

*Tsetse and Trypanosomiasis Information*

These results led to propose that a campaign against tsetse, undertaken by a development project called PAEOB (Projet d'Appui à l'Élevage dans l'Ouest du Burkina Faso), should be focussed on only 34 percent of the hydrographic network.

13674. **Sow, A., Sidibe, I., Desquesnes, M., Bengaly, Z. & Pangui, L.J., 2006.** The application of PCR-ELISA to the detection of *Trypanosoma congolense* type savannah (TCS) in bovine blood samples. *Tropical Biomedicine*, **23** (1): 123-129.

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PCR-ELISA was set up to detect strains of *Trypanosoma congolense* type savannah (TCS) in field samples of buffy coats. Results of PCR-ELISA and PCR were compared and the effectiveness of both techniques was also compared with Murray's method for the detection of TCS in 257 bovine buffy coats. The PCR products were labelled with digoxigenin (DIG-dUTP) during amplification cycles of the repetitive satellite DNA. A biotinylated DNA capture probe was used to detect the PCR products by ELISA in streptavidin coated microplates. Both the PCR-ELISA and PCR were more sensitive and more specific than Murray's method. Of the 257 buffy coats analysed by the three techniques, PCR-ELISA and PCR detected TCS in 98 and 97 buffy coats respectively, whereas Murray's method detected only 39 samples. PCR-ELISA and PCR had almost the same sensitivity and specificity. PCR-ELISA and PCR respectively detected TCS in 39.2 percent and 38.6 percent in all the 334 samples analysed by both techniques in this study.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **29**: 13621, 13695, 13696, 13699, 13703, 13704, 13706, 13707, 13711, 13731, 13871, 13873, 13878, 13881, 13984, 13987, 13906, 13923, 13943, 13944, 13970, 13977, 13978, 13999]

13675. **Batista, J.S., Riet-Correa, F., Teixeira, M.M., Madruga, C.R., Simoes, S.D. & Maia, T.F., 2006.** Trypanosomiasis by *Trypanosoma vivax* in cattle in the Brazilian semi-arid: Description of an outbreak and lesions in the nervous system. *Veterinary Parasitology*. **In press; corrected proof.**

Escola Superior de Agricultura de Mossoro, Av. Francisco Mota S/N, Br 110, Km 47, 59 Rio Grande do Norte, Brazil.

An outbreak of trypanosomiasis by *Trypanosoma vivax* is reported in the semi-arid of Paraíba, North eastern Brazil from May to August 2002. Sixty-four cows out of 130 were affected; 11 died and the other recovered after treatment with diminazene aceturate. Affected animals had fever, anaemia, weight loss, hypoglycaemia, increased serum levels of aspartate aminotransferase and, in nine cows, nervous signs. All cows with nervous signs died; six of them recovered after treatment, but the disease relapsed. Six cows aborted and one delivered a calf that died immediately after parturition. Thirty-two out of 100 calves were affected and five died. Nervous signs were not observed in the calves. Gross lesions were thickening of the meninges, enlarged lymph nodes and prominent white pulp of the spleen. The main

histological lesion was meningoencephalitis and malacia in the brain of cows with nervous signs. No antibodies against trypanosomes were found in 33 blood samples collected before the outbreak in the affected farm and in 29 samples collected at the same time in two other neighbouring farms. Until January 2003, all 89 animals tested had antibodies against *T. vivax*, suggesting the occurrence of sub clinical infections in cattle without clinical signs. Only two out of 85 serum samples collected on April 2004 were positive for *T. vivax* antibodies. Data obtained suggested that the semiarid region is non-endemic for trypanosomiasis and that disease occurred due to introduction of the parasite in a susceptible population after an apparent rise in the *Tabanus* spp. population.

13676. **Dhollander, S., Jallow, A., Mbodge, K., Kora, S., Sanneh, M., Gaye, M., Bos, J., Leak, S., Berkvens, D. & Geerts, S., 2006.** Equine trypanosomosis in the Central River Division of The Gambia: a study of veterinary gate-clinic consultation records. *Preventive Veterinary Medicine*, **75** (3-4): 152-162.

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The objective of this study was to provide epidemiological information of equine trypanosomosis in the Central River Division (CRD) of The Gambia. Therefore, 2,285 consultations records of equines, admitted in a gate-clinic at Sololo in CRD, were studied retrospectively. The data were recorded in the period between September 1995 and July 2002 and comprised consultations of 2,113 horses and 172 donkeys. "Trypanosome infection" was the most frequently diagnosed condition and accounted for 61 percent of the cases. Horses were more frequently diagnosed with trypanosome infections than donkeys ( $p < 0.001$ ), with an occurrence of 63 percent compared to 43 percent in donkeys. In both horses and donkeys, trypanosome infections were mainly due to *Trypanosoma congolense* (64 percent) and *T. vivax* (32 percent). There was no difference observed in the occurrence of trypanosome infections in male or female donkeys ( $p = 0.585$ ), but there were more female (67.8 percent) horses observed with trypanosome infections than male horses (60.7 percent;  $p = 0.003$ ). There was no difference observed in the occurrence of trypanosome infections in donkeys older or younger than 1 year ( $p = 0.130$ ), but older horses (63.2 percent  $> 1$  year) were observed with trypanosome infections than young horses (54.5 percent  $< 1$  year;  $p = 0.033$ ). The number of donkeys and horses with trypanosome infections decreased during the rainy season (June-September). The majority of equines that were admitted with trypanosome infections were severely anaemic. The average packed cell volume (PCV) declined with increasing parasitaemia ( $p = 0.006$ ). Seventy-four percent of the farmers' predictions of trypanosome infections in their equines were confirmed by darkground-microscopy. That proved that farmers had a fairly accurate knowledge of the diseases affecting their equines. The treatments executed at the gate-clinic were generally effective. The few (0.4 percent) relapses of the *T. vivax* infections that were previously treated with diminazene aceturate in this study were not sufficient to prove drug resistance. The study showed that the analysis of consultation records at a gate-clinic can provide complementary information to conventional epidemiological studies in the same research area.

13677. **Garcia, H., Garcia, M.E., Perez, G., Bethencourt, A., Zerpa, E., Perez, H. & Mendoza-Leon, A., 2006.** Trypanosomiasis in Venezuelan water buffaloes: association of packed cell volumes with seroprevalence and current trypanosome infection. *Annals of Tropical Medicine and Parasitology*, **100** (4): 297-305.

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The seroprevalence of trypanosomiasis and the prevalence of current trypanosome infection in water buffaloes from the most important livestock areas of Venezuela were evaluated by IFAT and the microhaematocrit centrifugation technique, respectively. The usefulness of a PCR-based assay for identifying the trypanosome species in the buffaloes was also evaluated. Of the 644 animals investigated, 40 (6.2 percent) were found infected with trypanosomes by blood centrifugation, and 196 (30.4 percent) were found positive for anti-trypanosome antibodies, by IFAT. The results of the PCR-based assay indicated that 92.5 percent of the animals with current infections were infected with *Trypanosoma vivax* and the rest with *T. theileri* (the first molecular confirmation of *T. theileri* in Venezuelan water buffaloes). The national programme to treat and prevent trypanosome infections in the buffaloes does not appear to be meeting with great success, even though it is focused on *T. vivax*. Although the level of parasitaemia was categorized as low for 28 (70 percent) of the infections detected (and packed-cell volumes appeared to be unassociated with the IFAT results, and uncorrelated in the infected animals with level of parasitaemia), the 40 infected buffaloes had a significantly lower mean packed-cell volume than the uninfected animals ( $P < 0.05$ ). Farmers should therefore be made aware of the probability of trypanosome-attributable losses in buffalo productivity.

13678. **Muhammad, G., Jabbar, A., Iqbal, Z., Athar, M. & Saqib, M., 2006.** A preliminary passive surveillance of clinical diseases of cart pulling camels in Faisalabad metropolis (Pakistan). *Preventive Veterinary Medicine*, **76** (3-4): 273-279.

Department of Veterinary Clinical Medicine and Surgery, University of Agriculture, Faisalabad 38040, Pakistan.

We identified clinical disorders of all 200 city-dwelling cart pulling male camels attending the Veterinary Teaching Hospital, University of Agriculture, Faisalabad, Pakistan during a 7-year period (1993-1999). Data were collected prospectively on a predesigned form and collated. Diagnoses of different diseases/disorders were based on clinical examination supplemented with relevant laboratory tests. A total of 463 entries of 34 different clinical diseases/disorders were recorded. Sarcoptic mange (35 percent of 200 camels) followed by anhidrosis (23 percent) and trypanosomiasis (19 percent) were the three most frequently encountered disorders. The body system most often involved was the integument (31 percent) followed by gastrointestinal (21 percent), locomotory (12 percent), thermoregulatory (6 percent), blood (6 percent), urogenital (6 percent), lymphatic (3 percent), nervous (3 percent), respiratory (3 percent) and ocular (3 percent).

13679. **Singla, L.D.; Aulakh, G. S.; Juyal, P.D. & Singh, J., 2004.** Bovine trypanosomosis in Punjab, India. In: *Proc. 11th International Conference of the Association of Institutions for Tropical Veterinary Medicine and 16th Veterinary Association Malaysia Congress*, Petaling Jaya, Malaysia, pp. 283-285.

Department of Veterinary Parasitology, College of Veterinary Science, Punjab Agricultural University, Ludhiana-141004, India.

Trypanosomosis (Surra), caused by mechanically transmitted blood protozoan *Trypanosoma evansi*, is a widely prevalent serious haemoprotozoan disease of domestic animals of considerable economic importance. The impact of "Surra" has been underestimated in bovines because they usually suffer from sub-clinical infection; however, various stress factors result in flaring up of dormant infection. In the present study the prevalence, clinical signs, haemato-pathological, biochemical, immunomodulatory and chemotherapeutic response in natural and experimental trypanosomosis in bovines is discussed. The percentage of prevalence of "Surra" was found to be 7.92, with more cases of subclinical nature in buffaloes than in cattle. An outbreak of dexamethasone flared up trypanosomosis was also reported. Haemato-biochemical changes revealed anaemia, leucocytosis, neutrophilia, lymphopaenia, increase in blood urea nitrogen, circulating immune complexes and immunoglobulins. Marked improvement in these parameters was observed after treatment. Experimentally dexamethasone immunomodulation of *T. evansi* infected male buffalo calves increased parasitaemia, intensity of clinical signs and mortality. Severe anaemia was observed in immunomodulated calves. The biochemical parameters were adversely affected. Higher levels of pyruvate corresponded with parasitaemia. Relapse of parasitaemia may be due to sequestering of parasites into central parenchyma and cerebrospinal fluid. Prognosis of "Surra" is good if diagnosed and treated with trypanocides in early stages of infection.

13680. **Sinshaw, A., Abebe, G., Desquesnes, M. & Yoni, W., 2006.** Biting flies and *Trypanosoma vivax* infection in three highland districts bordering Lake Tana, Ethiopia. *Veterinary Parasitology*, **142** (1-2): 35-46.

Bureau of Agriculture, Amhara National Regional State, P.O. Box 437, Bahir Dar, Ethiopia.

An epidemiological study was conducted to determine the prevalence of trypanosomosis in cattle, small ruminants and *Equidae*, and to identify biting flies; potential mechanical vectors of trypanosomes in the three districts of Bahir Dar Zuria, Dembia and Fogera, bordering Lake Tana, Ethiopia. About 1509 cattle, 798 small ruminants and 749 *Equidae* were bled for the prevalence study using the buffy-coat method and the measurement of the hematocrit value. Sixty-six NGU and 20 monoconical traps were deployed for the fly survey. The results indicated the presence of trypanosomes in 6.1 percent (92/1509) of the cattle with a maximum during the late rainy season (9.6 percent) than during the early dry season (3.6 percent) at Fogera district. Prevalence at the district level varied from 4 percent to 9.6 percent. Only one sheep (1/122) and one goat (1/676) were found positive for *T. vivax*-like trypanosomes and none of the *Equidae* was positive. All the

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trypanosomes encountered in cattle belong to the single species of *T. vivax*. The PCV was negatively associated with detection of *T. vivax* (21.6 percent in infected versus 25.4 percent in non-infected cattle). A total of 55,398 biting flies were caught of which 49,353 (89.08 percent) belong to *Stomoxys*, 4,715 (8.51 percent) to horse flies and 1,330 (2.4 percent) to *Chrysops* species. There was no tsetse fly. Species identification has indicated the presence of *Atylotus agrestis*, *Chrysops streptobalia*, *Stomoxys calcitrans*, *S. nigra*, *S. pulla*, *S. pallida*, *S. sitiens*, *S. taeniata*, *S. uruma*, *Haematopota lasiops* and *Hippobosca variegata*. The overall apparent density was 214.7 flies/trap/day. Seasonal comparison showed higher fly catches in the late rainy season than the early dry season. This study indicated that *T. vivax* infections culminate in cattle at the same time as mechanical vectors such as *Stomoxys* sp. and *Atylotus agrestis*. Therefore, attention towards *T. vivax* infection in cattle is essential to control the impact of the disease on productivity. A further study on biting flies is recommended.

#### (c) TRYPANOTOLERANCE

13681. **Jaitner, J., Njie, M., Corr, N. & Dempfle, L., 2006.** Milk production of West African Dwarf goats in the Gambia. *Tropical Animal Health and Production*, **38** (3): 261-266.

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Goats are important in the low-input systems of West Africa and their main importance lies in their role for income and saving. In addition, it is known that milk offtake for home consumption is also important. In order to obtain information about the real importance of milk offtake, a recording scheme was operated in 27 villages in the Central River Division of The Gambia from July 1998 until January 2000. Detailed information was obtained from about 1,500 kiddings. In the recording scheme, any sheep being milked as well as the goats of the International Trypanotolerance Centre nucleus flock were also recorded. In the villages, 36 percent of all lactations were used for milk offtake, but the fraction milked was lower for the first two lactations. The average length of lactation was 127 days and the average daily milk offtake was 0.18 litres. Goats are milked once a day and the residual milk is left for the kids. Milking starts about one week after parturition and stops when the goat becomes pregnant or the kid(s) die or the goat is drying off. The repeatability of the 90-day milk offtake was 0.24 +/- 0.09. Sixty-five percent of goat owner were women and a large fraction of goat owners also owned cattle. Goat milk was used exclusively for home consumption. It is concluded that in breeding and extension work more attention should be given to aspects of milk production.

13682. **Lemecha, H., Mulatu, W., Hussein, I., Rege, E., Tekle, T., Abdicho, S. & Ayalew, W., 2006.** Response of four indigenous cattle breeds to natural tsetse and trypanosomiasis challenge in the Ghibe valley of Ethiopia. *Veterinary Parasitology*, **141** (1-2): 165-176.

National Animal Health Research Center, Sebeta, Ethiopia.

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A comparative study on the response of four indigenous cattle breeds of Ethiopia, namely Abigar, Horro, Sheko and Gurage, to natural challenge of trypanosomiasis in the Tolley-Gullele area of the Ghibe valley has been undertaken from August 2000 until August 2004. Fifty female yearlings each of Horro, Sheko and Abigar and 31 of the Gurage were purchased from their natural habitats and introduced in to medium to high tsetse-trypanosomiasis challenge area of the Ghibe valley. While the natural habitats of first three breeds are naturally infested with tsetse flies and trypanosomiasis, that of the Gurage is known to be very minimal, if any, and hence the Gurage breed was used in this study as the known susceptible breed. During the study animal health, production performance and tsetse fly situation were monitored monthly. The Sheko breed has manifested very significantly ( $p < 0.001$ ) high overall average packed cell volume (PCV) values (25 percent) compared to that of Abigar (24 percent), Horro (23 percent) and Gurage (22 percent). It also had the lowest mean trypanosome prevalence rate of 9 percent against 23 percent of Horro, 26 percent of Abigar and 27 percent of Gurage, and the least number of Berenil treatments (1.36) compared to Abigar (4.0), Horro (4.6) and Gurage (6.7). While the Abigar manifested high sensitivity and frequent death to PCV depression, the Horro showed strong resilience to PCV depression and better response to Berenil treatment assistance. At this stage the Sheko breed was also found to be equal to the other breeds in its reproductive performance. These results need to be substantiated with further in-depth investigation including immune response, animal behaviour and environmental influences.

13683. **O'Gorman, G.M., Park, S.D., Hill, E.W., Meade, K.G., Mitchell, L.C., Agaba, M., Gibson, J.P., Hanotte, O., Naessens, J., Kemp, S.J. & Machugh, D.E., 2006.** Cytokine mRNA profiling of peripheral blood mononuclear cells (PBMC) from trypanotolerant and trypanosusceptible cattle infected with *Trypanosoma congolense*. *Physiological Genomics*. **In press; corrected proof.**

School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Dublin, Belfield, Ireland.

