14088. **Pitchford, W.S., 2007.** Improving accuracy of selection of young bulls by pastoralists. *Livestock Science*, **110** (1-2): 141-147.

International Livestock Research Institute, PO Box 30709 Nairobi, and the University of Adelaide, Roseworthy campus, Roseworthy SA 5371, Australia. [Wayne.Pitchford@adelaide.edu.au].

A key to maximising response to selection in pastoral cattle kept by groups such as the sub-Saharan Maasai is an accurate selection of young bulls. A breeding objective was developed based on weight, reproductive rate (days to calving), temperament, tick resistance and trypanotolerance. Accuracy of selection was defined as the correlation between the breeding objective and various selection indices. Accuracy was evaluated assuming availability of information on a range of traits (those in objective plus scrotal circumference) from individuals, parents, grandparents, half-sibs, progeny and genetic markers. Various scenarios that represent what could occur at the village level were tested. Just selecting on weight alone had an accuracy of 0.538. Additional measurements on the individual (including

repeated measures) had a large effect on accuracy. Records on relatives were less helpful than expected. Genetic markers for traits that are difficult to measure (days to calving and trypanotolerance) were helpful for improving accuracy. However, they are unlikely to be used in the near future because of cost and availability. An additional output from this study is simple selection indices that could be implemented immediately at the village level.

14089. **Thevenon, S., Dayo, G. K., Sylla, S., Sidibe, I., Berthier, D., Legros, H., Boichard, D., Eggen, A. & Gautier, M., 2007.** The extent of linkage disequilibrium in a large cattle population of western Africa and its consequences for association studies. *Animal Genetics*, **38** (3): 277-286.

UMR Trypanosomes, CIRAD, Montpellier, F-34398 France; UMR Trypanosomes, IRD, Montpellier, F-34398, France; and URBIO, CIRDES, Bobo-Dioulasso 01, Burkina Faso.

Several previous studies concluded that linkage disequilibrium (LD) in livestock populations from developed countries originated from the impact of strong selection. Here, we assessed the extent of LD in a cattle population from western Africa that was bred in an extensive farming system. The analyses were performed on 363 individuals in a *Bos indicus* x *Bos taurus* population using 42 microsatellite markers on BTA04, BTA07 and BTA13. A high level of expected heterozygosity (0.71), a high mean number of alleles per locus (9.7) and a mild shift in Hardy-Weinberg equilibrium were found. Linkage disequilibrium extended over shorter distances than what has been observed in cattle from developed countries. Effective population size was assessed using two methods; both methods produced large values: 1388 when considering heterozygosity (assuming a mutation rate of 10-3) and 2344 when considering LD on whole linkage groups (assuming a constant population size over generations). However, analysing the decay of LD as a function of marker spacing indicated a decreasing trend in effective population size over generations. This decrease could be explained by increasing selective pressure and/or by an admixture process. Finally, LD extended over small distances, which suggested that whole-genome scans will require a large number of markers. However, association studies using such populations will be effective.

(d) TREATMENT

[See also **30**: 14146, 14153, 14156, 14159, 14166]

14090. **Grace, D., Himstedt, H., Sidibe, I., Randolph, T. & Clausen, P. H., 2007**. Comparing FAMACHA((c)) eye colour chart and Hemoglobin Color Scale tests for detecting anemia and improving treatment of bovine trypanosomosis in West Africa. *Veterinary Parasitology,* **147** (1-2): 26-39.

Institute for Parasitology and Tropical Veterinary Medicine, Freie Universitaet Berlin, Konigsweg 67, 14163 Berlin, Germany.

African animal trypanosomosis (AAT) is considered the most important cattle disease in sub-Saharan Africa but its diagnosis in the field is difficult, resulting in inappropriate

treatments, excessive delay in treatments and under-treatment. A field study in West Africa investigated the usefulness of anaemia in the diagnosis of trypanosomosis. A total of 20,772 cattle blood samples were taken from 121 villages in 3 countries. The average packed cell volume (PCV) of trypanosomosis positive cattle was 23 percent, versus 28 percent for negative cattle. In a sub-set of animals, other causes of anaemia were investigated showing most of the anaemia burden was attributable to trypanosomosis. Anaemia was a reasonably accurate indicator of trypanosomosis in the study area, with a sensitivity of 56 percent and a specificity of 80 percent and a diagnostic odds ratio of 4.2, the highest of all the signs evaluated (anaemia, emaciation, staring coat, lymphadenopathy, fever, lacrimation and salivary or nasal discharge). Having confirmed the usefulness of anaemia as a predictor of trypanosomosis, two potential pen-side tests for anaemia were evaluated (the first reported trial of their use in cattle): firstly a colour chart developed for anaemia detection in sheep through visual inspection of conjunctival membranes (FAMACHA((c))) and secondly the Haemoglobin Colour Scale (HbCS) developed for assessing haemoglobin levels in human patients by comparing blood drops on filter paper with colour standards. In a population of cattle suspected by their owners to be sick with trypanosomosis (n=898) the sensitivity of the HbCS test was 56 percent and the specificity was 77 percent, while the sensitivity of the FAMACHA((c)) test was 95 percent and the specificity was 22 percent. The higher sensitivity but lower specificity suggest the FAMACHA((c)) may be useful as a screening test and the HbCS as a confirmatory test. The two tests were also evaluated in cattle randomly selected from the village herd. Using cut-off points to optimize test performance, the HbCS test had a sensitivity of 81 percent and a specificity of 62 percent (n=505 cattle), while the FAMACHA((c)) had a sensitivity of 92 percent and a specificity of 30 percent (n=298 cattle). Recommendations are made for the appropriate use of these tests in the West African region.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

14091. **Enyaru, J.C., Matovu, E., Nerima, B., Akol, M. & Sebikali, C., 2006**. Detection of *T. b. rhodesiense* trypanosomes in humans and domestic animals in south east Uganda by amplification of serum resistance-associated gene. *Annals of the New York Academy of Sciences,* **1081**: 311-319.

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The human serum resistance-associated (SRA) gene was identified in 28 (80 percent) of the 35 *T. b. rhodesiense* trypanosomes from parasitologically confirmed sleeping sickness cases, using the primers designed by Radwanska and in 27 (77.1 percent) of the same 35 *T. b. rhodesiense* trypanosomes using the primers designed by Gibson. However, about 20 percent of the 35 *T. b. rhodesiense* trypanosomes could not be detected by SRA-polymerase chain reaction (PCR) even when an aliquot of the first PCR was used in the second PCR, indicating that the gene may be absent in those trypanosomes or the trypanosomes could be having

another variant of SRA not detectable by these primers since three variants of SRA genes have so far been identified or the amount of trypanosomal DNA extracted from infected blood was too low to be detected. The trypanosome isolates that are SRA gene negative may indicate the presence of some *T. b. rhodesiense* trypanosomes with modified or lack SRA genes or simple loss of the SRA gene from the expression site in which it resides during antigenic variation. Analysis of trypanosomes derived from domestic animals showed that 79 (90.8 percent) of the 87 trypanosomes isolated from cattle were positive by *Trypanosoma brucei* (TBR)-PCR, indicating that they were *Trypanozoon* while 8 (9.2 percent) of the trypanosome isolates which were negative by TBR-PCR could be *T. vivax, T. congolense*, or *T. theileri*. When subjected to SRA-PCR, 10 (11.5 percent) of the 87 trypanosomes isolates derived from cattle were positive, indicating that there could be *T. b. rhodesiense* circulating in cattle, which is similar to the percentage of *T. b. rhodesiense* previously obtained in cattle in Serere, Soroti district.

14092. **Madruga, C. R., Araujo, F. R., Cavalcante-Goes, G., Martins, C., Pfeifer, I. B., Ribeiro, L. R., Kessler, R. H., Soares, C. O., Miguita, M., Melo, E. P., Almeida, R. F. & Lima, M. M., Jr., 2006.** The development of an enzyme-linked immunosorbent assay for *Trypanosoma vivax* antibodies and its use in epidemiological surveys. *Memórias do Instituto Oswaldo Cruz*, **101** (7): 801-807.

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There are data indicating that the distribution of *Trypanosoma vivax* in the Brazilian territory is expanding with potential to reach other areas, where the vectors are present. The detection of anti-trypanosomal antibodies in serum provides important information of the trypanosomal status in cattle herds. For this reason, an enzyme-linked immunosorbent assay (*Tv*-ELISA-Ab) with crude antigen from one Brazilian isolate of *T. vivax* was developed and evaluated. The sensitivity and specificity were respectively 97.6 and 96.9 percent. In the evaluation of cross-reactions, three calves inoculated with *T. evansi* trypimastigotes blood forms showed optical densities (OD) under the cut-off during the whole experimental period, except one at 45 days post-inoculation. With relation to *Babesia bovis, B. bigemina*, and *Anaplasma marginale*, which are endemic haemoparasites in the studied area, the cross-reactions were shown to be 5.7, 5.3, and 1.1 percent, respectively. The first serological survey of Pantanal and state of Para showed that *T. vivax* is widespread, although regions within both areas had significantly different prevalences. Therefore, this *Tv*-ELISA-Ab may be a more appropriate test for epidemiological studies in developing countries because the diagnostic laboratories in most countries may be able to perform an ELISA, which is not true for the polymerase chain reaction.

14093. **Monzon, C. M., 2006**. Characterisation of a monoclonal antibody against *Trypanosoma evansi* and its application for detecting circulating antibodies. *Revue Scientifique et Technique*, **25** (3): 1067-1074.

Facultad de Ciencias de la Salud, Universidad Nacional de Formosa, Consejo Nacional de Investigaciones Cientificas y Técnicas, Centro de Diagnóstico e Investigaciones Veterinarias de Formosa, Formosa, Argentina.

Monoclonal antibodies were obtained against *Trypanosoma evansi*. The 2-4F6 IgM monoclonal antibody (Mab) was chosen for the study because of its ability to detect antigens and its specificity (as it did not recognise *T. cruzi, T. equiperdum, Babesia equi* or *B. caballi*). The immunoblot test revealed that the 2-4F6 IgM Mab recognises epitopes in two antigenic bands, one measuring 85 kDa and the other 122 kDa. An immunoassay for antigen detection in serum using polyclonal antibodies for capture, the Mab 2-4F6 as primary antibody and an antimouse IgM as secondary antibody gave positive results in 10 of the 11 *Equidae* infected with *T. evansi*, whereas 20 controls gave negative results. These research results show that the Mab 2-4F6 and the antigen it recognises are useful in identifying *Equidae* infected with *T. evansi*.

14094. **Reyna-Bello, A., Eleizalde, M. C. & Silva, A. M., 2007**. Assessment of chromogen suitability in ELISA for the detection of anaplasmosis and trypanosomosis. *Journal of Immunoassay and Immunochemistry*, **28** (1): 1-11.

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Two different ELISAs were routinely performed in our laboratory to detect bovine trypanosomosis and anaplasmosis. The ELISA test for trypanosomosis involved the adsorption of a soluble fraction of parasites as the antigen; and, the ELISA for anaplasmosis was performed with a purified recombinant protein MSP5r adsorbed to the plate. With the purpose of assessing the merit of ABTS and TMB, we compared the absorbance obtained from positive and negative control sera from both assays. The results obtained, suggest that TMB is more adequate for recombinant antigens and that ABTS is preferred when partially purified antigenic extracts are used in the ELISA test.

(b) PATHOLOGY AND IMMUNOLOGY

14095. **Baral, T. N., De Baetselier, P., Brombacher, F. & Magez, S., 2007.** Control of *Trypanosoma evansi* infection is IgM mediated and does not require a type I inflammatory response. *Journal of Infectious Diseases*, **195** (10): 1513-1520.

Department of Cellular and Molecular Interactions, Vlaams Interuniversitair Instituut voor Biotechnologie, Laboratorium voor Cellulaire en Moléculaire Immunologie, Vrije Universiteit Brussel, Brussels, B-1050, Belgium. [tbaral@vub.ac.be].

Very recent reports have documented that *Trypanosoma evansi*, the etiological agent of the livestock disease "surra", can cause human trypanosomiasis. In contrast to trypanosomes causing human African trypanosomiasis, *T. evansi* has a wide geographic distribution and host range, yet information about the immunobiological aspects of *T. evansi* trypanosomiasis is limited. Here, we show that, although *T. evansi* causes the induction of tumour necrosis factor (TNF), interferon-gamma, and nitric oxide during the early stage of infection, none of these molecules are crucial for parasitaemia control and survival of the

infected animal. However, TNF and TNF receptor 2 affect the induction of late-stage anaemia. Using B cell- and immunoglobulin M (IgM)-deficient mice, we identified IgM as being crucial for parasitaemia control and host survival. Collectively, our results show that, compared with other trypanosomes, *T. evansi* displays a distinct host-parasite interaction profile, given that, despite an infection-associated induction of proinflammatory molecules, only IgM antibodies contribute significantly to parasite control.