To examine differences in cytokine profiles that may confer tolerance/susceptibility to bovine African trypanosomiasis, N'Dama (trypanotolerant,  $n = 8$ ) and Boran (trypanosusceptible,  $n = 8$ ) cattle were experimentally challenged with *Trypanosoma congolense*. Blood samples were collected over a 34 day period and RNA was extracted from peripheral blood mononuclear cells (PBMC). The expression levels of a panel of 14 cytokines were profiled over the time course of infection and between breeds. Messenger RNA (mRNA) transcript levels for the IL2, IL8 and IL1RN genes were significantly downregulated across the time course of infection in both breeds. There was an early increase in transcripts for genes encoding proinflammatory mediators (IFNG, IL1A, TNF and IL12) in N'Dama by 14 days post infection (dpi) compared to pre-infection levels that was not detected in the susceptible Boran breed. By the time of peak parasitaemia, a TH2-like cytokine environment was prevalent, which was particularly evident in the Boran. Increases in transcripts for the IL6 (29 and 34 dpi) and IL10 (21, 25 and 29 dpi) genes were detected that were higher in the Boran compared to N'Dama. These findings highlight the implications for using murine models to study the bovine immune response to trypanosomiasis, where in some cases cytokine expression patterns differ. Overall, these data suggest that the trypanotolerant N'Dama are more capable of responding very early in infection with

proinflammatory and TH1 type cytokines than the trypanosusceptible Boran and may explain why N'Dama control parasitaemia more efficiently than Boran during the early stages of infection.

(d) TREATMENT

[See also **29**: 13651, 13653, 13736, 13776, 13777, 13828, 13865, 13875, 13880, 13959, 13973, 14008, 14024]

13684. **Awa, D.N. & Ndamkou, C.N., 2006.** Response of *Trypanosoma vivax* and *Trypanosoma congolense* in zebu cattle in North Cameroon to prophylactic treatment with two formulations of isometamidium. *Preventive Veterinary Medicine*, **76** (1-2): 90-96.

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We tested the efficacy of two formulations of isometamidium in a tsetse-infested farm in North Cameroon from 20 August 2000 to 5 January 2001. A total of 90 adult cattle were used in three groups of 30 each corresponding to two treated and one untreated control. Drug efficacies were evaluated in terms of reduction of parasite incidence in the host's blood, maintenance of packed-cell volume (PCV) and weight gains. Both drugs reduced the incidence of parasites even though re-infections 2 weeks after treatment were common. PCV values were similar in both treated groups but higher than in the untreated control. Body weight changes followed a similar trend with the control losing weight from a mean of 427+/-119kg at the beginning to 398+/-93kg in 4 months. Weights increased from 375+/-76 and 396+/-110 to 396+/-69 and 418+/-112kg in the Veridium and Trypamidium groups, respectively. Efficacy was similar between the two formulations of isometamidium in the prophylaxis of bovine trypanosomosis. However, the presence of parasites in some animals barely 2 weeks after treatment suggested that either infections were not cleared or residual drug effects were not sufficient to prevent re-infections.

13685. **Clausen, P.H., 2005.** Studies on the diagnosis, development and distribution of drug-resistant trypanosomes in cattle herds in selected areas of East and West Africa. *Thesis, Freie Universitat Berlin*. 136 pp.

Institut für Parasitologie und Tropenveterinärmedizin, Königsweg 67, 14163 Berlin, Germany.

This study aimed to assess the development and distribution of drug-resistant trypanosomes in cattle herds in various sites in East Africa (Metekel, north west. Ethiopia; Upper Didessa Valley, west Ethiopia; Mukuno County, south east Uganda) and West Africa (province of Kenedougou, south west Burkina Faso). Longitudinal field studies were done to estimate the incidence of trypanocidal drug resistance in high risk areas. Several *in vivo* and *in vitro* tests were used to characterize the drug sensitivity of trypanosome field strains. The polymerase chain reaction was evaluated and its diagnostic potential to monitor the efficacy of prophylactic and curative treatments tested. Isometamidium resistance was widespread in *Trypanosoma congolense* in Metekel, Upper Didessa Valley and Kenedougou, but its

incidence varied between villages. Resistance to diminazene was also demonstrated in the various tests done. No resistance of trypanosomes to the drugs commonly used in cattle in Mukuno could be detected. The PCR proved to be a highly sensitive and specific tool to monitor the therapeutic and prophylactic efficacy and disease progression in bovine trypanosomiasis. Recommendations are given for measures to avoid or delay the development of resistance and to maintain the efficacy of currently available drugs. This thesis contains reprints of 14 papers published by the author, either as main or co-author, between 1992 and 2006 in the scientific literature.

13686. **Machila, N., Emongor, R., Shaw, A.P., Welburn, S.C., McDermott, J., Maudlin, I. & Eisler, M.C., 2006.** A community education intervention to improve bovine trypanosomiasis knowledge and appropriate use of trypanocidal drugs on smallholder farms in Kenya. *Agricultural Systems*. **In press; corrected proof.**

University of Edinburgh, Royal (Dick) School of Veterinary Studies, Centre for Tropical Veterinary Medicine, Kenya Agriculture Research Institute, Muguga, Kenya, and International Livestock Research Institute, Nairobi, Kenya.

This paper describes the development, design, dissemination and evaluation of a communication intervention designed to promote appropriate usage of trypanocidal drugs in trypanosomiasis endemic areas of western and coastal Kenya. Following a baseline study on current trypanosomiasis knowledge, attitudes and practices by smallholder farmers, a communication intervention strategy was developed involving dissemination through school children, village elders, animal health centres and Agrovets shops, and using layered messages in posters and leaflets. A participatory research approach was used to develop, design and assess the impact of animal health messages on the control of bovine trypanosomiasis for smallholder farmers in tsetse and trypanosomiasis endemic areas in Busia (two administrative divisions) and Kwale Districts (two administrative divisions) of Kenya. Communication intervention materials (in poster and leaflet formats) were developed and disseminated to residents in villages in one administrative division in each district (intervention area) while those from the other division in each district were not deliberately exposed to the animal health messages (control area). Several communication impact indicators were derived and these were measured 4–6 weeks after dissemination of the print media through questionnaires on trypanosomiasis knowledge administered to school children and cattle-keeping smallholders in the intervention and control study sites. School children's post-communication intervention trypanosomiasis signs knowledge was much higher than that observed during the pre-communication intervention survey. More trypanocides were named by school children during the post-intervention questionnaire survey compared to those known during the pre-intervention survey. The trypanosomiasis signs knowledge score obtained by the smallholder farmers exposed to the extension materials was higher than that obtained by those not exposed to them. Similarly, farmers' exposure to extension materials resulted in higher trypanocidal drug knowledge scores among exposed farmers than among those not exposed. These results indicate that over the period monitored, the routes (i.e. school children, village elders, animal health centres and Agrovets shops) and media (posters and leaflets) selected were effective in promoting a significant increase in knowledge of trypanosomiasis its causes and ways of dealing with it among livestock keepers.



13687. **Somda, J., Kamuanga, M., & Tollens, E., 2006.** Prospective analysis for community participation in trypanosomiasis control in The Gambia. *Tropical Animal Health and Production*, **38** (2): 103-111.

International Trypanotolerance Centre, Banjul, The Gambia, and Department of Agricultural and Environmental Economics, Katholieke Universiteit Leuven, Belgium; International Livestock Research Institute, Bobo Dioulasso, Burkina Faso; Department of Agricultural and Environmental Economics, Katholieke Universiteit Leuven, Belgium.

The shift towards community participation in the eradication of trypanosomiasis calls for the investigation of the underlying incentive structure for individuals in the community to cooperate in the provision of various control methods. Survey data were used to assess patterns of the community's demand for insecticide pour-ons and trypanocidal drugs and factors affecting individual demand in The Gambia. It was shown that insecticide pour-on formulations are strongly preferred. Similarly, farmers revealed a preference for community-based provision scheme. Factors affecting an individual farmer's decision to invest in either pour-on or trypanocidal drugs were highlighted. While there are many factors associated with farmers' decisions to invest in trypanosomiasis control methods and to participate in collective actions, the results indicate that farmers are ready to anticipate complete privatization of veterinary services through community-based schemes.

## 7. EXPERIMENTAL TRYPANOSOMIASIS

### (a) DIAGNOSTICS

[See also 29: 13619]

13688. **Agranoff, D., Stich, A., Abel, P. & Krishna, S., 2005.** Proteomic fingerprinting for the diagnosis of human African trypanosomiasis. *Trends in Parasitology*, **21** (4): 154-157.

Department of Cellular and Molecular Medicine (Infectious Diseases), St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK.

Papadopoulos *et al.* recently reported the discovery of a diagnostic serum proteomic signature for human African trypanosomiasis (HAT), using a combination of surface-enhanced laser desorption-ionization time-of-flight (SELDI-TOF) mass spectrometry and data-mining algorithms. This novel approach, coupled with biochemical characterization of the proteins that contribute to the signature, provides powerful new tools for the development of improved diagnostic tests, disease staging and identification of potential novel drug targets in HAT.

13689. **Aradaib, I.E. & Majid, A.A., 2006.** A simple and rapid method for detection of *Trypanosoma evansi* in the dromedary camel using a nested polymerase chain reaction. *Kinetoplastid Biology and Disease*, **5**: 2.

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A nested polymerase chain reaction (nPCR)-based assay, was developed and evaluated for rapid detection of *Trypanosoma evansi* in experimentally infected mice and naturally infected camels (*Camelus dromedarius*). Four oligonucleotide primers (TE1, TE2, TE3 and TE4), selected from nuclear repetitive gene of *T. evansi*, were designed and used for PCR amplifications. The first amplification, using a pair of outer primers TE1 and TE2, produced a 821-bp primary PCR product from *T. evansi* DNA. The second amplification, using nested (internal) pair of primers TE3 and TE4, produced a 270-bp PCR product. *T. evansi* DNAs extracted from blood samples of experimentally infected mice and naturally infected Sudanese breed of dromedary camels were detected by this nested PCR-based assay. The nested primers TE3 and TE4 increased the sensitivity of the PCR assay and as little as 10 fg of *T. evansi* DNA (equivalent to a single copy of the putative gene of the parasite) was amplified and visualized onto ethidium bromide-stained agarose gels. Amplification products were not detected when the PCR-based assay was applied to DNA from other blood parasites including *Thieleria annulata*, *Babesia bigemina* or nucleic acid free samples. Application of this nPCR-based assay to clinical samples resulted in direct detection of *T. evansi* from a variety of tissue samples collected from experimentally infected mice and blood from naturally infected camels. The described nPCR-based assay provides a valuable tool to study the epidemiology of *T. evansi* infection in camels and other susceptible animal populations.

13690. **Gonzales, J.L., Loza, A. & Chacon, E., 2006.** Sensitivity of different *Trypanosoma vivax* specific primers for the diagnosis of livestock trypanosomosis using different DNA extraction methods. *Veterinary Parasitology* **136** (2): 119-126.

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There are several *T. vivax* specific primers developed for PCR diagnosis. Most of these primers were validated under different DNA extraction methods and study designs leading to heterogeneity of results. The objective of the present study was to validate PCR as a diagnostic test for *T. vivax* trypanosomosis by means of determining the test sensitivity of different published specific primers with different sample preparations. Four different DNA extraction methods were used to test the sensitivity of PCR with four different primer sets. DNA was extracted directly from whole blood samples, blood dried on filter papers or blood dried on FTA cards. The results showed that the sensitivity of PCR with each primer set was highly dependant of the sample preparation and DNA extraction method. The highest sensitivities for all the primers tested were determined using DNA extracted from whole blood samples, while the lowest sensitivities were obtained when DNA was extracted from filter paper preparations. To conclude, the obtained results are discussed and a protocol for diagnosis and surveillance for *T. vivax* trypanosomosis is recommended.

13691. **Lejon, V., Claes, F., Verloo, D., Maina, M., Urakawa, T., Majiwa, P.A.O. & Buscher, P., 2005.** Recombinant RoTat 1.2 variable surface glycoprotein as

antigen for diagnosis of *Trypanosoma evansi* in dromedary camels. *International Journal for Parasitology*, **35** (4): 455-460.

Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium.

The transcript encoding a predominant *Trypanosoma evansi* variable surface glycoprotein RoTat 1.2 was cloned and expressed as a recombinant protein in *Spodoptera frugiperda* and *Trichoplusia ni* (insect) cells. Its potential as an antigen for specific detection of antibody in serum of dromedary camels affected by surra, was evaluated. In ELISA, the reactivity of the recombinant RoTat 1.2 VSG was similar to that of native RoTat 1.2 VSG. An indirect agglutination reagent was therefore prepared by coupling the recombinant RoTat 1.2 VSG onto latex particles. The performance of the latex agglutination test was evaluated on camel sera, and compared with the performance of CATT/*T. evansi* and LATEX/*T. evansi* tests, using the immune trypanolysis assay with *T. evansi* RoTat 1.2 as a reference test. The relative sensitivity and specificity of the latex coated with recombinant RoTat 1.2 VSG, using a 1:4 serum dilution, were respectively, 89.3 and 99.1 percent. No differences were observed between the performance of latex coated with recombinant RoTat 1.2 VSG and LATEX/*T. evansi* or CATT/*T. evansi*. Here, we describe the successful use of the recombinant RoTat 1.2 VSG for detection of specific antibodies induced by *T. evansi* infections.

13692. **Li, F.J., Gasser, R.B., Lai, D.H., Claes, F., Zhu, X.Q. & Lun, Z.R., 2006.** PCR approach for the detection of *Trypanosoma brucei* and *T. equiperdum* and their differentiation from *T. evansi* based on maxicircle kinetoplast DNA. *Molecular and Cellular Probes*. **In press; corrected proof.**

Center for Parasitic Organisms and State Key Laboratory of Biocontrol, School of Life Sciences, Zhongshan (Sun Yat-sen) University, Guangzhou 510275, PR China.

The goal of this study was to develop a PCR approach based on the sequence of maxicircle kinetoplast DNA (kDNA) of *Trypanosoma brucei* to distinguish *T. brucei*/*T. equiperdum* from *T. evansi* and to evaluate its diagnostic use for their detection in blood samples. Primers derived from the sequence of the maxicircle kDNA of *T. brucei*, encoding the NADH dehydrogenase subunit 5 (*nad5*) gene, were used to test the PCR-amplification from *T. brucei* (including *T. b. brucei* and *T. b. rhodesiense*), *T. equiperdum*, *T. evansi*, *T. vivax* and *T. congolense*. A primer pair to a nuclear DNA region incorporated into a separate PCR was employed to control for the presence of amplifiable genomic DNA (representing the subgenus *Trypanozoon*) in each sample subjected to the PCR. Products of approximately 395bp were amplified from all *T. brucei* and *T. equiperdum* samples tested using the *nad5*-PCR, but not from *T. evansi* DNA samples or any of the control samples representing *T. vivax*, *T. congolense*, or host. The current PCR approach allows the rapid differentiation of *T. brucei*/*T. equiperdum* from *T. evansi* and can detect the equivalent of 20-25 cells of *T. brucei* or *T. equiperdum* in purified genomic DNA or infected blood samples.

13693. **Thekiso, O.M.M., Inoue, N., Kuboki, N., Tuntasuvan, D., Bunnoy, W., Borisutsuwan, S., Igarashi, I. & Sugimoto, C., 2005.** Evaluation of loop-

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mediated isothermal amplification (LAMP), PCR and parasitological tests for detection of *Trypanosoma evansi* in experimentally infected pigs. *Veterinary Parasitology*, **130** (3-4): 327-330.

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

Six surra negative piglets (6-week-old) were infected with *Trypanosoma evansi* and two uninfected piglets were used as negative controls. Detection performances of various diagnostic tests (LAMP, PCR and parasitological tests) were compared by analysing blood samples collected weekly over a period of 11 weeks. With a two by two analysis without a gold standard, all methods were 100 percent specific. MI had the highest sensitivity of 65 percent, while LAMP, PCR, MHCT and TBS had sensitivities of 45, 33, 38 and 24 percent, respectively. However, when the analysis was done using MI as a gold standard, the sensitivity of MHCT was the highest at 53 percent followed by LAMP, PCR and TBS at 49, 44 and 35 percent, respectively. All methods gave high specificity above 60 percent. This study validates LAMP as an alternative method for the diagnosis of surra.

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13694. **Abenga, J.N., David, K., Ezebuio, C.O.G. & Lawani, F.A.G., 2005.** Observations on the tolerance of young dogs (puppies) to infection with *Trypanosoma congolense*. *African Journal of Clinical and Experimental Microbiology*, **6** (1): 28-33.

Nigerian Institute for Trypanosomiasis Research, PMB 2077, Kaduna, Nigeria.

Studies were undertaken to assess the susceptibility of young local dogs to infection with *Trypanosoma congolense*. Six puppies (7 weeks old) were used for the study. Although the puppies became parasitaemic 6 to 7 days post infection, they were tolerant to infection as the parasitaemia remained low throughout the first seven weeks of the eight-week observation period. The packed cell volume (PCV) also only dropped slightly during the last four weeks attaining the value of 25.6+3.8 ( $p>0.05$ ) by the eighth week while the mean body weight continued to increase. Similarly, the mean daily body temperature did not differ significantly from that of uninfected control. The significance of trypanotolerance in Nigerian local dogs is discussed.