14096. **Bhasin, K. K., Yu, J. M., Tward, A., Shih, D., Campbell, D. A. & Lusis, A. J., 2006.** *Trypanosoma congolense*: paraoxonase 1 prolongs survival of infected mice. *Experimental Parasitology*, **114** (3): 240-245.

Department of Medicine, University of California, Los Angeles, CA 90095, USA.

In vitro studies have suggested that a fraction of human high density lipoprotein (HDL), termed trypanosome lysis factor (TLF), can protect against trypanosome infection. We examined the involvement of two proteins located in the TLF fraction, apolipoprotein A-II (apoA-II) and paraoxonase 1 (PON1), against trypanosome infection. To test whether PON1 is involved in trypanosome resistance, we infected human PON1 transgenic mice, PON1 knockout mice, and wild-type mice with *Trypanosoma congolense*. When challenged with the same dosage of trypanosomes, mice overexpressing PON1 lived significantly longer than wild-type mice, and mice deficient in PON1 lived significantly shorter. In contrast, mice overexpressing another HDL associated protein, apoA-II, had the same survival as wild-type mice. Together, these data suggest that PON1 provides protection against trypanosome infection. *In vitro* studies using *T. brucei brucei* indicated that HDL particles containing PON1 and those depleted of PON1 did not differ in their lysis ability, suggesting that protection by PON1 is indirect. Our data are consistent with an *in vivo* role of HDL protection against trypanosome infection.

14097. **Harris, T. H., Mansfield, J. M. & Paulnock, D. M., 2007.** CpG oligodeoxynucleotide treatment enhances innate resistance and acquired immunity to African trypanosomes. *Infection and Immunity*, 75 (5): 2366-2373.

Department of Medical Microbiology and Immunology, University of Wisconsin-Madison School of Medicine and Public Health, Wisconsin 53706, USA.

Relative resistance to African trypanosomiasis is based on the development of a type I cytokine response, which is partially dependent on innate immune responses generated through MyD88 and Toll-like receptor 9 (TLR9). Therefore, we asked whether enhancement of the immune response by artificial stimulation with CpG oligodeoxynucleotide (ODN), a TLR9 agonist, would result in enhanced protection against trypanosomes. In susceptible BALB/c mice, relative resistance to infection was significantly enhanced by CpG ODN treatment and was associated with decreased parasite burden, increased cytokine production, and elevated parasite-specific B- and T-cell responses. In relatively resistant C57BL/6 mice, survival was not enhanced but early parasitaemia levels were reduced 100-fold and the majority of the parasites were nondividing, short stumpy (SS) forms. CpG ODN treatment of

lymphocyte-deficient C57BL/6-scid and BALB/cByJ-scid mice also enhanced survival and reduced parasitaemia, indicating that innate resistance to trypanosome infection can be enhanced. In C57BL/6-scid and BALB/cByJ-scid mice, the parasites were also predominantly SS forms during the outgrowth of parasitaemia. However, the effect of CpG ODN treatment on parasite morphology was not as marked in gamma interferon (IFN-gamma)-knockout mice, suggesting that downstream effects of IFN-gamma production may play a discrete role in parasite cell differentiation. Overall, these studies provide the first evidence that enhancement of resistance to African trypanosomes can be induced in susceptible animals in a TLR9-dependent manner and that CpG ODN treatment may influence the developmental life cycle of the parasites.

14098. **Li, S. Q., Fung, M. C., Reid, S. A., Inoue, N. & Lun, Z. R., 2007.** Immunization with recombinant beta-tubulin from *Trypanosoma evansi* induced protection against *T. evansi, T. equiperdum* and *T. brucei* infection in mice. *Parasite Immunology*, **29** (4): 191-199.

Centre for Parasitic Organisms, State Key Laboratory of Biocontrol and Key Laboratory for Tropical Diseases Control of the Ministry of Education, School of Life Sciences, Sun Yat-Sen (Zhongshan) University, Guangzhou, P.R. China.

The beta-tubulin gene of *Trypanosoma evansi* (STIB 806) was cloned and expressed in *Escherichia coli*. The predicted amino acid sequence of *T. evansi* beta-tubulin shows 100 percent, 99.8 percent, 99.1 percent, and 98.6 percent homology with *T. equiperdum, T. b. brucei, T. cruzi* and *T. danilewskyi*, respectively, but is diverse from that of *T. cyclops*, showing only 51.6 percent of homology. Recombinant beta-tubulin was expressed as inclusion bodies in *E. coli*. It was purified and renatured for immunological studies. Mice immunized with the renatured recombinant beta-tubulin were protected from lethal challenge with *T. evansi* STIB 806, *T. equiperdum* STIB 818 and *T. b. brucei* STIB 940, showing 83.3 percent, 70 percent and 76.7 percent protection, respectively. Serum collected from the rabbit immunized with recombinant beta-tubulin inhibited the growth of *T. evansi, T. equiperdum* and *T. b. brucei in vitro*. Serum from mice and rabbits immunized with recombinant beta-tubulin recognized only *T. evansi* beta-tubulin and not mouse beta-tubulin. The results of this study demonstrated that the recombinant *T. evansi* beta-tubulin is a potential candidate for the development of a vaccine to prevent animal trypanosomiasis caused by these three trypanosome species.

14099. **Namangala, B., Sugimoto, C. & Inoue, N., 2007.** Effects of exogenous transforming growth factor beta on *Trypanosoma congolense* infection in mice. *Infection and Immunity*, **75** (4): 1878-1885.

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan.

The socioeconomic implications of trypanosomosis in sub-Saharan Africa and the limitations of its current control regimes have stimulated research into alternative control methods. Considering the pro- and anti-inflammatory properties of transforming growth factor beta1 (TGF-beta1) and its potential to enhance immunity against protozoan parasites,

we examined the effects of intraperitoneally delivered TGF-beta1 in C57BL/6 mice infected with *Trypanosoma congolense*, the haemoprotozoan parasite causing nagana in cattle. A triple dose of 10 ng TGF-beta1 significantly reduced the first parasitaemic peak and delayed mortality of infected mice. Furthermore, exogenous TGF-beta1 significantly decreased the development of trypanosome-induced anaemia and splenomegaly. The apparent TGF-beta1-induced antitrypanosome protection, occurring mainly during the early stage of infection, correlated with an enhanced parasite antigen-specific Th1 cell response characterized by a skewed type I cytokine response and a concomitant stronger antitrypanosome immunoglobulin G2a antibody response. Infected TGF-beta1-pretreated mice exhibited a significant reduction in the trypanosome-induced hyperexpansion of B cells. Furthermore, evidence is provided herein that exogenous TGF-beta1 activates macrophages that may contribute to parasite control. Collectively, these data indicate that exogenous TGF-beta1 is immunostimulative, inducing partial protection against *T. congolense* infection, possibly through mechanisms involving innate immune responses.