13695. **Adamu, S., Fatihu, M.Y., Useh, N.M., Mamman, M., Sekoni, V.O. & Esievo, K.A., 2006.** Sequential testicular and epididymal damage in Zebu bulls experimentally infected with *Trypanosoma vivax*. *Veterinary Parasitology*. **In press; corrected proof.**

Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

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Six Zebu bulls aged between 31 and 34 months exhibiting good libido were used to study sequential testicular and epididymal damage in *Trypanosoma vivax* infection. Three bulls were infected with *T. vivax*, while the other three served as controls. All infected bulls became parasitaemic by day 5 post-infection and developed clinical trypanosomosis with rapidly developing anaemia. Representative bulls, one from each of the infected and control groups, were sacrificed on days 14, 28 and 56 post-infection. Testes and epididymides from these animals were studied histopathologically after processing and staining with haematoxylin and eosin (H and E). Testicular degeneration developed in all the infected bulls characterized by depletion of spermatogenic cells and destruction of interstitial tissue. The most severe testicular degeneration occurred in the bull that was sacrificed 56 days post-infection. Epididymal sperm reserves were 36 percent, 4 percent and 0 percent, respectively, in infected bulls that were sacrificed on days 14, 28 and 56 post-infection. The 0 percent epididymal sperm reserve may suggest complete cessation of spermatogenesis. It was concluded from this study that *T. vivax* infection of Zebu bulls could cause severe testicular and epididymal damage that may result in infertility or even sterility of the affected animals at early infection stages not previously thought.

13696. **Akinwale, O.P., Nock, I.H., Esievo, K.A.N., Edeghere, H.U. & Olukosi, Y.A., 2006.** Study on the susceptibility of Sahel goats to experimental *Trypanosoma vivax* infection. *Veterinary Parasitology*, **137** (3-4): 210-213.

Public Health Division, Nigerian Institute of Medical Research, PMB2013  
Yaba, Lagos, Nigeria.[pheabian@yahoo.co.uk].

Sahel goats, also known as Borno whites are found in the northern semi-arid, tsetse free Sahel region of Nigeria. They are transported alongside cattle from this zone to all other zones in the country, including the tsetse-infested zones, for commercial purposes and are kept for some time in these tsetse-infested zones until they are sold. This study therefore assessed the susceptibility of this breed of goats to trypanosome infection and its response to treatment with Berenil. Six bucks were inoculated intravenously with *Trypanosoma vivax* through the jugular vein while two served as uninfected control. The mean pre-patent period was 4.5 days and increasing parasitaemia followed the establishment of infection. Onset of parasitaemia was associated with increase in rectal temperature in all the infected goats and the temperature peak coincided with the only parasitaemic peak second week post-infection. The infected goats were treated with Berenil (Hoechst, Germany) 3.5 mg/kg body weight at 4 weeks post-infection. The packed cell volume (PCV) continued to fall from a mean of 30.73 pre-infection to a mean 13.21 at 1 week post-treatment. Deaths were recorded for 4 of the infected goats 1 week post-treatment while the remaining two died 2 weeks post-treatment, not responding to treatment.

13697. **Al-Mohammed, H.I., 2006.** Parasitological and immunological studies on rats experimental infected with Saudi Arabian strain of *Trypanosoma evansi*. *Journal of the Egyptian Society of Parasitology*, **36** (2): 363-371.

College of Medicine, King Faisal University, P.O. Box 55017, Al-Ahsa, 31982,  
Saudi Arabia. [hamdan@kfu.edu.sa]

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Surra, an enzootic disease caused by *Trypanosoma evansi*, is one of the most important trypanosomiasis in Kingdom of Saudi Arabia. The state of parasitaemia in relation to the corresponding humoral response in experimentally infected Wister rats for 15 days were investigated. The prepatent period was found to be 5 days. The disease was characterized by intermittent fluctuation of parasitaemia and a significant difference in the level of parasitaemia ( $P > 0.05$ ) was detected in specimens of day 7, 9, 13 & 14. There was a difference in the mean number of blood parasites in relation to sex throughout the 15 days of the study. This difference was statistically significant ( $p < 0.05$ ). Using indirect haemagglutination serological test, almost all inoculated rats displayed specific antibodies of diagnostic value on day 7 after infection ranging between 1/80-1/160. Thereafter, antibody titres increased progressively to reach very high positive dilutions of  $> 2,048$  in all animals at the end of study on day 15. No sex difference could be observed in both serological specimens of 7 & 15 days. Also no correlation was observed between the state of parasitaemia and the serological titres in infected rats.

13698. **Bhasin, K.K., Yu, J.M., Tward, A., Shih, D., Campbell, D.A. & Lulis, 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.

13699. **Biryomumaisho, S. & Katunguka-Rwakishaya, E., 2006.** The pathogenesis of anaemia in goats experimentally infected with *Trypanosoma congolense* or *Trypanosoma brucei*: use of the myeloid:erythroid ratio. *Veterinary Parasitology*. **In press; corrected proof.**

Department of Veterinary Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda.

The present study examined the development of anaemia in Small East African goats experimentally infected with *Trypanosoma congolense* or *Trypanosoma brucei*. Experimental

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goats received a primary trypanosome challenge on day 0, treated with diminazene aceturate on day 49 and received a secondary trypanosome challenge on day 77 of the 136-day experiment. Both primary and secondary challenges were characterised by reduced peripheral erythrocyte counts, fall in packed cell volume (PCV), hypohaemoglobinaemia and reductions in the myeloid:erythroid ratios (M:E) compared with the uninfected goats. The progressive reduction in the M:E ratios denoted increased erythropoiesis in response to increased destruction of erythrocytes in blood by infecting trypanosomes or their products. The more rapid fall in M:E ratio in *T. congolense* infections shows that this parasite causes more severe clinical pathological effects in goats than *T. brucei*.

13700. **Budovsky, A., Prinsloo, I. & El-On, J., 2006.** Pathological developments mediated by cyclophosphamide in rats infected with *Trypanosoma lewisi*. *Parasitology International*, **55** (4): 237-242.

Department of Microbiology and Immunology, Ben-Gurion University of the Negev, Israel.

*Trypanosoma lewisi* is an obligatory, flagellated parasite of the rat. Despite the fact that naturally the rats overcome the disease, a lethal infection can be induced by the administration of an immunosuppressive agent, i.e. cyclophosphamide (Cy). In the Cy treated infected rats (CyI) the severity of the trypanosome infection was demonstrated in the internal organs, in the following order: lungs>liver>heart>spleen>kidney. The parasites were not detected in the brain. The accumulation of the parasites in the lungs led to the development of hemorrhagic inflammatory foci. The rupture of blood vessels was accompanied by lymphocyte infiltrations into the damaged tissues and multiple foci of oedema around the blood vessels. In most cases the lungs were dark brown in colour due to intra-alveolar haemorrhages. The spleen of the CyI rats showed general deformation of the tissue's architecture, migration of macrophages and cell depletion due to the Cy action. The liver showed inflammatory hemorrhagic foci associated with massive destruction of the parenchyma. In spite of the heavy parasite (>50 percent) developed in the CyI rats the brain remained free of parasites, which might explain the non-virulent character of this parasite compared to the African trypanosomes.

13701. **Caljon, G., Van Den Abbeele, J., Stijlemans, B., Coosemans, M., De Baetselier, P. & Magez, S., 2006.** Tsetse fly saliva accelerates the onset of *Trypanosoma brucei* infection in a mouse model associated with a reduced host inflammatory response. *Infection and Immunity*, **74** (11): 6324-6330.

Unit of Cellular and Molecular Immunology, Flanders Interuniversity Institute for Biotechnology (VIB), Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium. [gucaljon@vub.ac.be]

Tsetse flies (*Glossina* sp.) are the vectors that transmit African trypanosomes, protozoan parasites that cause human sleeping sickness and veterinary infections in the African continent. These blood-feeding dipteran insects deposit saliva at the feeding site that enables the blood-feeding process. Here we demonstrate that tsetse fly saliva also accelerates the onset of a *Trypanosoma brucei* infection. This effect was associated with a reduced

inflammatory reaction at the site of infection initiation (reflected by a decrease of interleukin-6 [IL-6] and IL-12 mRNA) as well as lower serum concentrations of the trypanocidal cytokine tumour necrosis factor. Variant-specific surface glycoprotein-specific antibody isotypes immunoglobulin M (IgM) and IgG2a, implicated in trypanosome clearance, were not suppressed. We propose that tsetse fly saliva accelerates the onset of trypanosome infection by inhibiting local and systemic inflammatory responses involved in parasite control.

13702. **Ching, S., He, L., Lai, W. & Quan, N., 2005.** IL-1 type I receptor plays a key role in mediating the recruitment of leukocytes into the central nervous system. *Brain, Behavior, and Immunity*, **19** (2): 127-137.

Department of Oral Biology, Ohio State University, Columbus, OH 43210-1094, USA.

This study investigates the role of type I IL-1 receptor (IL-1R1) in mediating the recruitment of leukocytes into the brain parenchyma in mice. Chronic infection with *Trypanosoma brucei* resulted in the recruitment of T cells, but no other cell types, into the brain. This did not occur in IL-1R1-knockout mice. Thus, IL-1R1 appears to be important for the recruitment of leukocytes across the blood-brain barrier.

13703. **Dargantes, A.P., Campbell, R.S.F., Copeman, D.B. & Reid, S.A., 2005.** Experimental *Trypanosoma evansi* infection in the goat. II. Pathology. *Journal of Comparative Pathology*, **133** (4): 267-276.

College of Veterinary Medicine, Central Mindanao University, Musuan 8710, Bukidnon, Philippines.

Infection of male goats aged 8-10 months with 5,000 or 50,000 organisms of a Mindanao strain of *Trypanosoma evansi* was observed over a period of 90 days. The infection induced clinical disease which was lethal, especially at the higher dose rate. Lesions were more acute in goats that received the higher dose. Gross and microscopical changes were not pathognomonic, except in the presence of demonstrable trypanosomes. At necropsy, a combination of lymphadenopathy, splenomegaly, hepatomegaly, testicular enlargement, anaemic signs and consolidation of the anterior lobes of the lungs was suggestive of surra. Testicular changes, especially aspermia, indicated probable infertility. The cytopathology of the lungs, liver, intestine, kidneys, testes, bone marrow, brain and other organs was immunological in nature, characterized by mononuclear infiltration of interstitial tissues, with minor cellular damage and the presence of trypanosomes. B- and T- cell responses were observed in the lymphatic system, but the findings indicated immunosuppression in the lymph nodes, spleen and bone marrow during the third month after infection. Exudative inflammatory changes were mild. It is suggested that the cytopathology of most haemophilic trypanosomal infections is predominantly an immunological process.

13704. **Dargantes, A.P., Reid, S.A. & Copeman, D.B., 2005.** Experimental *Trypanosoma evansi* infection in the goat. I. Clinical signs and clinical pathology. *Journal of Comparative Pathology*, **133** (4): 261-266.



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College of Veterinary Medicine, Central Mindanao University, Musuan 8710, Bukidnon, Philippines.

A strain of *Trypanosoma evansi* isolated from an equine case of surra in Mindanao, Philippines was used to infect intravenously two groups (A and B) of five male goats aged 8-10 months. Animals of groups A and B received 5,000 and 50,000 trypanosomes, respectively, and five further animals (group C) served as uninfected controls. Four of the 10 infected goats died 8-78 days after inoculation. Group C goats gained weight (mean 22.8 g/day) while infected goats in groups A and B lost weight (means of 21.4 and 45.0 g/day, respectively). Parasitaemia fluctuated regularly between peaks and troughs, with repeated periods of about 6 days during which no trypanosomes were detected in the blood. Clinical signs and clinico-pathological changes in infected goats were not pathognomonic in the absence of parasites in the blood, and leucocytosis was not a reliable indicator of infection. It was concluded that in endemic areas fluctuating fever, progressive emaciation, anaemia, coughing, testicular enlargement and diarrhoea are suggestive of surra; confirmation, however, may necessitate examination of blood every few days for trypanosomes, and possibly other diagnostic tests.

13705. **Egbe-Nwiyi, T.N., Nwaosu, A. & Gazdama, M.A., 2005.** The effects of oral manganese chloride supplementation on the severity of *Trypanosoma brucei* and *Trypanosoma congolense* infections in rats. *Veterinarski Arhiv*, **75**: 541-547.

Department of Veterinary Physiology and Pharmacology, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.

Eighty healthy adult albino rats of both sexes were used in two experiments to study the effects of manganese chloride supplementation on the severity of *Trypanosoma brucei* and *Trypanosoma congolense* infections. In each experiment, forty rats were divided into four groups of 10 each: A. infected unsupplemented; B. infected supplemented; C. uninfected unsupplemented control; d. Uninfected supplemented control. Aqueous solution (5 percent) of MnCl<sub>2</sub> was administered daily using stomach tube to each rat at 50 mg/kg body weight in groups b and d from 10 days before infection to the end of the experiment. Each rat in groups a and b was infected by intraperitoneal injection of 1x10<sup>6</sup> trypanosomes (*T. brucei* or *T. congolense*) in diluted donor blood. The prepatent periods were shorter (P<0.05) in *T. brucei* than *T. congolense* infections, and shorter (P<0.05) in infected unsupplemented than in infected supplemented rats. The infected unsupplemented groups had higher (P<0.05) parasitaemia and more severe anaemia than the infected supplemented groups. Therefore, oral manganese chloride supplementation in rats appeared to reduce the severity of trypanosome infections by delaying the onset of parasitaemia, reducing the levels of parasitaemia and accompanying anaemia.

13706. **Faye, D., Fall, A., Leak, S., Losson, B. & Geerts, S., 2005.** Influence of an experimental *Trypanosoma congolense* infection and plane of nutrition on milk production and some some biochemical parameters in West African Dwarf goats. *Acta Tropica*, **93** (3): 247-257.

International Trypanotolerance Centre, PMB 14, Banjul, The Gambia.

The interactions of trypanosomiasis and plane of nutrition on health and productivity of multiparous and primiparous West African Dwarf (WAD) does were studied in a multi-factorial experiment including diet (supplementation or basal diet).

13707. **Hilali, M., Abdel-Gawad, A., Nassar, A. & Abdel-Wahab, A., 2006.** Hematological and biochemical changes in water buffalo calves (*Bubalus bubalis*) infected with *Trypanosoma evansi*. *Veterinary Parasitology*. **In press, corrected proof.**

Parasitology Department, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.

Four water buffalo calves (*Bubalus bubalis*) were each inoculated intravenously with 106 *T. evansi* (camel isolate) and the fifth calf kept as non-infected control. The blood and sera of all calves were examined every 4 days during the first month post-inoculation (pi) and then once weekly until the end of the experiment (88 days pi). They were examined for haematological and biochemical changes, liver and kidney function tests. Haemoglobin concentration (Hb percent), packed cell volume (PCV) and red blood cell count were significantly decreased. Total leucocytic count, lymphocytes and monocytes showed significant increase. Liver function tests revealed significant elevation in the activity of lactate dehydrogenase enzyme (LDH), globulin, total bilirubin and indirect bilirubin while alkaline phosphatase enzyme showed significant decrease. Kidney function tests revealed significant decrease of both creatinine and urea.

13708. **Kagira, J.M.M., N. W., Thuita, J. K., Ngotho, M. & Hau, J., 2005.** Influence of cyclophosphamide on the haematological profile of laboratory bred African soft-furred rats (*Mastomys natalensis*). *Scandinavian Journal of Laboratory Animal Science*, **32** (3): 153-158.

Kenya Agricultural Research Institute, Trypanosomiasis Research Centre (KARI-TRC), P.O. Box 362, Kikuyu, Kenya

The African soft-furred rat (*Mastomys natalensis*) has been shown to be a possible model for propagation of *Trypanosoma brucei gambiense*. This study aimed at determining the baseline biological reference values and reproductive data of a laboratory bred *Mastomys natalensis* colony, which was established at TRC. In addition, the effect of cyclophosphamide (an immunosuppressant) treatment (s) on the haematological profile was investigated. The mean gestation period was 23 days and the mean litter size was eight. At birth, the pups weighed  $2.4 \pm 0.23$  g and the weights increased to  $78.0 \pm 10.6$  g in males and  $53.9 \pm 4.5$  g in females by 90 days. The mean haematological values were significantly ( $p < 0.05$ ) higher in adults than juveniles. However, there was no statistical difference of haematological values between the sexes. Cyclophosphamide treatment caused a macrocytic hypochromic anaemia, which was noted 24 hours after treatment and was more severe in animals treated more than once. Thus, in studies involving a disease that causes anaemia, repeated cyclophosphamide treatment should be limited. Our study is a contribution to the clinical and biological characterization of the disease pattern in this preferred rodent model of *T. b. gambiense*.

13709. **Agez, S., Radwanska, M., Drennan, M., Fick, L., Baral, T.N., Brombacher, F. & De Baetselier, P., 2006.** Interferon-gamma and nitric oxide in combination with antibodies are key protective host immune factors during *Trypanosoma congolense* Tc13 infections. *Journal of Infectious Diseases*, **193** (11): 1575-1583.

Laboratory of Cellular and Molecular Immunology, Department of Molecular and Cellular Recognition, Flanders Interuniversity Institute for Biotechnology, Vrije Universiteit Brussel, Brussels, Belgium.

The control of chronic *Trypanosoma congolense* trypanosomiasis was analyzed using several gene-deficient mouse strains. First, interferon (IFN)-gamma receptor (IFN-gamma-R)-deficient mice were used to show that IFN- gamma -mediated immune activation is crucial for parasitaemia control. Second, infections in major histocompatibility complex (MHC) class II-deficient mice indicate that this molecule is needed for initiation of IFN-gamma and subsequent tumour necrosis factor (TNF) production. Downstream of IFN-gamma-R signalling, inducible NO synthase (iNOS)-dependent trypanosome killing occurs, as is shown by the hypersusceptible phenotype of iNOS-deficient mice. Besides proinflammatory responses, B cells and, more specifically, immunoglobulin (Ig) G antibodies are crucial for parasite killing. Hence, parasitaemia control is abolished in B cell-deficient mice, whereas IgM-deficient mice control the infection as efficiently as do wild-type mice. In addition, splenectomized mice that have a normal IgM response but an impaired IgG2a/3 response fail to control *T. congolense* infection. Collectively, these results suggest that host protective immunity against *T. congolense* is critically dependent on the combined action of the proinflammatory mediators/effectors IFN- gamma , TNF, and NO and antiparasite IgGs.

13710. **Merschjohann, K. & Steverding, D., 2006.** *In vitro* growth inhibition of bloodstream forms of *Trypanosoma brucei* and *Trypanosoma congolense* by iron chelators. *Kinetoplastid Biology and Disease*, **5**: 3.

Department of Parasitology, Ruprecht-Karls-University, 69120 Heidelberg, Germany. [dsteverding@hotmail.com].

African trypanosomes exert significant morbidity and mortality in man and livestock. Only a few drugs are available for the treatment of trypanosome infections and therefore, the development of new anti-trypanosomal agents is required. Previously it has been shown that bloodstream-form trypanosomes are sensitive to the iron cheater deferoxamine. In this study the effect of 13 iron cheaters on the growth of *Trypanosoma brucei*, *T. congolense* and human HL-60 cells was tested *in vitro*. With the exception of 2 compounds, all cheaters exhibited anti-trypanosomal activities, with 50 percent inhibitory concentration (IC50) values ranging between 2.1 - 220  $\mu$ M. However, the iron cheaters also displayed cytotoxicity towards human HL-60 cells and therefore, only less favourable selectivity indices compared to commercially available drugs. Interfering with iron metabolism may be a new strategy in the treatment of trypanosome infections. More specifically, lipophilic iron-chelating agents may serve as lead compounds for novel anti-trypanosomal drug development.

13711. **Morales, I., de Leon, M., Morales, M., Dalla, F. & Gutierrez, C., 2006.** Ocular lesions associated with *Trypanosoma evansi* in experimentally infected goats. *Veterinary Parasitology*, **141** (3-4): 325-329.

Veterinary Faculty, University of Las Palmas, 35416 Arucas, Las Palmas, Canary Islands, Spain.

Ocular lesions associated with *Trypanosoma* spp. infection have been described in man and many animal species. However, loss of vision has not been demonstrated in humans presenting Chagas disease or in animals affected by different trypanosome species. In order to assess the possible ocular disorders caused by *Trypanosoma evansi* infection, six goats were inoculated with  $1 \times 10^5$  *T. evansi* and maintained for 12 months and four goats were used as control. The inoculated animals became positive at serological and parasitological tests at 1-month post-inoculation and showed a subclinical course of the disease. Unilateral superficial corneal ulceration and retinochoroiditis were observed in two inoculated animals. Data from ocular neurologic examination and electroretinography showed no significant differences between inoculated and non-inoculated goats. It could be concluded that *Trypanosoma evansi* can produce ocular lesion but without apparent loss of vision in goats.

13712. **Morty, R.E., Bulau, P., Pelle, R., Wilk, S. & Abe, K., 2006.** Pyroglutamyl peptidase type I from *Trypanosoma brucei*: a new virulence factor from African trypanosomes that de-blocks regulatory peptides in the plasma of infected hosts. *Biochemical Journal*, **394** (3): 635-645.

Department of Internal Medicine, University Hospital Giessen and Marburg, Aulweg 123, D-35392 Giessen, Germany. [rory.morty@innere.med.uni-giessen.de]

Peptidases of parasitic protozoans are emerging as novel virulence factors and therapeutic targets in parasitic infections. A trypanosome-derived aminopeptidase that exclusively hydrolysed substrates with Glp (pyroglutamic acid) in P1 was purified 9248-fold from the plasma of rats infected with *Trypanosoma brucei brucei*. The enzyme responsible was cloned from a *T. brucei brucei* genomic DNA library and identified as type I PGP (pyroglutamyl peptidase), belonging to the C15 family of cysteine peptidases. We showed that PGP is expressed in all life cycle stages of *T. brucei brucei* and is expressed in four other bloodstream-form African trypanosomes. Trypanosome PGP was optimally active and stable at bloodstream pH, and was insensitive to host plasma cysteine peptidase inhibitors. Native purified and recombinant hyper-expressed trypanosome PGP removed the N-terminal Glp blocking groups from TRH (thyrotrophin-releasing hormone) and GnRH (gonadotropin-releasing hormone) with a  $k(\text{cat})/K(\text{m})$  value of 0.5 and 0.1  $\text{s}^{-1} \times \mu\text{M}^{-1}$  respectively. The half-life of TRH and GnRH was dramatically reduced in the plasma of trypanosome-infected rats, both *in vitro* and *in vivo*. Employing an activity-neutralizing anti-trypanosome PGP antibody, and pyroglutamyl diazomethyl ketone, a specific inhibitor of type I PGP, we demonstrated that trypanosome PGP is entirely responsible for the reduced plasma half-life of TRH, and partially responsible for the reduced plasma half-life of GnRH in a rodent model of African trypanosomiasis. The abnormal degradation of TRH and GnRH, and perhaps other

neuropeptides N-terminally blocked with a pyroglutamyl moiety, by trypanosome PGP, may contribute to some of the endocrine lesions observed in African trypanosomiasis.

13713. **Naessens, J., Kitani, H., Nakamura, Y., Yagi, Y., Sekikawa, K. & Iraqi, F., 2005.**  
TNF- $\alpha$  mediates the development of anaemia in a murine *Trypanosoma brucei rhodesiense* infection, but not the anaemia associated with a murine *Trypanosoma congolense* infection. *Clinical and Experimental Immunology*, **139**: 405-410.

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Development of anaemia in inflammatory diseases is cytokine-mediated. Specifically, the levels of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), produced by activated macrophages, are correlated with severity of disease and anaemia in infections and chronic disease. In African trypanosomiasis, anaemia develops very early in infection around the time when parasites become detectable in the blood. Since the anaemia persists after the first waves of parasitaemia when low numbers of trypanosomes are circulating in the blood, it is generally assumed that anaemia is not directly induced by a parasite factor, but might be cytokine-mediated, as in other cases of anaemia accompanying inflammation. To clarify the role of TNF- $\alpha$  in the development of anaemia, blood parameters of wild type (TNF- $\alpha$ +/+), TNF- $\alpha$ -null (TNF- $\alpha$ -/-) and TNF- $\alpha$ -hemizygous (TNF- $\alpha$ -/+) trypanotolerant mice were compared during infections with the cattle parasite *Trypanosoma congolense*. No differences in PCV, erythrocyte numbers or haemoglobin were observed between TNF- $\alpha$ -deficient and wild type mice, suggesting that the decrease in erythrocytes was not mediated by TNF- $\alpha$ . Erythropoetin (EPO) levels increased during infection and no significant differences in EPO levels were observed between the three mouse strains. In contrast, during an infection with the human pathogen *Trypanosoma brucei rhodesiense*, the number of red blood cells in TNF- $\alpha$ -deficient mice remained significantly higher than in the wild type mice. These data suggest that more than one mechanism promotes the development of anaemia associated with trypanosomiasis.

13714. **Nikolskaia, O.V., Kim, Y.V., Kovbasnjuk, O., Kim, K.J. & Grab, D.J., 2006.**  
Entry of *Trypanosoma brucei gambiense* into microvascular endothelial cells of the human blood-brain barrier. *International Journal for Parasitology*, **36** (5): 513-519.

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Using an *in vitro* model of the human blood-brain barrier consisting of human brain microvascular endothelial cells, we recently demonstrated that *Trypanosoma brucei gambiense* bloodstream-forms efficiently cross these cells via a paracellular route while *Trypanosoma brucei brucei* crosses these cells poorly. Using a combination of techniques that include fluorescence activated cell sorting, confocal and electron microscopy, we now show that some *T. b. gambiense* blood stream form parasites have the capacity to enter human brain microvascular endothelial cells. The intracellular location of the trypanosomes

was demonstrated in relation to the endothelial cell plasma membrane and to the actin cytoskeleton. These parasites may be a terminal stage within a lysosomal compartment or they may be viable trypanosomes that will be able to exit the brain microvascular endothelial cells. This process may provide an additional transcellular route by which the parasites cross the blood-brain barrier.

13715. **Nikolskaia, O.V., Kim, Y.V., Lonsdale-Eccles, J.D., Fukuma, T., Scharfstein, J. & Grab, D.J., 2006.** Blood-brain barrier traversal by African trypanosomes requires calcium signaling induced by parasite cysteine protease. *Journal of Clinical Investigation*, **116** (10): 2739-2747.

Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, USA.

In this study we investigated why bloodstream forms of *Trypanosoma brucei gambiense* cross human brain microvascular endothelial cells (BMECs), a human blood-brain barrier (BBB) model system, at much greater efficiency than do *T. b. brucei*. After noting that *T. b. gambiense* displayed higher levels of cathepsin L-like cysteine proteases, we investigated whether these enzymes contribute to parasite crossing. First, we found that *T. b. gambiense* crossing of human BMECs was abrogated by N-methylpiperazine-urea-phenomethylalanine-vinylsulfone-benzene (K11777), an irreversible inhibitor of cathepsin L-like cysteine proteases. Affinity labelling and immunochemical studies characterized brucipain as the K11777-sensitive cysteine protease expressed at higher levels by *T. b. gambiense*. K11777-treated *T. b. gambiense* failed to elicit calcium fluxes in BMECs, suggesting that generation of activation signals for the BBB is critically dependant on brucipain activity. Strikingly, crossing of *T. b. brucei* across the BBB was enhanced upon incubation with brucipain-rich supernatants derived from *T. b. gambiense*. The effects of the conditioned medium, which correlated with ability to evoke calcium fluxes, were cancelled by K11777, but not by the cathepsin B inhibitor CA074. Collectively, these *in vitro* studies implicate brucipain as a critical driver of *T. b. gambiense* transendothelial migration through the human BBB.

13716. **Nishimura, K., Yanase, T., Araki, N., Ohnishi, Y., Kozaki, S., Shima, K., Asakura, M., Samosomsuk, W. & Yamasaki, S., 2006.** Effects of polyamines on two strains of *Trypanosoma brucei* in infected rats and *in vitro* culture. *Journal of Parasitology*, **92** (2): 211-217.

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We studied the effects of polyamines, which are necessary for proliferation and antioxidantation in *Trypanosoma brucei gambiense* Wellcome strain (WS) and *Trypanosoma brucei brucei* ILtat 1.4 strain (IL). No difference was found in activity of ornithine decarboxylase (ODC), a key enzyme in polyamine synthesis in trypanosomes, in both strains maintained *in vitro*; higher ( $P < 0.05$ ) ODC values were found in IL *in vivo*. However, WS *in vivo* exhibited higher proliferation rates with higher spermidine content and decreased host

survival times than IL. The *in vitro* proliferation and polyamine contents of WS increased with the addition of polyamine to the 1-difluoromethylornithine culture medium, but not IL. These results suggested that WS uses extracellular polyamine for proliferation. In the *in vitro* culture, WS was less tolerant of hydrogen peroxide (oxidative stress) than IL, and malondialdehyde levels in WS were higher than in IL. The expression of trypanothione synthetase mRNA in WS *in vitro* was higher than in IL. These results suggest that IL is dependent on the synthesis of polyamines for proliferation and reduction of oxidative stress, whereas WS is dependent on the uptake of extracellular polyamines. A thorough understanding of the differences in the metabolic capabilities of various trypanosomes is important for the design of more effective medical treatments.

13717. **Oli, M.W., Cotlin, L.F., Shiflett, A.M. & Hajduk, S.L., 2006.** Serum resistance-associated protein blocks lysosomal targeting of trypanosome lytic factor in *Trypanosoma brucei*. *Eukaryotic Cell*, **5** (1): 132-139.

Global Infectious Disease Program, Josephine Bay Paul Center, Marine Biological Laboratory, 7 MBL Street, 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 (apoA-I), apolipoprotein L-I (apoL-I), and haptoglobin-related protein (Hpr) provides this innate protection against *T. b. brucei* infection. This HDL subfraction, called trypanosome lytic factor (TLF), kills *T. b. brucei* following receptor binding, endocytosis, and lysosomal localization. *Trypanosoma brucei rhodesiense*, which is morphologically and physiologically indistinguishable from *T. b. brucei*, is resistant to TLF-mediated killing and causes human African sleeping sickness. Human infectivity by *T. b. rhodesiense* correlates with the evolution of a resistance-associated protein (SRA) that is able to ablate TLF killing. To examine the mechanism of TLF resistance, we transfected *T. b. brucei* with an epitope-tagged SRA gene. Transfected *T. b. brucei* expressed SRA mRNA at levels comparable to those in *T. b. rhodesiense* and was highly resistant to TLF. In the SRA-transfected cells, intracellular trafficking of TLF was altered, with TLF being mainly localized to a subset of SRA-containing cytoplasmic vesicles but not to the lysosome. These results indicate that the cellular distribution of TLF is influenced by SRA expression and may directly determine the organism's susceptibility to TLF.

13718. **Orhue, N.N., Nwanze, E.A.C., & Okafor, A., 2005.** Serum total protein, albumin and globulin levels in *Trypanosoma brucei*-infected rabbits: Effect of orally administered *Scoparia dulcis*. *African Journal of Biotechnology*, **4** (10): 1152-1155.

University of Benin, Department of Biochemistry, PMB 1154, Benin, Nigeria.

The effects of orally administered *Scoparia dulcis* on *Trypanosoma brucei*-induced changes in serum total protein, albumin and globulin were investigated in rabbits over a period of twenty eight days. Results obtained show that infection resulted in

hyperproteinaemia, hyperglobulinaemia and hypoalbuminaemia. However these lesions were less severe ( $p < 0.05$ ) in the infected and treated group relative to their untreated counterparts. We speculate that the herb may be involved in modulating the severity of these trypanosome associated lesions by some yet undefined mechanisms.

13719. **Ouwe-Missi-Oukem-Boyer, O., Mezui-Me-Ndong, J., Boda, C., Lamine, I., Labrousse, F., Bisser, S. & Bouteille, B., 2006.** The vervet monkey (*Chlorocebus aethiops*) as an experimental model for *Trypanosoma brucei gambiense* human African trypanosomiasis: a clinical, biological and pathological study. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **100** (5): 427-436.

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It has long been known that the vervet monkey, *Chlorocebus (C.) aethiops*, can be infected with *Trypanosoma rhodesiense*, but this model has not been described for *T. gambiense*. In this study, we report the development of such a model for human African trypanosomiasis. Twelve vervet monkeys infected with *T. gambiense* developed chronic disease. The duration of the disease ranged between 23 and 612 days (median 89 days) in five untreated animals. Trypanosomes were detected in the blood within the first 10 days post-infection and in the cerebrospinal fluid, with a median delay of 120 days ( $n = 4$ , range 28-348 days). Clinical changes included loss of weight, adenopathy, and in some cases eyelid oedema and lethargy. Haematological alterations included decreases in haemoglobin level and transitory decreases in platelet count. Biological modifications included increased gamma globulins and total proteins and decreased albumin. Pathological features of the infection were presence of Mott's cells, inflammatory infiltration of either mononuclear cells or lymphocytes and plasma cells in the brain parenchyma, and astrocytosis. These observations indicate that the development of the disease in vervet monkeys is similar to human *T. gambiense* infection. We conclude that *C. aethiops* is a promising experimental primate model for the study of *T. gambiense* trypanosomiasis.