14100. **Oluyinka, O. O., Mairo, I. H., Ajanusi, J. A., David, O., Sekoni, V. & Nok, A. J., 2007.** Semen sialic acid surge and modulation of alpha-L-fucosidase activity: possible link to loss in reproductive capacity during trypanosomiasis. *Cell Biochemistry and Function.* **In press, corrected proof.**

Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria.

The profiles of semen sialic acid and the enzyme alpha-L-fucosidase were studied in rams undergoing chronic infection by *Trypanosoma congolense*. Our data showed a significant surge in the level of sialic acid with parasitaemia. The pattern followed a polynomial function we had reported for erythrocyte sialic acid in mice undergoing acute infection by *T. congolense*. The activity of the enzyme alpha-fucosidase decreased progressively with approximately 60 percent decrease at the end of the 14 weeks of infection. Representative semen samples from the control and infected rams were subjected to kinetic characterization. While the uninfected semen sample showed two active pH peaks at 4.5-5.5 and at 6.8-7.2, respectively, there was an apparent shift to only a single pH optimum at 4.5-5.5 for the pathological semen. The fucosidases from both sources were optimally active at 35 ^{0}C albeit with contrasting activation energies (E (a)) with values 20.58 and 35 kJ/mol for the control and infected semen, respectively. Kinetic studies using methylumbelliferyl-beta-fucoside (4MU-Fuc) as substrate gave K (M) and V (max) values of 3.25 µM and 14.6 µM min^{-1} mg^{-1}, respectively for the control semen. The values for the infected semen were 18.25 µM and 10.5 µM min^{-1} mg^{-1}, respectively. The significance of these results is discussed as they relate to loss in reproductive capacity in trypanosomosis.

14101. **Omer, O. H., Mousa, H. M. & Al-Wabel, N., 2007**. Study on the antioxidant status of rats experimentally infected with *Trypanosoma evansi*. *Veterinary Parasitology*, **145** (1-2): 142-145.

Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Buraidah, Saudi Arabia. [osamaom@hotmail.com].

The antioxidant status of rats experimentally infected with *Trypanosoma evansi* isolated from a camel was studied using established parasitological, haematological and biochemical methods. The results indicated that infections in all rats resulted in a fulminating parasitaemia. Changes in blood parameters in *T. evansi*-infected rats indicated leukocytosis and a macrocytic hypochromic anaemia. A degree of anisocytosis was also observed. The activities of plasma glucose-6-phosphate dehydrogenase and glutathione peroxidase in whole blood of infected rats were significantly higher ($p<0.05$ and $p<0.001$, respectively) compared with control. No statistically significant difference was observed in the activity of superoxide dismutase in infected and control rats. Results obtained indicated that trypanosomosis caused oxidative stress and induced antioxidant enzymes.

14102. **Shi, M. Q., Wang, C. R., Wei, G. J., Pan, W. L., Appleyard, G. & Tabel, H., 2006.** Experimental African trypanosomiasis: lack of effective CD1d-restricted antigen presentation. *Parasite Immunology*, **28** (12): 643-647.

Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada.

BALB/c mice are highly susceptible to African trypanosomiasis, whereas C57BL/6 mice are relatively resistant. Other investigators have reported that the synthesis of IgG antibodies to purified membrane form of variant surface glycoprotein (mfVSG) of *Trypanosoma brucei* is CD1 restricted. In this study, we examine the role of the CD1d/NKT cell pathway in susceptibility and resistance of mice to infection by African trypanosomes. Administration of anti-CD1d antibodies to *Trypanosoma congolense*-infected BALB/c mice neither affects the parasitemia nor the survival time. Correspondingly, CD1d(-/-) and CD1d(+/+) BALB/c mice infected with *T. congolense* or *T. brucei* show no differences in either parasitaemia or survival time. The course of disease in relative resistant C57BL/6 mice infected with *T. congolense* is also not affected by the absence of CD1d. Parasitaemia, survival time, and plasma levels of IgG2a and IgG3 parasite-specific antibodies in infected CD1d(-/-) C57BL/6 are not different from those of infected CD1d(+/+) C57BL/6 mice. We conclude that CD1d-restricted immune responses do not play an important role in susceptibility/resistance of mice infected with virulent African trypanosomes. We speculate that virulent trypanosomes have an evasion mechanism that prevents the induction of a parasite-specific, CD1d-restricted immune response by the host.

14103. **Shi, M. Q., Wei, G. J. & Tabel, H., 2007.** *Trypanosoma congolense* infections: MHC class II-restricted immune responses mediate either protection or disease, depending on IL-10 function. *Parasite Immunology*, **29** (2): 107-111.

Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada.

BALB/c mice are highly susceptible and C57BL/6 relatively resistant to *Trypanosoma congolense* infections. Here we show that relatively resistant wild-type B6 mice infected with *T. congolense* survive significantly longer (> 200 days) than infected major histocompatibility complex (MHC) class II-deficient B6 mice (approximately 50 days). We

also show that blocking of the interleukin-10 (IL-10) receptor induces early death of wild-type B6 mice infected with *T. congolense* (approximately 10 days), but does not affect the survival of infected MHC class II-deficient B6 mice. We conclude that MHC class II-restricted immune responses mediate protection and, when IL-10 function is impaired, MHC class II-restricted immune responses mediate early mortality in otherwise resistant B6 mice. Thus, in *T. congolense* infections, MHC class II-restricted immune responses mediate either protection or disease, depending on IL-10 function.

14104. **Shiflett, A. M., Faulkner, S. D., Cotlin, L. F., Widener, J., Stephens, N. & Hajduk, S. L., 2007.** African trypanosomes: intracellular trafficking of host defence molecules. *Journal of Eukaryotic Microbiology*, **54** (1): 18-21.

Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA.

Trypanosoma brucei brucei is the causative agent of Nagana in cattle and can infect a wide range of mammals but is unable to infect humans because it is susceptible to the innate cytotoxic activity of normal human serum. A minor subfraction of human high-density lipoprotein (HDL), containing apolipoprotein A-I (APOA1), apolipoprotein L-I (APOL1) and haptoglobin-related protein (HPR) provides this innate protection against *T. b. brucei* infection. Both HPR and APOL1 are cytotoxic to *T. b. brucei* but their specific activities for killing increase several hundred-fold when assembled in the same HDL. This HDL is called trypanosome lytic factor (TLF) and kills *T. b. brucei* following receptor binding, endocytosis, and lysosomal localization. Trypanosome lytic factor is activated in the acidic lysosome and facilitates lysosomal membrane disruption. Lysosomal localization is necessary for *T. b. brucei* killing by TLF. *Trypanosoma brucei rhodesiense*, which is indistinguishable from *T. b. brucei*, is resistant to TLF killing and causes human African sleeping sickness. Human infectivity by *T. b. rhodesiense* correlates with the evolution of a human serum resistance associated protein (SRA) that is able to ablate TLF killing. When *T. b. brucei* is transfected with the SRA gene it becomes highly resistant to TLF and human serum. In the SRA transfected cells, intracellular trafficking of TLF is altered and TLF mainly localizes to a subset of SRA containing cytoplasmic vesicles but not to the lysosome. These findings indicate that the cellular distribution of TLF is influenced by SRA expression and may directly determine susceptibility.

14105. **Vincendeau, P. & Bouteille, B., 2006.** Immunology and immunopathology of African trypanosomiasis. *Anais da Academia Brasiliera de Ciências*, **78** (4): 645-665.

Laboratoire de Parasitologie, Université de Bordeaux II, Bordeaux, France. [philippe.vincendeau@parasito.u-bordeaux2.fr].

Major modifications of immune system have been observed in African trypanosomiasis. These immune reactions do not lead to protection and are also involved in immunopathology disorders. The major surface component (variable surface glycoprotein, VSG) is associated with escape to immune reactions, cytokine network dysfunctions and autoantibody production. Most of our knowledge results from experimental trypanosomiasis. Innate resistance elements have been characterised. In infected mice, VSG preferentially

stimulates a Th 1-cell subset. A response of gamma delta and CD8 T cells to trypanosome antigens was observed in trypanotolerant cattle. An increase in CD5 B cells, responsible for most serum IgM and production of autoantibodies has been noted in infected cattle. Macrophages play important roles in trypanosomiasis, in synergy with antibodies (phagocytosis) and by secreting various molecules (radicals, cytokines, prostaglandins). Trypanosomes are highly sensitive to TNF-alpha, reactive oxygen and nitrogen intermediates. TNF-alpha is also involved in cachexia. IFN-gamma acts as a parasite growth factor. These various elements contribute to immunosuppression. Trypanosomes have learnt to use immune mechanisms to their own profit. Recent data show the importance of alternative macrophage activation, including arginase induction. L-ornithine produced by host arginase is essential to parasite growth. All these data reflect the deep insight into the immune response associated with trypanosomes and might suggest interference therapeutic approaches.

14106. **Yang, C., Suo, X., Huang, X., Zhang, G., Jia, Y., Wang, Q. & Shen, J., 2007.** Protection of mice against homologous or heterologous infections with antiserum mixture to the predominant variable antigen type repertoire of *Trypanosoma evansi* YNB stock. *Experimental Parasitology*, **116** (1): 53-58.

Parasitology Laboratory, College of Veterinary Medicine, China Agricultural University, Beijing 100094, China.

The objective of this study was to test a hypothesis that the predominant variable antigen type (VAT) repertoire of a single stock of *Trypanosoma evansi* was limited and small. It was further assumed that six rabbits could produce all antibodies against the predominant VAT repertoire of a stock of *T. evansi* and the antiserum mixture from the six rabbits containing all the antibodies could completely protect mice against any homologous stock infections and partially protect mice against some heterologous stock infections. Mice were each intraperitoneally infected with 100 parasites of clone-derived and non-clone-derived populations of the YNB stock, Kazakhstan strain or Vietnam strain of *T. evansi*, and treated with the antiserum mixture when trypanosomes had been detected in the blood. All of the 10 mice infected with either non-clone-derived or clone-derived populations of the YNB stock survived, and some (4/10) of mice infected with the heterologous Kazakhstan strain survived, while all those (10/10) infected with the heterologous Vietnam strain died. These results support the hypothesis that the predominant VAT repertoire of a single stock of *T. evansi* was limited and small, and have important implications in the consideration of treating human trypanosomosis due to drug resistant strains with antiserum mixture.

(c) CHEMOTHERAPEUTICS

14107. **Boibessot, I., Tettey, J. N. A., Skellern, G. G., Watson, D. G. & Grant, M. H., 2006**. Metabolism of isometamidium in hepatocytes isolated from control and inducer-treated rats. *Journal of Veterinary Pharmacology and Therapeutics*, **29** (6): 547-553.

Bioengineering Unit, University of Strathclyde, Glasgow, UK.

Little is known about the metabolism and mechanism of action of the trypanocide, isometamidium (ISM), the major drug used for prophylaxis of trypanosomiasis. We have investigated its metabolism and distribution in isolated rat hepatocytes using liquid chromatography-mass spectrometry and confocal laser scanning microscopy (CLSM). Two putative metabolites were formed, which were proposed to be a mono-acetyl derivative and an oxidized metabolite (SII). This is the first demonstration of the hepatic metabolism of ISM, as previous *in vivo* studies were hampered by dose-limiting toxicity and insensitive analytical methods. The intrinsic fluorescence of the drug enabled its intracellular uptake to be followed by CLSM. It is taken up rapidly into the nucleolus, nuclear membrane and endoplasmic reticulum within 5 min., and retained in the nucleus for at least 24 h. Persistent binding of ISM to cellular macromolecules may contribute to its prophylactic effect in vivo. Pretreatment of rats with 3-methylcholanthrene, phenobarbitone (PB) or the widely used pyrethroid pesticide, deltamethrin, resulted in an increase in metabolism of ISM to the proposed SII after 1 h incubation with hepatocytes. 3-methylcholanthrene was the most potent inducer, causing a maximal 19.5-fold induction of SII formation after exposure of hepatocytes to ISM for 1 h compared with formation by control hepatocytes. In comparison, at the 1 h timepoint deltamethrin pre-treatment caused a 10.2-fold induction, and PB only 8.2 fold.