13720. **Pan, W., Ogunremi, O., Wei, G., Shi, M. & Tabel, H., 2006.** CR3 (CD11b/CD18) is the major macrophage receptor for IgM antibody-mediated phagocytosis of African trypanosomes: Diverse effect on subsequent synthesis of tumor necrosis factor [alpha] and nitric oxide. *Microbes and Infection*, **8** (5): 1209-1218.

Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK S7N 5B4, Canada.

Immunoglobulin M (IgM) antibodies to the variant surface glycoproteins (VSG) of African trypanosomes are the first and predominant class of anti-trypanosomal antibodies in the infected host. They are a major factor in controlling waves of parasitaemia, but not in long-term survival. The macrophage receptor(s) that enables phagocytosis of IgM anti-VSG-coated African trypanosomes is unknown. We assessed whether complement receptor CR3 (CD11b/CD18) might be involved in mediating phagocytosis of *Trypanosoma congolense*.



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We show that murine complement C3 fragments are deposited onto *T. congolense* when the trypanosomes are incubated with IgM anti-VSG and fresh mouse serum. In the presence of fresh mouse serum, there is significantly and markedly less phagocytosis of IgM-opsonized *T. congolense* by CD11b-deficient macrophages compared to phagocytosis by wild-type macrophages (78 percent fewer *T. congolense* are ingested per macrophage). Significantly less tumour necrosis factor (TNF)-[alpha] (38 percent less), but significantly more nitric oxide (NO) (63 percent more) are released by CD11b-deficient macrophages that have engulfed trypanosomes than by equally treated wild-type macrophages. We conclude that CR3 is the major, but not the only, receptor involved in IgM anti-VSG-mediated phagocytosis of *T. congolense* by macrophages. We further conclude that IgM anti-VSG-mediated phagocytosis of *T. congolense* enhances synthesis of disease-producing TNF-[alpha] and inhibits synthesis of parasite-controlling NO. We suggest that signalling of inhibition of NO synthesis is mediated via CR3.

13721. **Pays, E., 2006.** The variant surface glycoprotein as a tool for adaptation in African trypanosomes. *Microbes and Infection*, **8** (3): 930-937.

Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, 12, rue des Profs. Jeener et Brachet, B6041 Gosselies, Belgium.

African trypanosomes (*Trypanosoma brucei*) are flagellated protozoan parasites that infect a wide variety of mammals, causing nagana in cattle and sleeping sickness in humans. These organisms can cause prolonged chronic infections due to their ability to successively expose different antigenic variants of the variant surface glycoprotein (VSG). The genomic loci where the VSG genes are expressed are telomeric and contain polycistronic transcription units with several genes that are involved in adaptation of the parasite to the host. At least three of these genes, which respectively encode the two subunits of the heterodimeric receptor for transferrin and a protein conferring resistance to the human trypanolytic factor apolipoprotein L-I, share the same origin as the VSG. The high recombination potential of the telomeric VSG expression sites, coupled to their dynamic mono-allelic expression control, provides trypanosomes with a powerful capacity for adaptation to their hosts.

13722. **Pays, E., Vanhollebeke, B., Vanhamme, L., Paturiaux-Hanocq, F., Nolan, D.P. & Perez-Morga, D., 2006.** The trypanolytic factor of human serum. *Nature Reviews Microbiology*, **4** (6): 477-486.

Laboratory of Molecular Parasitology, Institute of Molecular Biology and Medicine (IBMM), Université Libre de Bruxelles, 12 rue des Professeurs Jeener et Brachet, B-6041 Gosselies, Belgium. [epays@ulb.ac.be]

African trypanosomes (the prototype of which is *Trypanosoma brucei brucei*) are protozoan parasites that infect a wide range of mammals. Human blood, unlike the blood of other mammals, has efficient trypanolytic activity, and this needs to be counteracted by these parasites. Resistance to this activity has arisen in two subspecies of *Trypanosoma brucei* - *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* - allowing these parasites to infect humans, and this results in sleeping sickness in East Africa and West Africa, respectively. Study of the mechanism by which *T. b. rhodesiense* escapes lysis by

human serum led to the identification of an ionic-pore-forming apolipoprotein - known as apolipoprotein L1 - that is associated with high-density-lipoprotein particles in human blood. In this article, we argue that apolipoprotein L1 is the factor that is responsible for the trypanolytic activity of human serum.

13723. **Penchenier, L., Alhadji, D., Bahebegue, S., Simo, G., Laveissiere, C. & Cuny, G., 2005.** Spontaneous cure of domestic pigs experimentally infected by *Trypanosoma brucei gambiense*. Implications for the control of sleeping sickness. *Veterinary Parasitology*, **133** (1): 7-11.

Laboratoire de Recherches et de Coordination sur les Trypanosomes, UR035 (IRD) CIRAD, TA207/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France. [Penchenier@mpl.ird.fr].

The existence of a pig reservoir for human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* complicates the fight against this disease. This study reports results obtained from pigs, which were inoculated with the blood of a person, suffering from HAT in Cameroon. The pigs were reared and kept in the shelter from all contact with *Glossina*, and monitored for 188 days. The seroconversion was checked by agglutination assays for trypanosomiasis (CATT 1.3 and LATEX/*T.b.gambiense*). The parasitaemia was measured by quantitative buffy coat method (QBC) and by polymerase chain reaction method (PCR). In addition, growth was recorded as well as blood counting and blood formulas. The results showed that the pigs were trypanotolerant and cure themselves in less than 6 months. It is concluded that sterilisation of this reservoir could be achieved by tsetse-control measures in 1 year. It confirms the strategy to complement screening and treatment of HAT with tsetse fly control measures.

13724. **Semballa, S., Okomo-Assoumou, M.C., Holzmuller, P., Buscher, P., Magez, S., Lemesre, J.L., Daulouede, S., Courtois, P., Geffard, M. & Vincendeau, P., 2006.** Identification of a tryptophan-like epitope borne by the variable surface glycoprotein (VSG) of African trypanosomes. *Experimental Parasitology*. **In press; corrected proof**.

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Antibodies (Ab) directed against a tryptophan-like epitope (WE) were previously detected in patients with human African trypanosomiasis (HAT). We investigated whether or not these Ab resulted from immunization against trypanosome antigen(s) expressing a WE. By Western blotting, we identified an antigen having an apparent molecular weight ranging from 60 to 65kDa, recognized by purified rabbit anti-WE Ab. This antigen, present in trypomastigote forms, was absent in procyclic forms and *Trypanosoma cruzi* trypomastigotes. Using purified variable surface glycoproteins (VSG) from various trypanosomes, we showed that VSG was the parasite antigen recognized by these rabbit Ab. Anti-WE and anti-VSG Ab were purified from HAT sera by affinity chromatography. Immunoreactivity of purified antibodies eluted from affinity columns and of depleted fractions showed that WE was one of the epitopes borne by VSG. These data underline the existence of an invariant WE in the structure of VSG from several species of African trypanosomes.

13725. **Shi, M.W., G.J., Pan, W.L., Tabel, H., 2005.** Impaired Kupffer cells in highly susceptible mice infected with *Trypanosoma congolense*. *Infection and Immunity*, **73** (12): 8393-8396.

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In highly susceptible BALB/c mice infected with *Trypanosoma congolense*, the total number of Kupffer cells in the liver remains constant; however, their mean size increases fivefold towards the terminal stage. About 25 percent of Kupffer cells undergo apoptosis. We suggest that development of an impairment of the macrophage system might be a major mechanism for inefficient elimination of trypanosomes.

13726. **Shi, M., Wei, G., Pan, W. & Tabel, H., 2006.** Experimental African trypanosomiasis: a subset of pathogenic, IFN- gamma -producing, MHC class II-restricted CD4+ T cells mediates early mortality in highly susceptible mice. *Journal of Immunology*, **176** (3):1724-32.

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

Infections of highly susceptible BALB/c mice with virulent strains of *Trypanosoma congolense* or *Trypanosoma brucei* result in rapid death (8 days). We have previously shown that this mortality is IFN- gamma dependent. In this study we show that IFN- gamma is produced predominantly by CD3+Thy1.2+TCR beta +CD4+ T cells shortly before the death of infected mice. Mortality may therefore be dependent on IFN- gamma -producing CD4+ T cells. Surprisingly, infected CD4+/+ and CD4-/- BALB/c mice have similar parasitaemia and survival time. In infected CD4-/- mice, the production of both IFN- gamma and IL-10 is very low, suggesting that both cytokines are predominantly produced by CD4+ T cells and that the outcome of the disease might depend on the balance of their effects. Infected BALB/c mice partially depleted of CD4+ T cells or MHC class II function have lower parasitaemia and survive significantly longer than infected normal BALB/c mice or infected BALB/c mice whose CD4+ T cells are fully depleted. Partial depletion of CD4+ T cells markedly reduces IFN- gamma secretion without a major effect on the production of IL-10 and parasite-specific IgG2a Abs. Based on our previous and current data, we conclude that a subset of a pathogenic, MHC class II-restricted CD4+ T cells (Tp cells), activated during the course of *T. congolense* infection, mediates early mortality in infected BALB/c mice via excessive synthesis of IFN- gamma. IFN- gamma, in turn, exerts its pathological effect by enhancing the cytokine release syndrome of the macrophage system activated by the phagocytosis of parasites. We speculate that IL-10-producing CD4+ T cells might counteract this effect.

13727. **Shiflett, A.M., Bishop, J.R., Pahwa, A. & Hajduk, S.L., 2005.** Human high density lipoproteins are platforms for the assembly of multi-component innate immune complexes. *Journal of Biological Chemistry*, **280** (38): 32578-32585.

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Human innate immunity to non-pathogenic species of African trypanosomes is provided by human high density lipoprotein (HDL) particles. Here we show that native human HDLs containing haptoglobin-related protein (Hpr), apolipoprotein L-I (apoL-I) and apolipoprotein A-I (apoA-I) are the principal antimicrobial molecules providing protection from trypanosome infection. Other HDL subclasses containing either apoA-I and apoL-I or apoA-I and Hpr have reduced trypanolytic activity, whereas HDL subclasses lacking apoL-I and Hpr are non-toxic to trypanosomes. Highly purified, lipid-free Hpr and apoL-I were both toxic to *Trypanosoma brucei brucei* but with specific activities at least 500-fold less than those of native HDLs, suggesting that association of these apolipoproteins within the HDL particle was necessary for optimal cytotoxicity. These studies show that HDLs can serve as platforms for the assembly of multiple synergistic proteins and that these assemblies may play a critical role in the evolution of primate-specific innate immunity to trypanosome infection.

13728. **Suzuki, T., Ueta, Y.Y., Inoue, N., Xuan, X., Saitoh, H. & Suzuki, H., 2006.** Beneficial effect of erythropoietin administration on murine infection with *Trypanosoma congolense*. *American Journal of Tropical Medicine and Hygiene*, **74** (6): 1020-1025.

Research Unit for Functional Genomics, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.

The effect of erythropoietin treatment on *Trypanosoma congolense* infection in mice was studied. Survival rates of mice were dramatically improved by treatment with recombinant human erythropoietin (r-hu-EPO; 5,000 U/kg) when infected with 1,000 cells of *T. congolense* IL3000 ( $P < 0.05$ ). All the untreated mice infected with *T. congolense* IL3000 died by day 9 of infection; however, 100 percent, 50 percent, and 25 percent of the mice treated with r-hu-EPO for 8 days survived to day 20, day 40, and day 60 of the parasitological infection, respectively. Anti-8-hydroxy-2'-deoxyguanosine antibody, a biomarker for oxidative damage of DNA, yielded positive reactions in the cytoplasm of the parasites recovered from the mice treated with r-hu-EPO. These results, taken together, indicate that erythropoietin administration is effective for the treatment of *T. congolense* infection.

13729. **Tsujimura, Y., Watarai, S., Uemura, A., Ohnishi, Y. & Kodama, H., 2005.** Effect of anti-ganglioside antibody in experimental *Trypanosoma brucei* infection in mice. *Research in Veterinary Science*, **78** (3): 245-247.

Laboratory of Veterinary Immunology, Division of Veterinary Science, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 599-8531, Japan.

The effect of antibody against ganglioside antigen on *Trypanosoma brucei* parasites was examined *in vitro* and *in vivo* using anti-ganglioside GM1 (AGM-1) monoclonal

antibody. The antibody showed complement-dependent cytotoxicity against *T. brucei* with mouse complement. Furthermore, mice given AGM-1 were challenged intraperitoneally with *T. brucei*. Although all non-treated control mice died within six days after infection, all of AGM-1-injected mice had survived by six days post-infection. These data suggest that antibody against ganglioside antigen on *T. brucei* has potential in protection against *T. brucei* infection.

13730 **Utz, S., Roditi, I., Kunz Renggli, C., Almeida, I.C., Acosta-Serrano, A. & Butikofer, P., 2006.** *Trypanosoma congolense* procyclins: unmasking cryptic major surface glycoproteins in procyclic forms. *Eukaryotic Cell*, **5** (8): 1430-1440.

Institute of Biochemistry & Molecular Medicine, University of Bern, Bern, Switzerland.

In the tsetse fly, the protozoan parasite *Trypanosoma congolense* is covered by a dense layer of glycosylphosphatidylinositol (GPI)-anchored molecules. These include a protease-resistant surface molecule (PRS), which is expressed by procyclic forms early in infection, and a glutamic acid- and alanine-rich protein (GARP), which appears at later stages. Since neither of these surface antigens is expressed at intermediate stages, we investigated whether a GPI-anchored protein of 50 to 58 kDa, previously detected in procyclic culture forms, might constitute the coat of these parasites. We therefore partially purified the protein from *T. congolense* Kilifi procyclic forms, obtained an N-terminal amino acid sequence, and identified its gene. Detailed analyses showed that the mature protein consists almost exclusively of 13 heptapeptide repeats (EPGENGT). The protein is densely N glycosylated, with up to 13 high-mannose oligosaccharides ranging from Man(5)GlcNAc(2) to Man(9)GlcNAc(2) linked to the peptide repeats. The lipid moiety of the glycosylphosphatidylinositol is composed of sn-1-stearoyl-2-lyso-glycerol-3-HPO (4)-1-(2-O-acyl)-d-myo-inositol. Heavily glycosylated proteins with similar repeats were subsequently identified in *T. congolense* Savannah procyclic forms. Collectively, this group of proteins was named *T. congolense* procyclins to reflect their relationship to the EP and GPEET procyclins of *T. brucei*. Using an antiserum raised against the EPGENGT repeat, we show that *T. congolense* procyclins are expressed continuously in the fly midgut and thus form the surface coat of cells that are negative for both PRS and GARP.

13731. **Valera, Z., Parra, O., Alvarado, M., Barboza, G., Escalona, F. & Ramírez, R., 2005.** Effect of experimental *Trypanosoma vivax* infection on hematological parameters of sheep. *Revista Científica, Facultad de Ciencias Veterinarias, Universidad del Zulia*, **15** (5): 412-420.

Facultad de Ciencias Veterinarias, Universidad del Zulia, Maracaibo, Edo. Zulia, Venezuela.

Eight crossbred West African sheep aged 6 months to one year were randomly divided into two groups. The experimental sheep (n=4) were inoculated intravenously with 2 ml of fresh blood containing  $1.3 \times 10^6$  trypanosomes/ml from a positive ewe. The remaining four animals served as controls. The effects of the parasite on body temperature, parasitaemia, haematocrit, haemoglobin, leukocytes and total serum proteins were evaluated for 90 days.

All the infected animals were positive for the parasite 2 days post-inoculation, developing undulating parasitaemia and severe anaemia which persisted until the end of the experiment. The course of experimental infection with *T. vivax* showed two phases. The first phase was observed during the first four weeks post-infection, where the infected sheep developed high levels of parasitaemia and decreased ( $P<0.005$ ) haematocrit and haemoglobin level and total leukocyte count. The second phase was evident from the fifth week post-infection. During this period, the infected animals showed levels of parasitaemia lower than the preceding phase, remittent fever, persistence of low haematocrit and haemoglobin values, as well as, significant increase ( $P<0.05$ ) in serum proteins. The changes in haematological parameters and body temperature were related to the appearance of trypanosomes in the circulation and to the intensity of parasitaemia.

(c) CHEMOTHERAPEUTICS

[See also 29: 13615]

13732. **Anene, B.E., Ezeokonkwo, R.C., Mmesirionye, T.I., Tettey, J.N.A., Brock, J.M., Barrett, M.P. & De Koning, H.P., 2006.** A diminazene-resistant strain of *Trypanosoma brucei brucei* isolated from a dog is cross-resistant to pentamidine in experimentally infected albino rats. *Parasitology*, **132** (1): 127-133.

R.C. Ezeokonkwo: University of Nigeria, Faculty of Veterinary Medicine, Nsukka, Enugu State, Nigeria.