14108. **Fijolek, A., Hofer, A. & Thelander, L., 2007.** Expression, purification, characterization, and *in vivo* targeting of trypanosome CTP synthetase for treatment of African sleeping sickness. *Journal of Biological Chemistry*, **282** (16): 11858-11865.

Department of Medical Biochemistry and Biophysics, Umea University, SE-901 87 Umea, Sweden. [artur.fijolek@medchem.umu.se].

African sleeping sickness is a fatal disease caused by two parasite subspecies: *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. We previously reported that trypanosomes have extraordinary low CTP pools compared with mammalian cells. Trypanosomes also lack salvage of cytidine/cytosine making the parasite CTP synthetase a potential target for treatment of the disease. In this study, we have expressed and purified recombinant *T. brucei* CTP synthetase. The enzyme has a higher K (m) value for UTP than the mammalian CTP synthetase, which in combination with a lower UTP pool may account for the low CTP pool in trypanosomes. The activity of the trypanosome CTP synthetase is irreversibly inhibited by the glutamine analogue acivicin, a drug extensively tested as an antitumor agent. There is a rapid uptake of acivicin in mice both given intraperitoneally and orally by gavage. Daily injection of acivicin in trypanosome-infected mice suppressed the infection up to one month without any significant loss of weight. Experiments with cultured bloodstream *T. brucei* showed that acivicin is trypanocidal if present at 1 mμM concentration for at least 4 days. Therefore, acivicin may qualify as a drug with "desirable" properties, i.e. cure within 7 days, according to the current Target Product Profiles of WHO and DNDi.

14109**. Gudin, S., Quashie, N. B., Candlish, D., Al-Salabi, M. I., Jarvis, S. M., Ranford-Cartwright, L. C. & de Koning, H. P., 2006.** *Trypanosoma brucei*: A survey of pyrimidine transport activities. *Experimental Parasitology*, **114** (2): 118-125.

Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Biomedical Research Center, Glasgow G12 8TA, UK., and University of Westminster, 115 New Cavendish Street, London W1W 6UW, UK. [H.de-Koning@bio.gla.ac.uk].

Purine uptake has been studied in many protozoan parasites in the last few years, and several of the purine transporters have been cloned. In contrast, very little is known about the salvage of preformed pyrimidines by protozoa, and no pyrimidine transporters have been cloned, yet chemotherapy based on pyrimidine nucleobases and nucleosides has been as effective as purine antimetabolites in the treatment of infectious and neoplastic disease. Here, we surveyed the presence of pyrimidine transporters in *Trypanosoma brucei brucei*. We could not detect any mediated uptake of thymine, thymidine or cytidine, but identified a very high-affinity transporter for cytosine, designated C1, with a Km value of 0.048 ± 0.009 μM. We also confirmed the presence of the previously reported U1 uracil transporter and found it capable of mediating uridine uptake as well, with a Km of 33 ± 5 μM. A higher-affinity U2 uridine transporter (Km = 4.1 ± 2.1 μM) was also identified, but efficiency of the C1 and U2-mediated transport was low. Pyrimidine antimetabolites were tested as potential trypanocidal agents and only 5-fluorouracil was found to be effective. This drug was efficiently taken up by bloodstream forms of *T. b. brucei*.

14110. **Jaeger, T. & Flohe, L., 2006**. The thiol-based redox networks of pathogens: unexploited targets in the search for new drugs. *Biofactors*, **27** (1-4): 109-120.

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Hydroperoxide metabolism in diverse pathogens is reviewed under consideration of involved enzymes as potential drug targets. The common denominator of the peroxidase systems of *Trypanosoma, Leishmania, Plasmodium*, and *Mycobacterium* species is the use of NAD(P)H to reduce hydroperoxides including peroxynitrite via a flavin-containing disulfide reductase, a thioredoxin (Trx)-related protein and a peroxidase that operates with thiol catalysis. In *Plasmodium falciparum*, thioredoxin- and glutathione dependent systems appear to be linked via glutaredoxin and plasmoredoxin to terminal thioredoxin peroxidases belonging to both, the peroxiredoxin (Prx) and glutathione peroxidase (GPx) family. In *Mycobacterium tuberculosis*, a catalase-type peroxidase is complemented by the typical 2-C-Prx AhpC that, in contrast to most bacteria, is reduced by TrxC, and an atypical 2-C-Prx reduced by TrxB or C. A most complex variation of the scheme is found in trypanosomatids, where the unique redox metabolite trypanothione reduces the thioredoxin-related tryparedoxin, which fuels Prx- and GPx-type peroxidases as well as ribonucleotide reductase. In *Trypanosoma brucei* and *Leishmania donovani* the system has been shown to be essential for viability and virulence by inversed genetics. It is concluded that optimum efficacy can be expected from inhibitors of the most upstream components of the redox cascades. For trypanosomatids attractive validated drug targets are trypanothione reductase and trypanothione synthetase; for mycobacteria thioredoxin reductase appears most appealing, while in *Plasmodium* simultaneous inhibition of both the thioredoxin and the glutathione pathway appears advisable to avoid mutual substitution in co-substrate supply to the peroxidases. Financial and organisational needs to translate the scientific progress into

applicable drugs are discussed under consideration of the socio-economic impact of the addressed diseases.

14111. **Luscher, A., Nerima, B. & Maser, P., 2006**. Combined contribution of TbAT1 and *Tb*MRPA to drug resistance in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*, **150** (2): 364-366.

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No abstract available.

14112. **Martyn, D. C., Jones, D. C., Fairlamb, A. H. & Clardy, J., 2007.** High-throughput screening affords novel and selective trypanothione reductase inhibitors with anti-trypanosomal activity. *Bioorganic and Medicinal Chemistry Letters,* **17** (5): 1280-1283.

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA.

Trypanothione reductase (TR), an enzyme that buffers oxidative stress in trypanosomatid parasites, was screened against commercial libraries containing approximately 134,500 compounds. After secondary screening, four chemotypes were identified as screening positives with selectivity for TR over human glutathione reductase. Thirteen compounds from these four chemotypes were purchased, and their *in vitro* activity against TR and *Trypanosoma brucei* is described.