Trypanosomiasis is a major cause of mortality for dogs in Nigeria and treatment with diminazene aceturate has steadily become less effective, either as a result of low quality of the locally available diminazene preparations or of drug resistance. To investigate these alternatives, samples of locally obtained drugs were analysed for diminazene aceturate content and a strain of *Trypanosoma brucei brucei* was isolated from a diminazene-refractory dog in Nsukka, south-eastern Nigeria, and used to infect albino rats. The quality of diminazene aceturate-based preparations was variable, with two preparations containing less than 95 percent of the stated active compound. Rats infected with *T. brucei* isolated from the dog were treated 7 and 10 days after infection either with 7 mg/kg diminazene aceturate (intraperitoneally, once) or with 4 mg/kg pentamidine isethionate (intramuscularly, 7 consecutive days). Relapse rates were 100 percent for both trypanocides in the groups of rat treated 10 days post-infection, and 83 percent and 50 percent of rats treated 7 days after infection relapsed to diminazene aceturate and pentamidine isethionate, respectively. Careful consideration of physiological parameters showed that pentamidine was only marginally superior to diminazene aceturate as applied in this study. It was concluded that dogs in Nigeria are infected with genuinely diminazene aceturate-resistant trypanosomes that appear to be cross-resistant to pentamidine isethionate.

13733. **Ariyanayagam, M.R.O., S. L., Guther, M. L. S. & Fairlamb, A. H., 2005.** Phenotypic analysis of trypanothione synthetase knockdown in the African trypanosome. *Biochemical Journal*, **391** (2): 425-432.

*Tsetse and Trypanosomiasis Information*

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Trypanothione plays a pivotal role in defence against chemical and oxidant stress, thiol redox homeostasis, ribonucleotide metabolism and drug resistance in parasitic kinetoplastids. In *Trypanosoma brucei*, trypanothione is synthesized from glutathione and spermidine by a single enzyme, TryS (trypanothione synthetase), with glutathionylspermidine as an intermediate. To examine the physiological roles of trypanothione, tetracycline-inducible RNA interference was used to reduce expression of TryS. Following induction, TryS protein was reduced >10-fold and growth rate was reduced 2-fold, with concurrent 5-10-fold decreases in glutathionylspermidine and trypanothione and an up to 14-fold increase in free glutathione content. Depleted trypanothione levels were associated with increases in sensitivity to arsenical, antimonial and nitro drugs, implicating trypanothione metabolism in their mode of action. Escape mutants arose after 2 weeks of induction, with all parameters, including growth, returning to normal. Selective inhibitors of TryS are required to fully validate this novel drug target.

13734. **Athri, P., Wenzler, T., Ruiz, P., Brun, R., Boykin, D.W., Tidwell, R. & Wilson, W.D., 2006.** 3D QSAR on a library of heterocyclic diamidine derivatives with antiparasitic activity. *Bioorganic and Medicinal Chemistry*, **14** (9): 3144-3152.

Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA.

African trypanosomes, *Trypanosoma brucei rhodesiense* (TBR) and *Trypanosoma brucei gambiense* (TBG), affect hundreds of thousands of lives in tropical regions of the world. The toxicity of the diamidine pentamidine, an effective drug against TBG, necessitates the design of better drugs. An orally effective prodrug of the diamidine, furamidine (DB75), presently scheduled for phase III clinical trials, has excellent activity against TBG with toxicity lower than that of pentamidine. As part of an effort to develop additional and improved diamidines against African trypanosomes, 3D QSAR analyses have been conducted with furamidine and a set of 25 other structurally related compounds. The results have been used as a guide to design compounds that potentially have better activity against African trypanosomes.

13735. **Azema, L., Lherbet, C., Baudoin, C. & Blonski, C., 2006.** Cell permeation of a *Trypanosoma brucei* aldolase inhibitor: Evaluation of different enzyme-labile phosphate protecting groups. *Bioorganic and Medicinal Chemistry Letters*, **16** (13): 3440-3.

Laboratoire SPCMIB, Groupe de Chimie Organique Biologique, Université Paul Sabatier UMR CNRS 5068, 118 route de Narbonne, 31062 Toulouse Cedex 4, France.

13736. **Barrett, M.P. & Gilbert, I.H., 2006.** Targeting of toxic compounds to the Trypanosome's interior. *Advances in Parasitology*, **63**: 125-183.

*Tsetse and Trypanosomiasis Information*

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Drugs can be targeted into African trypanosomes by exploiting carrier proteins at the surface of these parasites. This has been clearly demonstrated in the case of the melamine-based arsenical and the diamidine classes of drug that are already in use in the treatment of human African trypanosomiasis. These drugs can enter via an aminopurine transporter, termed P2, encoded by the TbAT1 gene. Other toxic compounds have also been designed to enter via this transporter. Some of these compounds enter almost exclusively through the P2 transporter, and hence loss of the P2 transporter leads to significant resistance to these particular compounds. It now appears, however, that some diamidines and melaminophenylarsenicals may also be taken up by other routes (of yet unknown function). These too may be exploited to target new drugs into trypanosomes. Additional purine nucleoside and nucleobase transporters have also been subverted to deliver toxic agents to trypanosomes. Glucose and amino acid transporters too have been investigated with a view to manipulating them to carry toxins into *Trypanosoma brucei*, and recent work has demonstrated that aquaglyceroporins may also have considerable potential for drug-targeting. Transporters, including those that carry lipids and vitamins such as folate and other pterins also deserve more attention in this regard. Some drugs, for example suramin, appear to enter via routes other than plasma-membrane-mediated transport. Receptor-mediated endocytosis has been proposed as a possible way in for suramin. Endocytosis also appears to be crucial in targeting natural trypanocides, such as trypanosome lytic factor (TLF) (apolipoprotein L1), into trypanosomes and this offers an alternative means of selectively targeting toxins to the trypanosome's interior. Other compounds may be induced to enter by increasing their capacity to diffuse over cell membranes; in this case depending exclusively on selective activity within the cell rather than selective uptake to impart selective toxicity. This review outlines studies that have aimed to exploit trypanosome nutrient uptake routes to selectively carry toxins into these parasites.

13737. **Baral, T.N., Magez, S., Stijlemans, B., Conrath, K., Vanhullebeke, B., Pays, E., Muyldermans, S. & De Baetselier, P., 2006.** Experimental therapy of African trypanosomiasis with a nanobody-conjugated human trypanolytic factor. *Nature Medicine*, **12** (5): 580-584.

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High systemic drug toxicity and increasing prevalence of drug resistance hampers efficient treatment of human African trypanosomiasis (HAT). Hence, development of new highly specific trypanocidal drugs is necessary. Normal human serum (NHS) contains apolipoprotein L-I (apoL-I), which lyses African trypanosomes except resistant forms such as *Trypanosoma brucei rhodesiense*. *T. b. rhodesiense* expresses the apoL-I-neutralizing serum resistance-associated (SRA) protein, endowing this parasite with the ability to infect humans



and cause HAT. A truncated apoL-I (Tr-apoL-I) has been engineered by deleting its SRA-interacting domain, which makes it lytic for *T. b. rhodesiense*. Here, we conjugated Tr-apoL-I with a single-domain antibody (nanobody) that efficiently targets conserved cryptic epitopes of the variant surface glycoprotein (VSG) of trypanosomes to generate a new manmade type of immunotoxin with potential for trypanosomiasis therapy. Treatment with this engineered conjugate resulted in clear curative and alleviating effects on acute and chronic infections of mice with both NHS-resistant and NHS-sensitive trypanosomes.

13738. **Bazin, M.A., Loiseau, P.M., Bories, C., Letourneux, Y., Rault, S. & El Kihel, L., 2006.** Synthesis of oxysterols and nitrogenous sterols with antileishmanial and trypanocidal activities. *European Journal of Medical Chemistry*, **41** (10): 1109-1116.

Centre d'Études et de Recherche sur le Médicament de Normandie, UFR des Sciences Pharmaceutiques, 5, rue Vaubenard, 14032 Caen cedex, France.

13739. **Berger, H., Seebacher, W., Saf, R., Kaiser, M., Brun, R. & Weis, R., 2006.** Antiprotozoal activities of new bis-chlorophenyl derivatives of bicyclic octanes and aza-nonanes. *Bioorganic and Medicinal Chemistry Letters*, **16** (20): 5457-5461.

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens-University, Universitätsplatz 1, A-8010 Graz, Austria.

13740. **Bi, X., Lopez, C., Bacchi, C.J., Rattendi, D. & Woster, P.M., 2006.** Novel alkylpolyaminoguanidines and alkylpolyaminobiguanides with potent antitrypanosomal activity. *Bioorganic and Medicinal Chemistry Letters*, **16** (12): 3229-3232.

Department of Pharmaceutical Sciences, Wayne State University, 259 Mack Ave, Detroit, MI 48202, USA.

13741. **Bizimana, N., Tietjen, U., Zessin, K.-H., Diallo, D., Djibril, C., Melzig, M.F. & Clausen, P.-H., 2006.** Evaluation of medicinal plants from Mali for their *in vitro* and *in vivo* trypanocidal activity. *Journal of Ethnopharmacology*, **103** (3): 350-356.

Institute for Parasitology und International Animal Health, Freie Universität Berlin, Königsweg 67, D-14163 Berlin, Germany.

13742. **Boda, C.E., Enanga, B., Courtioux, B., Breton, J.C. & Bouteille, B., 2006.** Trypanocidal activity of methylene blue: Evidence for *in vitro* efficacy and *in vivo* failure. *Chemotherapy*, **52** (1): 16-19.

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13743. **Bosch, J., Robien, M.A., Mehlin, C., Boni, E., Riechers, A., Buckner, F.S., Van Voorhis, W.C., Myler, P.J., Worthey, E.A., DeTitta, G., Luft, J.R., Lauricella, A., Gulde, S., Anderson, L.A., Kalyuzhnyi, O., Neely, H.M., Ross, J., Earnest, T.N., Soltis, M., Schoenfeld, L., Zucker, F., Merritt, E.A., Fan, E., Verlinde, C.L. & Hol, W.G., 2006.** Using fragment cocktail crystallography to assist inhibitor design of *Trypanosoma brucei* nucleoside 2-deoxyribosyltransferase. *Journal of Medicinal Chemistry*, **49** (20): 5939-5946.

Department of Biochemistry, Division of Infectious Disease, and Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195, USA.

13744. **Braddock, M., 2006.** Overcoming resistance with designer immunotoxins. *Expert Opinion on Pharmacotherapy*, **7** (10): 1409-1412.

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Normal human serum contains apolipoprotein L-I (apoL-I), which lyses African trypanosomes. Resistant forms, such as *Trypanosoma brucei rhodesiense* express apoL-I-neutralising serum resistance-associated protein, which enables this parasite to infect humans and cause human African trypanosomiasis. This paper describes the construction of a mutant apoL-I conjugated to a nanobody that targets the variant surface glycoprotein of trypanosomes. Treatment with this engineered immunotoxin has resulted in both alleviating and curative effects on chronic and acute infections of mice with normal human serum-resistant and -sensitive trypanosomes.

13745. **Brigotti, M., Alfieri, R.R., Petronini, P.G. & Carnicelli, D., 2006.** Inhibition by suramin of protein synthesis *in vitro*. Ribosomes as the target of the drug. *Biochimie*, **88** (5): 497-503.

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Suramin, a drug widely used both as a therapeutic agent and in research, inhibits translation in eukaryotic cell-free systems from rabbit reticulocyte lysate (IC<sub>50</sub>)=142-241 μM). Suramin affects both initiation (block of 43S pre-initiation complex formation) and elongation (impairment of poly (U) translation). The drug induces an increase in the pools of ribosomal subunits and the formation of high molecular weight ribosomal complexes, thus causing the disappearance of polysomes. Ribosomes isolated from suramin-treated translating mixtures are inactivated. [<sup>3</sup>H]Suramin binds to ribosomes and to isolated 60S and 40S ribosomal subunits (116, 106 and 3 binding sites, respectively) showing higher affinity for the small subunit (K<sub>d</sub>)=2 μM).

13746. **Chackal-Catoen, S., Miao, Y., Wilson, W.D., Wenzler, T., Brun, R. & Boykin, D.W., 2006.** Dicationic DNA-targeted antiprotozoal agents: naphthalene replacement of benzimidazole. *Bioorganic and Medicinal Chemistry*, **14** (22): 7434-7445.

Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303-3083, USA.

13747. **Dardonville, C., Barrett, M.P., Brun, R., Kaiser, M., Taniou, F. & Wilson, W.D., 2006.** DNA binding affinity of bisguanidine and bis (2-aminoimidazoline) derivatives with *in vivo* antitrypanosomal activity. *Journal of Medicinal Chemistry*, **49** (12): 3748-3752.

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A new antitrypanosomal hit compound that cures an acute (STIB 900) mouse model of *Trypanosoma brucei rhodesiense* trypanosomiasis is described. This bis (2-aminoimidazolinium) dicationic compound proved to be an excellent DNA minor groove binder, suggesting a possible mechanism for its trypanocidal activity. From these studies, the 4, 4'-diaminodiphenylamine skeleton emerged as a good scaffold for antitrypanosomal drugs.

13748. **Deterding, A., Dungey, F.A., Thompson, K.A. & Steverding, D., 2005.** Antitrypanosomal activities of DNA topoisomerase inhibitors. *Acta Tropica*, **93** (3): 311-316.

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

Only four drugs are available for chemotherapy of human African sleeping sickness with undesirable toxic side effects. The development of new anti-trypanosomal drugs is therefore urgently required. In this study, 15 DNA topoisomerase inhibitors, including approved anti-cancer drugs, were tested for *in vitro* activity against bloodstream forms of *Trypanosoma brucei* and human leukaemia HL-60 cells. All compounds exhibited anti-trypanosomal activity, with ED50 values ranging between 3 nM and 30 µM, and MIC values between 100 nM and >100 µM. The trypanocidal activities of the most effective DNA topoisomerase inhibitors, aclarubicin, doxorubicin and mitoxantrone, were comparable with those of commercial anti-trypanosomal drugs. These data support the use of DNA topoisomerase inhibitors as lead compounds for anti-trypanosomal drug development.

13749. **Egbe-Nwiyi, T.N., Igbokwe, I.O. & Onyeyili, P.A., 2005.** Diminazene aceturate resistance on the virulence of *Trypanosoma brucei* for rats. *Journal of Comparative Pathology*, **133** (4): 286-288.

Department of Veterinary Physiology and Pharmacology, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.

13750. **Espinoza-Fonseca, L.M. & Trujillo-Ferrara, J.G., 2006.** Toward a rational design of selective multi-trypanosomatid inhibitors: A computational docking study. *Bioorganic and Medicinal Chemistry Letters*, **16** (24): 6288-6292.

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA; Escuela Superior de Medicina del Instituto Politécnico Nacional, Apartado Postal 42-161, C.P. 11340, México City, México; Departamento de Bioquímica, Escuela Superior de Medicina del Instituto Politécnico Nacional, Apartado Postal 42-161, C.P. 11340, Mexico City, México.

Compound V7, a benzothiazole which was recently found as selective inhibitor of trypanosomal TIMs, was docked into TIMs from *Trypanosoma cruzi*, *Trypanosoma brucei*, *Entamoeba histolytica*, *Plasmodium falciparum*, yeast, and human. Structural analyses revealed the importance of the accessibility to the two aromatic clusters located at the dimer's interface for the selective inhibition of trypanosomal TIMs. Thus, it was found that different accessibilities of the protein interface of TIMs play an important role in the inhibitory activity of benzothiazoles. These findings will contribute to the rational development and improvement of benzothiazoles to be used as multi-trypanosomatid inhibitors.

13751. **Flores-Holguin, N. & Glossman-Mitnik, D., 2005.** CHIH-DFT determination of the electrical, optical, and magnetic properties and NICS aromaticity of megalzol. *Journal of Molecular Structure*, **717** (1-3): 1-3.

Grupo de Química Computacional, Simulación y Modelado Molecular and Programa Institucional de Nanotecnología, CIMAV, S.C., Miguel de Cervantes 120, Complejo Industrial Chihuahua, Chihuahua, Chih. 31109, México.

13752. **Fujii, N., Mallari, J.P., Hansell, E.J., Mackey, Z., Doyle, P., Zhou, Y.M., Gut, J., Rosenthal, P.J., McKerrow, J.H. & Guy, R.K., 2005.** Discovery of potent thiosemicarbazone inhibitors of rhodesain and cruzain. *Bioorganic and Medicinal Chemistry Letters*, **15** (1): 121-123.

Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, USA.

13753. **Gonzalez-Rey, E., Chorny, A. & Delgado, M., 2006.** VIP: an agent with license to kill infective parasites. *Annals of the New York Academy of Science*, **1070**: 303-308.