14113. **Mathis, A. M**., **Bridges, A. S**., **Ismail, M. A**., **Kumar, A**., **Francesconi, I**., **Anbazhagan, M**., **Hu, Q**., **Tanious, F. A**., **Wenzler, T**., **Saulter, J**., **Wilson, W. D**., **Brun, R**., **Boykin, D. W**., **Tidwell, R. R**. & **Hall, J. E**., **2007**. Diphenyl furans and aza analogues: Effects of structural modification on *in vitro* activity, DNA binding, and accumulation and distribution in trypanosomes. *Antimicrobial Agents and Chemotherapy.* **In press, corrected proof**.

Division of Molecular Pharmaceutics, School of Pharmacy, University of North Carolina Chapel Hill, Chapel Hill, NC; Department of Pathology and Laboratory Medicine, UNC School of Medicine, University of North Carolina, Chapel Hill, NC; Department of Chemistry, Georgia State University, Atlanta, GA., USA; Swiss Tropical Institute, Basel, Switzerland.

Human African trypanosomiasis is a devastating disease with only a few treatment options, including pentamidine. Diamidine compounds such as pentamidine, DB75, and DB820 are potent antitrypanosomal compounds. Previous investigations have shown that diamidines accumulate to high concentrations in trypanosomes. However, the mechanism of action of this class of compounds remains unknown. A long-hypothesized mechanism of action has been binding to DNA and interference with DNA-associated enzymes. The fluorescent diamidines, DB75 and DB820, have been shown to localize not only in the DNA

containing nucleus and kinetoplast of trypanosomes, but also to the acidocalcisomes. Here we investigate two series of analogues of DB75 and DB820 with varying *in vitro* antitrypanosomal activity to determine whether any correlation exists between trypanosome accumulation, distribution and *in vitro* activity. Despite wide ranges of *in vitro* antitrypanosomal activity, all of the compounds investigated accumulated to mM concentrations in trypanosomes over 8 h. Interestingly, some of the less potent compounds accumulated to concentrations much higher than more potent compounds. All of the compounds were localized to one or both of the DNA containing nucleus or kinetoplast, and many were also found in the acidocalcisomes. Accumulation in the nucleus and kinetoplast should be important to the mechanism of action of these compounds. The acidocalcisomes also may play a role in the mechanism of action of these compounds. This investigation suggests that the extent of accumulation alone is not responsible for killing trypanosomes and that organelle specific accumulation may not predict *in vitro* activity.

14114. **Mbaya, A. W., Nwosu, C. O. & Onyeyili, P. A., 2007.** Toxicity and anti-trypanosomal effects of ethanolic extract of *Butyrospermum paradoxum* (*Sapotaceae*) stem bark in rats infected with *Trypanosoma brucei* and *Trypanosoma congolense*. *Journal of Ethnopharmacology*, **111** (3): 526-530.

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The ethanolic extract of *Butyrospermum paradoxum* stem bark, commonly used in the traditional treatment of various diseases including animal and human trypanosomosis in north-eastern Nigeria, was tested for toxicity and anti-trypanosomal efficacy in rats infected with *Trypanosoma congolense* and *Trypanosoma brucei*. Following intra-peritoneal administration, the extract induced behavioural changes, morbidity and mortality in the rats. The symptoms observed included anorexia, dehydration, depression, prostration, coma and death. These symptoms were noted at high doses (>800mg/kg) only. At necropsy, the pathological lesions were mainly congestion and oedema of the lungs, bronchi, bronchioles and kidneys, hepatomegally and focal necrosis of the liver cells. The severity of the symptoms and lesions were dose related. The intra-peritoneal LD (50) of the extract was 820mg/kg. The extract produced anti-trypanosomal effect through the complete suppression or delay in parasite establishment with reduction in the level of parasitaemia and the severity of the attendant disease as well as enhanced survival of the rats infected with *Trypanosoma congolense* and *Trypanosoma brucei*. The results suggest that the folkloric medicinal application of the extracts of *Butyrospermum paradoxum* has a pharmacological basis.

14115. **Midgley, I., Fitzpatrick, K., Taylor, L. M., Houchen, T. L., Henderson, S. J., Wright, S. J., Cybulski, Z. R., John, B. A., McBurney, A., Boykin, D. W. & Trendler, K. L., 2007.** Pharmacokinetics and metabolism of the prodrug DB289 (2,5-bis[4-(N-methoxyamidino)phenyl]furan monomaleate) in rat and monkey and its conversion to the antiprotozoal/antifungal drug DB75 (2,5-bis(4-guanylphenyl)furan dihydrochloride). *Drug Metabolism and Disposition*, **35** (6): 955-967.

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DB289 (pafuramidine maleate; 2,5-bis[4-(N-methoxyamidino)phenyl]furan monomaleate) is a prodrug of DB75 (furamidine dihydrochloride; 2,5-bis(4-guanylphenyl)furan dihydrochloride), an aromatic dication related to pentamidine that has demonstrated good efficacy against African trypanosomiasis, *Pneumocystis carinii* pneumonia, and malaria, but lacks adequate oral availability. The pharmacokinetics and metabolism of ^{14}C-DB289 have been investigated in rat and monkey after oral and intravenous administration. Oral doses were well absorbed (approximately 50-70 percent) and effectively converted to DB75 in both species but subject to first-pass metabolism and hepatic retention, limiting its systemic bioavailability to 10 to 20 percent. Clearance of DB289 approximated the liver plasma flow and its large volume of distribution was consistent with extensive tissue binding. Plasma protein binding of DB289 was 97 to 99 percent in four animal species and humans, but that of DB75 was noticeably less and more species- and concentration-dependent. Together, prodrug and active metabolite accounted for less than 20 percent of the plasma radioactivity after an oral dose, but DB75 was the major radiochemical component in key organs such as brain and liver and was largely responsible for the persistence of ^{14}C in the body. The predominant route of excretion of radioactivity was via the feces, although biliary secretion was not particularly extensive. High-performance liquid chromatography and liquid chromatography-mass spectrometry investigations showed that the formation of DB75 from the prodrug involved the sequential loss of the two N-methoxy groups, either directly or by O-demethylation followed by reduction of the resulting oxime to the amidine. It was estimated that almost half of an oral dose of DB289 to rats and about one-third of that to monkeys was metabolized to DB75.

14116. **Muth, M., Hoerr, V., Glaser, M., Ponte-Sucre, A., Moll, H., Stich, A. & Holzgrabe, U., 2007.** Antitrypanosomal activity of quaternary naphthalimide derivatives. *Bioorganic and Medicinal Chemistry Letters*, **17** (6): 1590-1593.

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Sleeping sickness caused by *Trypanosoma brucei gambiense* and *rhodesiense* is fatal if left untreated. Due to the toxicity of drugs currently used and the emerging resistance against these drugs new lead compounds are urgently needed. Within the frame of a broad screening programme for drugs with antitrypanosomal activity, some highly potent tertiary and quaternary mono- and bisnaphthalimides being active in the lower micromolar and nanomolar range of concentration have been identified. These compounds are easily available via a two- or three-step microwave-driven synthesis with high yield.

14117. **Nakata, H., 2007**. Mitogen-activated protein kinase signaling is involved in suramin-induced neurite outgrowth in a neuronal cell line. *Biochemical and Biophysical Research Communications*, **355** (3): 842-848.

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Suramin is a well-known antitrypanosomal drug and a novel experimental agent for the treatment of several cancers. Previous study showed that suramin is an activator of extracellular signal-regulated kinase (ERK1/2) signaling in several cell lines including Chinese hamster ovary cells, although the physiological relevance of this activation remains uncertain. Here, it was shown that suramin enhances neurite outgrowth concomitant with activation of ERK1/2 in Neuro-2a cells, a neuronal cell line. These neurite outgrowth and ERK1/2 activation were significantly inhibited by PD98059, an inhibitor of mitogen-activated protein kinase, as well as by activation of endogenous adenosine A2A receptors. The suramin-induced phosphorylation of ERK1/2 was also inhibited by inhibitors of Src family kinases. This attenuation of ERK1/2 activity was accompanied by a significant decrease in suramin-induced neurite outgrowth. These results suggest that suramin activates the Src/ERK1/2 signaling pathway that induces neurite outgrowth, both of which are negatively regulated by cAMP produced in response to activation of endogenous adenosine A2A receptors.

14118. **Ndjakou Lenta, B., Vonthron-Senecheau, C., Fongang Soh, R., Tantangmo, F., Ngouela, S., Kaiser, M., Tsamo, E., Anton, R. & Weniger, B., 2007**. *In vitro* antiprotozoal activities and cytotoxicity of some selected Cameroonian medicinal plants. *Journal of Ethnopharmacology*, **111** (1): 8-12.

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Eight extracts from seven selected Cameroonian medicinal plants, traditionally used to treat malaria and other protozoal diseases, were tested *in vitro* for their antiprotozoal activities against *Plasmodium falciparum* K1 chloroquine-resistant strain, *Leishmania donovani, Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense*, protozoa responsible for malaria, visceral leishmaniasis, Chagas disease and African trypanosomiasis, respectively. The most active extract against *Plasmodium falciparum* K1 strain and *Trypanosoma brucei rhodesiense* was the methanolic extract of *Albizia zygia* (*Fabaceae*) stem bark with IC(50) values of 1.0 µg/ml and 0.2 µg/ml, respectively. Five extracts showed IC (50) values below 5mµg/ml against *Leishmania donovani*, with the methanolic seed extract of *Harungana madagascarensis* showing the highest activity, but only the methanolic extract of *Albizia zygia* showed activity against *Trypanosoma cruzi.* Cytotoxicity and selectivity indexes were estimated for the most active extracts. The best ratio of cytotoxicity to antiplasmodial activity (SI(a)=14) was established for the methanolic leaf extract of *Symphonia globulifera* (*Clusiaceae*), while the methanolic stem bark extract of *Albizia zygia* showed the best ratio of cytotoxicity to antitrypanosomal activity (SI(b)=22.5).

14119. **Ogbadoyi, E. O., Abdulganiy, A. O., Adama, T. Z. & Okogun, J. I., 2007**. *In vivo* trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma brucei brucei*. *Journal of Ethnopharmacology*, **112** (1): 85-89.

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Chemotherapy of African trypanosomiasis still remains far from being satisfactory. There is the urgent need for therapeutic agents that are effective, affordable and accessible to the rural poor in Africa who bear most of the disease burden. Root preparations of *Annona senegalensis* Pers. is claimed by traditional medicine practitioners to be effective in the treatment of sleeping sickness. Validation of this claim, evaluation of the therapeutic effects of other parts of the plant, and standardization of the preparations are necessary in order to fully exploit the chemotherapeutic potentials of this plant. We have evaluated the chemotherapeutic effects of extracts of the leaves, whole root, root and stem bark of the plant in experimental trypanosomiasis. Crude and partially purified aqueous extracts of the leaves, at a dose of 200mg/kg body weight per day completely cured experimental *Trypanosoma brucei brucei* infection in mice. Sub-inoculation of blood and cerebrospinal fluid drawn from the cured mice into healthy mice failed to produce any infection within 60 days of post-inoculation. Treatment of healthy mice with the crude extract before infection did not prevent establishment of infection. Administration of 5000 mg/kg body weight of the crude extract did not lead to fatality in mice. Preliminary phytochemical screening showed the presence of tannin, phlobatanin, and saponin.

14120. **Rosselli, F. P., Albuquerque, C. N. & Da Silva, A. B., 2006.** A chemometric study of megazol derivatives with activity against *Trypanosoma equiperdum*. *SAR QSAR in Environmental Research*, **17** (6): 533-547.

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The AM1 semiempirical method was employed to study megazol and 13 of its analogues where their activity against *Trypanosoma equiperdum* was obtained from *in vitro* tests. Several molecular properties (descriptors or variables) were calculated for the 14 compounds studied to be correlated with the biological activity. For a practical analysis of large data sets, it is necessary to reduce the dimensionality and select the most relevant descriptors related to the biological activity under study. For this purpose, the following chemometric methods were employed: principal component analysis (PCA), hierarchical cluster analysis (HCA), K-nearest neighbour (KNN), stepwise discriminant analysis (SDA) and soft independent modelling of class analogy (SIMCA). These methods showed that the descriptors molecular electronic energy (Eelet), charge on the first nitrogen at substituent 2 (qN), volume of substituent at C5 position (V-S5), dihedral angle (D3) and bond length between atom C4 and its substituents (L4) are responsible for the separation between active and inactive compounds against *T. equiperdum*.

14121. **Turnipseed, S. B., Clark, S. B., Andersen, W. C., Karbiwnyk, C. M., Miller, K. E. & Hurlbut, J. A., 2006**. Confirmation of diminazene diaceturate in bovine plasma using electrospray liquid chromatography-mass spectrometry. *Journal of Chromatography*, **844** (1): 127-133.