Instituto de Parasitología y Biomedicina, CSIC, Avd. Conocimiento, PT Ciencias de la Salud, Granada 18100, Spain. [elenag@ipb.csic.es]

Antimicrobial peptides are small, cationic, and amphipathic peptides of variable length, sequence, and structure. They are effector molecules of innate immunity with microbicidal and both pro- or anti-inflammatory activities. Vasoactive intestinal polypeptide (VIP) and the structurally related pituitary adenylate cyclase-activating polypeptide (PACAP) are well-

known immunomodulators. On the basis of their cationic and amphipathic structures, resembling antimicrobial peptides, we propose that their immune role could also include a direct lethal effect against pathogens. We thus investigated the potential antiparasitic activities of VIP and PACAP against the African trypanosome *Trypanosoma brucei* (*T. brucei*). Both peptides killed the bloodstream (infective) form but not the insect (noninfective) form of the parasite. VIP and PACAP caused complete destruction of the parasite integrity through a mechanism involving their entry and accumulation into the cytosol. These results provide the basis for further studies of these and other structurally related peptides as alternative treatments for parasitic diseases mainly with associated drug resistances.

13754. **Goringer, H.U., Homann, M., Zacharias, M. & Adler, A., 2006.** RNA aptamers as potential pharmaceuticals against infections with African trypanosomes. *Handbook of Experimental Pharmacology*, **173**: 375-393.

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Considerable progress has been made over the past 10 years in the development of nucleic acid-based drug molecules using a variety of different technologies. One approach is a combinatorial technology that involves an iterative Darwinian-type in vitro evolution process, which has been termed SELEX for "systematic evolution of ligands by exponential enrichment". The procedure is a highly efficient method of identifying rare ligands from combinatorial nucleic acid libraries of very high complexity. It allows the selection of nucleic acid molecules with desired functions, and it has been instrumental in the identification of a number of synthetic DNA and RNA molecules, so-called aptamers that recognize ligands of different chemical origin. Aptamers typically bind their target with high affinity and high specificity and have successfully been converted into pharmaceutically active compounds. Here we summarize the recent examples of the SELEX technique within the context of identifying high-affinity RNA ligands against the surface of the protozoan parasite *Trypanosoma brucei*, which is the causative agent of sleeping sickness.

13755. **Gros, L., Lorente, S.O., Jimenez, C.J., Yardley, V., Rattray, L., Wharton, H., Little, S., Croft, S.L., Ruiz-Perez, L.M., Gonzalez-Pacanowska, D. & Gilbert, I.H., 2006.** Evaluation of azasterols as anti-parasitics. *Journal of Medicinal Chemistry*, **49** (20): 6094-6103.

Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3XF, UK.

13756. **Gros, L., Castillo-Acosta, V.M., Jimenez Jimenez, C., Sealey-Cardona, M., Vargas, S., Manuel Estevez, A., Yardley, V., Rattray, L., Croft, S.L., Ruiz-Perez, L.M., Urbina, J.A., Gilbert, I.H. & Gonzalez-Pacanowska, D., 2006.** New azasterols against *Trypanosoma brucei*: role of 24-sterol methyltransferase in inhibitor action. *Antimicrobial Agents and Chemotherapy*, **50** (8): 2595-2601.

Welsh School of Pharmacy, Cardiff University, UK.

13757. **Hoet, S., Stevigny, C., Herent, M.F. & Quetin-Leclercq, J., 2006.** Antitrypanosomal compounds from the leaf essential oil of *Strychnos spinosa*. *Planta Medica*, **72** (5): 480-482.

Laboratoire de Pharmacognosie, Unité d'Analyse Chimique et Physico-Chimique des Médicaments, Université Catholique de Louvain, Bruxelles, Belgium.

13758. **Hu, Y. & Aksoy, S., 2005.** An antimicrobial peptide with trypanocidal activity characterized from *Glossina morsitans morsitans*. *Insect Biochemistry and Molecular Biology*, **35** (2): 105-115.

Department of Epidemiology and Public Health, Section of Vector Biology, Yale University, School of Medicine, 60 College St., 606 LEPH, New Haven, CT 06510, USA.

Prior studies in trypanosome infected *Glossina morsitans morsitans* have shown induced expression and synthesis of several antimicrobial peptides in fat body tissue. Here, we have expressed one of these peptides, Attacin (GmAttA1) in *Drosophila* (S2) cells *in vitro*. We show that the purified recombinant protein (recGmAttA1) has strong antimicrobial activity against *Escherichia coli*-K12, but not against the enteric gram-negative symbiont of tsetse, *Sodalis glossinidius*. The recGmAttA1 also demonstrated inhibitory effects against both the mammalian bloodstream form and the insect stage *Trypanosoma brucei in vitro* (minimal inhibitory concentration MIC50 0.075  $\mu$ M). When blood meals were supplemented with purified recGmAttA1 during the course of parasite infection, the prevalence of trypanosome infections in tsetse midgut was significantly reduced. Feeding fertile females GmAttA1 did not affect the fecundity or the longevity of mothers, nor did it affect the hatchability of their offspring. We discuss a paratransgenic strategy, which involves the expression of trypanocidal molecules such as recGmAttA1 in the midgut symbiont *Sodalis in vivo* to reduce trypanosome transmission.

13759. **Hui, X., Desrivot, J., Bories, C., Loiseau, P.M., Franck, X., Hocquemiller, R. & Figadere, B., 2006.** Synthesis and antiprotozoal activity of some new synthetic substituted quinoxalines. *Bioorganic and Medicinal Chemistry Letters*, **16** (4): 815-20.

Laboratoire de Pharmacognosie et Groupe Chimiothérapie Antiparasitaire (associé au CNRS-BioCIS), Faculté de Pharmacie, Université de Paris-Sud, rue J.B. Clement, 92296 Chatenay-Malabry, France.

13760. **Ismail, M.A., Arafa, R.K., Brun, R., Wenzler, T., Miao, Y., Wilson, W.D., Generaux, C., Bridges, A., Hall, J.E. & Boykin, D.W., 2006.** Synthesis, DNA affinity, and antiprotozoal activity of linear dicationic: Terphenyl diamidines and analogues. *Journal of Medicinal Chemistry*, **49** (17): 5324-5332.

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13761. **Ismail, M.A., Batista-Parra, A., Miao, Y., Wilson, W.D., Wenzler, T., Brun, R. & Boykin, D.W., 2005.** Dicationic near-linear biphenyl benzimidazole derivatives as DNA-targeted antiprotozoal agents. *Bioorganic and Medicinal Chemistry*, **13** (24): 6718-6726. 8b

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A series of near-linear biphenyl benzimidazole diamidines were synthesized from their respective diamidoximes, through the bis-O-acetoxyamidoxime, followed by hydrogenation in glacial acetic acid/ethanol in the presence of Pd-C. The compounds were quite active *in vitro* versus *Trypanosoma brucei rhodesiense*, giving IC<sub>50</sub> values ranging from 3 to 37 nM. These compounds were even more active versus *Plasmodium falciparum*, exhibiting IC<sub>50</sub> values ranging from 0.5 to 23 nM. The compounds showed moderate to good activity *in vivo* in the STIB900 model for acute African trypanosomiasis. The most active compounds gave 3 out of 4 cures on an i.p. dosage of 20 mg/kg.

13762. **Jagielski, A.K., Kryskiewicz, E. & Bryla, J., 2006.** Suramin-induced reciprocal changes in glucose and lactate synthesis in renal tubules contribute to its hyperglycaemic action. *European Journal of Pharmacology*, **537** (1-3): 205-209.

Department of Metabolic Regulation, Institute of Biochemistry, Warsaw  
University, I. Miecznikowa 1, 02-096 Warsaw, Poland.

13763. **Juyal, P.D., Singh, S., Singla, L.D. & Sood, N., 2005.** Effect of bleomycin hydrochloride on the course of *Trypanosoma evansi* infection in Swiss albino mice. *Journal of Parasitic Diseases*, **29** (2): 153-155.

Department of Veterinary Parasitology, College of veterinary Science, Punjab  
Agricultural University, Ludhiana-141004, India.

Bleomycin hydrochloride was used as trypanocide (5 mg per kg s/c) against experimental *T. evansi* (cattle strain) infection in Swiss albino mice. It was observed that 2 (24, 48 h) and 3 (24, 48, 72 h) consecutive injections of bleomycin had trypanocidal effect for a transient period of one and 4 days respectively, and subsequently reappearance of the trypanosomes occurred in peripheral blood. The increase in the number of treatments reduced the parasitaemia as indicated both by intensity as well the number of trypanosomes/ml of the tail blood. The survival period in treated groups was prolonged up to 12 days when compared with that of untreated control group. Histopathological changes were suggestive of mild hepatotoxicity.

13764. **Khabnadideh, S., Pez, D., Musso, A., Brun, R., Perez, L.M.R., Gonzalez-Pacanowska, D. & Gilbert, I.H., 2005.** Design, synthesis and evaluation of 2,4-

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diaminoquinazolines as inhibitors of trypanosomal and leishmanial dihydrofolate reductase. *Bioorganic and Medicinal Chemistry*, **13** (7): 2637-2649.

Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3XF, UK.

13765. **Kuboki, N., Yokoyama, N., Kojima, N., Sakurai, T., Inoue, N. & Sugimoto, C., 2006.** Efficacy of dipalmitoylphosphatidylcholine liposome against African trypanosomes. *Journal of Parasitology*, **92** (2): 389-393.

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

We demonstrate here that dipalmitoylphosphatidylcholine (DPPC) liposome has an antitrypanosomal effect, especially against the bloodstream forms (BSFs) of African trypanosomes (*Trypanosoma congolense*, *T. brucei rhodesiense*, and *T. brucei brucei*). The DPPC liposome significantly decreased the *in vitro* percentage of viable and motile BSF African trypanosomes but only marginally reduced the percentage of viable and motile procyclic form (PCF) of trypanosomes. The DPPC liposome absorption was much more pronounced to BSF than to PCF trypanosomes. Administration of the DPPC liposome showed a slight but significant reduction in the early development of parasitemia in *T. congolense*-infected mice. These results suggest that parasites were killed by specific binding of the DPPC liposome to the trypanosomes. This work demonstrates for the first time that a liposome has antitrypanosomal activity.

13766. **Lamas, M.C., Villaggi, L., Nocito, I., Bassani, G., Leonardi, D., Pascutti, F., Serra, E. & Salomon, C.J., 2006.** Development of parenteral formulations and evaluation of the biological activity of the trypanocide drug benznidazole. *International Journal of Pharmaceutics*, **307** (2): 239-243.

Area Tecnologia Farmaceutica, Facultad de Ciencias Bioquímicas y Farmaceuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina.

One of the drugs most frequently used for the treatment of Chagas disease is benznidazole (BZL). It is practically insoluble in water (0.4 mg/ml), which precludes the preparation of liquid dosage forms, in particular, parenteral formulations. Thus, the aim of this work was to investigate the solubilization of BZL at two pH values using various cosolvents such as ethyl alcohol, propylene glycol, polyethylene glycol 400, benzyl alcohol, diethylene glycol monoethyl ether (Transcutol) and surfactants such as polysorbates (Tween) 40 and 80, and sodium dioctyl sulfosuccinate (AOT). Solvent systems based on PEG 400, with the addition of ethyl alcohol and/or potassium biphthalate buffer solution, increased the BZL solubility up to 10 mg/ml. These alcoholic vehicles showed no toxicity against parasite when assayed at 1 percent. Physical and chemical stability studies showed that the formulations were stable for at least 1.5 years. In agreement with the biological activity



results, the selected formulations are suitable for further clinical studies. Moreover, increasing the aqueous solubility of BZL reduced the problems of *in vitro* testing techniques and bioassays leading to more reliable results and/or reproducibility.

13767. **Lanteri, C.A., Stewart, M.L., Brock, J.M., Alibu, V.P., Meshnick, S.R., Tidwell, R.R. & Barrett, M.P., 2006.** Roles for the *Trypanosoma brucei* P2 transporter in DB75 uptake and resistance. *Molecular Pharmacology*, **70** (5): 1585-1592.

Division of Infection and Immunity, Institute of Biomedical and Life Sciences, The Glasgow Biomedical Research Centre, University of Glasgow, Glasgow G12 8QQ, UK. [m.barrett@bio.gla.ac.uk].

13768. **Lorente, S.O., Jimenez, C.J., Gros, L., Yardley, V., de Luca-Fradley, K., Croft, S.L., J, A.U., Ruiz-Perez, L.M., Pacanowska, D.G. & Gilbert, I.H., 2005.** Preparation of transition-state analogues of sterol 24-methyl transferase as potential anti-parasitics. *Bioorganic and Medicinal Chemistry*, **13** (18): 5435-5453.

Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3XF, UK.

13769. **Mackey, Z.B., Baca, A.M., Mallari, J.P., Apsel, B., Shelat, A., Hansell, E.J., Chiang, P. K., Wolff, B., Guy, K.R., Williams, J. & McKerrow, J. H., 2006.** Discovery of trypanocidal compounds by whole cell HTS of *Trypanosoma brucei*. *Chemical Biology and Drug Design*, **67** (5): 355-363.

Department of Pathology and the Sandler Center for Basic Research in Parasitic Diseases, University of California, QB3 1700 4th St, San Francisco, CA 94158, USA.

One potentially rapid and cost-effective approach to identifying and developing new trypanocidal drugs would be high throughput-screening of existing drugs already approved for other uses, as well as clinical candidates in late development. We have developed an ATP-bioluminescence assay that could be used to rapidly and efficiently screen compound libraries against trypanosomes in a high throughput-screening format to validate this notion. We screened a collection of 2,160 FDA-approved drugs, bioactive compounds and natural products to identify hits that were cytotoxic to cultured *Trypanosoma brucei* at a concentration of 1  $\mu$ M or less. This meant that any hit identified would be effective at a concentration readily achievable by standard drug dosing in humans. From the screen, 35 hits from seven different drug categories were identified. These included the two approved trypanocidal drugs, suramin and pentamidine, several other drugs suspected but never validated as trypanocidal, and 17 novel trypanocidal drugs.

13770. **Masocha, W., Rottenberg, M.E. & Kristensson, K., 2006.** Minocycline impedes African trypanosome invasion of the brain in a murine model. *Antimicrobial Agents and Chemotherapy*, **50** (5): 1798-1804.

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Department of Neuroscience, Retzius vag 8, Karolinska Institutet, SE-171 77 Stockholm, Sweden.[willias.masocha@ki.se]

Passage of *Trypanosoma brucei* across the blood-brain barrier (BBB) is a hallmark of late-stage human African trypanosomiasis. In the present study we found that daily administration of minocycline, a tetracycline antibiotic, impedes the penetration of leukocytes and trypanosomes into the brain parenchyma of *T. brucei brucei*-infected C57BL/6 mice. The trypanosome-induced astrocytic and microglial reactions were reduced in the minocycline-treated mice, as were the levels in the brain of transcripts encoding adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and endothelial-leukocyte adhesion molecule 1 (E-selectin); the inflammatory cytokines tumor necrosis factor alpha, interleukin-1alpha (IL-1alpha), IL-1beta, IL-6, and gamma interferon; and matrix metalloprotease 3 (MMP-3), MMP-8, and MMP-12. Loss of weight occurring during infection with *T. b. brucei* was not observed after treatment of the mice with minocycline; these mice also survived longer than nontreated mice. Invasion of trypanosomes and leukocytes into the brain parenchyma most likely triggered the loss of weight and death of infected animals, since minocycline did not affect the growth of *T. b. brucei* either *in vitro* or *in vivo* or the levels of the transcripts encoding the cytokines and MMPs in the spleen. In conclusion, our data show that *T. b. brucei* invasion of the brain is related to that of leukocytes and that minocycline can ameliorate the disease in trypanosome-infected mice.

13771. **Mathis, A.M., Holman, J.L., Sturk, L.M., Ismail, M.A., Boykin, D.W., Tidwell, R.R. & Hall, J.E., 2006.** Accumulation and intracellular distribution of antitrypanosomal diamidine compounds DB75 and DB820 in African trypanosomes. *Antimicrobial Agents and Chemotherapy*, **50** (6): 2185-2191.

Division of Molecular Pharmaceutics, School of Pharmacy, University of North Carolina, Chapel Hill, Chapel 27599, USA.

13772. **Miller, D.M.S., Swan, G. E., Lobetti, R. G. & Jacobson, L. S., 2005.** The pharmacokinetics of diminazene aceturate after intramuscular administration in healthy dogs. *Journal of the South African Veterinary Association*, **76** (3): 146-150.

Department of Companion Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa.

The pharmacokinetics of diminazene aceturate following intramuscular (i.m.) administration at 4.2 mg/kg was evaluated in 8 healthy German Shepherd dogs. The results of this study indicate that diminazene is rapidly distributed and sequestered into the liver, followed by a slower terminal phase during which diminazene is both redistributed to the peripheral tissues and/or renally excreted. It is recommended that diminazene administered i.m. at 4.2 mg/kg should not be repeated within a 21-day period.

- 13773 **Muscia, G.C., Bollini, M., Carnevale, J.P., Bruno, A.M. & Asís, S.E. 2006.** Microwave-assisted Friedländer synthesis of quinolines derivatives as potential antiparasitic agents. *Tetrahedron Letters*, **47** (50): 8811-8815.

Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 (1113) Buenos Aires, Argentina. [elizabet@ffyb.uba.ar]

13774. **Orhan, I., Aslan, M., Sener, B., Kaiser, M. & Tasdemir, D., 2006.** *In vitro* antiprotozoal activity of the lipophilic extracts of different parts of Turkish *Pistacia vera* L. *Phytomedicine*, **13** (9-10): 735-739.

Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, TR-06330 Ankara, Turkey.

13775. **Parveen, S.K., Khan, M.O.F., Austin, S.E., Croft, S.L., Yardley, V., Rock, P. & Douglas, K.T., 2005.** Antitrypanosomal, antileishmanial, and antimalarial activities of quaternary arylalkylammonium 2-amino-4-chlorophenyl phenyl sulfides, a new class of trypanothione reductase inhibitor, and of N-acyl derivatives of 2-amino-4-chlorophenyl phenyl sulfide. *Journal of Medicinal Chemistry*, **48** (25): 8087-8097.

K.T. Douglas: University of Manchester, School of Pharmacy and Pharmaceutical Science, Oxford Rd, Manchester M13 9PL, Lancs., England.

13776. **Pradines, B., Pages, J.M. & Barbe, J., 2005.** Chemosensitizers in drug transport mechanisms involved in protozoan resistance. *Current Drug Targets: Infectious Disorders*, **5** (4): 411-431.

Unité de Recherche en Biologie et Épidémiologie Parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Marseille, France.

The emergence and spread of antiparasitic drug resistance pose a severe and increasing public health threat. Failures in prophylaxis or those in treatment with quinolines, hydroxynapthoquinones, sesquiterpenic lactones, antifolate drugs, arsenic and antimony containing drugs, sulfamides induce reemergence of parasite-related morbidity and mortality. Resistance is often associated with alteration of drug accumulation into parasites, which results from a reduced uptake of the drug, an increased efflux or, a combination of the two processes. Resistance to quinolines, artemisinin derivatives and arsenicals and expression of an active efflux mechanism are more or less correlated in protozoa like *Plasmodium* spp., *Leishmania* spp., and *Trypanosoma* spp. Various parasite candidate genes have been proposed to be involved in drug resistance, each concerned in membrane transport. Genes encoding membrane glycoproteins, orthologue to the P-glycoproteins identified in MDR human cancer cells, have been described in these resistant pathogens in addition to various membrane proteins involved in drug transport. Several compounds have demonstrated, in the past decade, promising capability to reverse the drug resistance in parasite isolates *in vitro*, in animal models and for human malaria. These drugs belong to different pharmacological

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classes such as calcium channel blockers, tricyclic antidepressants, antipsychotic calmodulin antagonists, histamine H1-receptor antagonists, analgesic antipyretic drugs, non-steroidal anti-inflammatory drugs, and to different chemical classes such as synthetic surfactants, alkaloids from plants used in traditional medicine, pyrrolidinoaminoalkanes and derivatives, and anthracene derivatives. Here, are summarized the molecular bases of antiparasitic drug resistance emphasizing recent developments with compounds acting on trans-membrane proteins involved in drug efflux or uptake.

13777. **Puri, U.K., Sharma, S.K., Kaur, P. & Juyal, P. D., 2006.** Pharmacokinetics of suramin in uninfected and experimental *Trypanosoma evansi* infected buffalo calves. *Indian Journal of Animal Sciences*, **76** (7): 495-497.

Punjab Agricultural University, Ludhiana, Punjab 141004, India.

The pharmacokinetic pattern of suramin was studied in buffalo calves experimentally infected with *Trypanosoma evansi*. Ten male buffalo calves were divided into two groups of equal number. Group 1 was inoculated with  $4.06 \times 10^7$  trypanosome/calf and treated with suramin (0.5 g/45 kg b.w. i.v.), while Group 2 was left uninfected but treated with the same dose of suramin i.v. The level of suramin in the plasma was determined by spectrophotometry. One minute after administration, the plasma level of suramin was  $103.8 \pm 1.63$  and  $160.5 \pm 3.60$  ug/ml, which declined to  $8.29 \pm 0.19$  and  $10.9 \pm 0.88$  µg/ml 7 days after administration in infected and uninfected buffalo calves, respectively. No concentration of the drug was found in the plasma seven days after treatment. The pharmacokinetics of suramin following intravenous administration was calculated by 3-compartment open model. There were significantly lower plasma levels up to 6 h after administration and lower values of distribution,  $t_{1/2\alpha}$ , AUC and CP levels in infected than uninfected buffalo calves. The significant difference between various pharmacokinetic parameters of *T. evansi* infected and uninfected buffalo calves may be due to the depletion of the drug, pathophysiological alteration and circulation changes that accompany trypanosome infection.

13778. **Rapp, M., Haubrich, T.A., Perrault, J., Mackey, Z.B., McKerrow, J.H., Chiang, P.K. & Wnuk, S.F., 2006.** Antitrypanosomal activity of 5'-deoxy-5'-(iodomethylene) adenosine and related 6-N-cyclopropyladenosine analogues. *Journal of Medical Chemistry*, **49** (6): 2096-2102.

Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33199, USA.

13779. **Reguera, R.M., Tekwani, B.L. & Balana-Fouce, R., 2005.** Polyamine transport in parasites: a potential target for new antiparasitic drug development. *Comparative Biochemistry and Physiology*, **140** (2): 151-164.

Department of Pharmacology and Toxicology (INTOXCAL), University of Leon, Campus de Vegazana (s/n), 24071 Leon, Spain.

The metabolism of the naturally occurring polyamines - putrescine, spermidine and spermine - is a highly integrated system involving biosynthesis, uptake, degradation and interconversion. Metabolic differences in polyamine metabolism have long been considered to be a potential target to arrest proliferative processes ranging from cancer to microbial and parasitic diseases. Despite the early success of polyamine inhibitors such as alpha-difluoromethylornithine (DFMO) in treating the latter stages of African sleeping sickness, in which the central nervous system is affected, they proved to be ineffective in checking other major diseases caused by parasitic protozoa, such as Chagas' disease, leishmaniasis or malaria. In the use and design of new polyamine-based inhibitors, account must be taken of the presence of up-regulated polyamine transporters in the plasma membrane of the infectious agent that are able to circumvent the effect of the drug by providing the parasite with polyamines from the host. This review contains information on the polyamine requirements and molecular, biochemical and genetic characterization of different transport mechanisms in the parasitic agents responsible for a number of the deadly diseases that afflict underdeveloped and developing countries.

13780. **Rodenko, B., Detz, R.J., Pinas, V.A., Lambertucci, C., Brun, R., Wanner, M.J. & Koomen, G.J., 2006.** Solid phase synthesis and antiprotozoal evaluation of di- and tri-substituted 5'-carboxamidoadenosine analogues. *Bioorganic and Medical Chemistry*, **14** (5): 1618-1629.

Van't Hoff Institute for Molecular Sciences, Universiteit van Amsterdam, the Netherlands.

13781. **Salem, M.M. & Werbovetz, K.A., 2006.** Isoflavonoids and other compounds from *Psoralea argyrea* with antiprotozoal activities. *Journal of Natural Products*, **69** (1): 43-9.

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

13782. **Seebacher, W., Schlapper, C., Brun, R., Kaiser, M., Saf, R. & Weis, R., 2005.** Antiprotozoal activities of new bicyclo[2.2.2]octan-2-imines and esters of bicyclo[2.2.2]octan-2-ols. *European Journal of Pharmaceutical Sciences*, **24** (4): 281-289.

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

13783. **Seebacher, W., Schlapper, C., Brun, R., Kaiser, M., Saf, R. & Weis, R., 2006.** Synthesis of new esters and oximes with 4-aminobicyclo[2.2.2]octane structure and evaluation of their antitrypanosomal and antiplasmodial activities. *European Journal of Medicinal Chemistry*, **41** (8): 970-977.

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

13784. **Seebacher, W., Weis, R., Kaiser, M., Brun, R. & Saf, R., 2005.** Synthesis of 2-azabicyclo [3.2.2] nonanes from bicycle [2.2.2] octan-2-ones and their activities against *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum* K1. *Journal of Pharmacy and Pharmaceutical Sciences*, **8** (3): 578-585.

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

New 2-azabicyclo [3.2.2] nonanes were prepared from antiprotozoal bicycle [2.2.2] octan-2-ones to investigate the influence of the replacement of the rigid bicyclo-octane structure by the more flexible bicyclo-nonane system on the antiplasmodial and antitrypanosomal activity. The 2-azabicyclo [3.2.2] nonanes were synthesized via a one-step procedure from bicycle [2.2.2] octan-2-ones and tested for their activities against *Trypanosoma b. rhodesiense* and *Plasmodium falciparum* K1 (resistant to chloroquine and pyrimethamine) using *in vitro* microplate assays. Due to their promising *in vitro* antiprotozoal activity and their low cytotoxicity, 2-azabicyclo [3.2.2] nonanes should serve as lead compounds for further modifications.

13785. **Staroverov, S.A., Pristensky, D.V., Yermilov, D.N., Gabalov, K.P., Zhemerichkin, D.A., Sidorkin, V.A., Shcherbakov, A.A., Shchyogolev, S.Y. & Dykman, L.A., 2006.** The effectivity analysis of accumulation of liposomal, micellar, and water-soluble forms of diminazene in cells and in organs. *Drug Delivery*, **13** (5): 351-355.

Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia.

13786. **Steverding, D., Pemberton, A.J., Royle, H., Spackman, R.W. & Rivett, A.J., 2006.** Evaluation of the anti-trypanosomal activity of tyropeptin A. *Planta Medica*, **72** (8): 761-763.

Biomedical Research Centre, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK. [dsteverding@hotmail.com]

The natural compound tyropeptin A, a new peptidyl aldehyde proteasome inhibitor, was tested for its trypanocidal activity *in vitro* using culture-adapted bloodstream forms of *Trypanosoma brucei*. The concentrations of tyropeptin A required to reduce the growth rate by 50 percent and to kill all cells were 10 and 100 times lower for bloodstream-form trypanosomes than for human leukaemia HL-60 cells, respectively. Enzymatic analysis showed that the trypsin-like activity of the trypanosome proteasome and the chymotrypsin-like activity of the mammalian proteasome are particularly sensitive to inhibition by tyropeptin A. The results suggest that natural compounds targeting the trypsin-like activity of

the proteasome may serve as leads for rational drug development of novel anti-trypanosomal agents.

13787. **Steverding, D., Spackman, R.W., Royle, H.J. & Glenn, R.J., 2005.** Trypanocidal activities of trileucine methyl vinyl sulfone proteasome inhibitors. *Parasitology Research*, **95** (1): 73-76.

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

Previous studies have shown that proteasome inhibitors are novel agents for chemotherapy of human African trypanosomiasis or sleeping sickness. In this study, five peptide trileucine methyl vinyl sulfones with different N-terminal substituents were tested for their trypanocidal activities *in vitro* using culture-adapted bloodstream forms of *Trypanosoma brucei*. Two inhibitors displayed promising anti-trypanosomal activities with ED50 values in the sub-micromolar range. Higher trypanocidal activity of the compounds generally corresponded to a higher  $k_{obs}$  value for inhibition of the trypsin-like activity but not for the inhibition of the chymotrypsin-like activity of the proteasome. These data suggest that inhibitors with strong activity against the trypsin-like activity of the proteasome are the rational choice for future anti-sleeping sickness drug development.

13788. **Stewart, M.B., Boussard, C., Brun, R., Gilbert, I.H. & Barrett, M.P., 2005.** Interaction of monobenzamidine-linked trypanocides with the *Trypanosoma brucei* P2 aminopurine transporter. *Antimicrobial Agents and Chemotherapy*, **49** (12): 5169-5171.

M.P. Barrett: University of Glasgow, Institute of Medical and Life Sciences, Division of Infection and Immunity, Glasgow G12 8QQ, Lanark, Scotland.

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Center for Cellular and Molecular Neurobiology, University of Liege, Liege, Belgium.

13790. **Tasdemir, D., Brun, R., Perozzo, R. & Donmez, A.A., 2005.** Evaluation of antiprotozoal and plasmodial enoyl-ACP reductase inhibition potential of Turkish medicinal plants. *Phytotherapy Research*, **19** (2): 162-166.

Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Switzerland. [deniz@oci.unizh.ch].

A total of 58 extracts of different polarity were prepared from various organs of 16 species of Turkish plants and screened for their antitrypanosomal, antileishmanial and antiplasmodial activities. No significant activity was observed against *Trypanosoma cruzi*, whereas many extracts showed appreciable trypanocidal potential against *T. brucei*

*rhodesiense*, with the CHCl<sub>3</sub> soluble portion of *Phlomis kurdica* being the most active (IC<sub>50</sub> 2.7 ug/ml).

13791. **Tasdemir, D., Kaiser, M., Brun, R., Yardley, V., Schmidt, T.J., Tosun, F. & Ruedi, P., 2006.** Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: *in vitro*, *in vivo*, structure-activity relationship, and quantitative structure-activity relationship studies. *Antimicrobial Agents Chemotherapy*, **50** (4): 1352-1364.

Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. [deniz@oci.unizh.ch]

13792. **Tsuda, A., Witola, W.H., Konnai, S., Ohashi, K. & Onuma, M., 2006.** The effect of TAO expression on PCD-like phenomenon development and drug resistance in *Trypanosoma brucei*. *Parasitology International*, **55** (2): 135-142.

Laboratory of Infectious Disease, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.

13793. **Ullmann, H., Meis, S., Hongwiset, D., Marzian, C., Wiese, M., Nickel, P., Communi, D., Boeynaems, J.M., Wolf, C., Hausmann, R., Schmalzing, G. & Kassack, M.U., 2005.** Synthesis and structure-activity relationships of suramin-derived P2Y<sub>11</sub> receptor antagonists with nanomolar potency. *Journal of Medicinal Chemistry*, **48** (22): 7040-7048.

Pharmaceutical Institute, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany.

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Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK.

13795. **Vega, M.C., Montero-Torres, A., Marrero-Ponce, Y., Rolon, M., Gomez-Barrio, A., Escario, J.A., Aran, V.J., Nogal, J.J., Meneses-Marcel, A. & Torrens, F., 2006.** New ligand-based approach for the discovery of antitrypanosomal compounds. *Bioorganic and Medicinal Chemistry Letters*, **16** (7): 1898-1904.

Department of Parasitology, Faculty of Pharmacy, Universidad Complutense de Madrid, 28040 Madrid, Spain.

13796. **Vicik, R., Hoerr, V., Glaser, M., Schultheis, M., Hansell, E., McKerrow, J.H., Holzgrabe, U., Caffrey, C.R., Ponte-Sucre, A. & Moll, H., 2006.** Aziridine-2,3-dicarboxylate inhibitors targeting the major cysteine protease of *Trypanosoma*



*brucei* as lead trypanocidal agents. *Bioorganic and Medicinal Chemistry Letters*, **16** (10): 2753-2757.

Institute of Pharmacy and Food Chemistry, University of Wuerzburg, Am Hubland, D-97074 Wuerzburg, Germany.

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Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens-University, Universitätsplatz 1, A-8010 Graz, Austria. [robert.weis@uni-graz.at]

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Pharmacognosie et Biomolécules Naturelles Actives, UMR no 7081, Faculté de Pharmacie, Université Louis Pasteur Strasbourg, Illkirch, France.

13799. **Wu, D., George, T.G., Hurh, E., Werbovetz, K.A. & Dalton, J.T., 2006.** Pre-systemic metabolism prevents *in vivo* antikinetoplastid activity of N1, N4-substituted 3,5-dinitro sulfanilamide, GB-II-150. *Life Sciences*. **In press; corrected proof.**

Division of Pharmaceutics, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA.

13800. **Wurochekke, A.U.J., James, D. B., Bello, M. I. & Ahmodu, A., 2005.** Trypanocidal activity of the leaf of *Guira senegalensis* against *Trypanosoma brucei brucei* infection in mice. *Journal of Medical Sciences (Pakistan)*, **5** (4): 333-336.

Department of Biochemistry, Federal University of Technology, Yola, Nigeria.

*In vitro* and *in vivo* trypanocidal activity of the leaf extract of *Guira senegalensis* against *Trypanosoma brucei brucei* has been investigated. Extract obtained from fresh leaves heated with methanol had highest *in vitro* activity against the parasite at concentration of 8.3 mg ml<sup>-1</sup> of blood. Dried leaf methanolic extract also had *in vitro* activity at the same concentration after 30 min of incubation. Treatment with 100 mg/kg/day of the fresh leaf extract for five days tends to ameliorate the disease condition but did not clear the parasitaemia and pack cell volume values were not significantly affected. All other animals treated with the extract higher than the 100 mg/kg/day died before the infected controls. Addition of glycerol as an adjuvant did not show effect. The plant may be a promising trypanocide.

13801. **Yabu, Y., Suzuki, T., Nihei, C., Minagawa, N., Hosokawa, T., Nagai, K., Kita, K. & Ohta, N., 2006.** Chemotherapeutic efficacy of ascofuranone in *Trypanosoma vivax*-infected mice without glycerol. *Parasitology International*, **55** (1): 39-43.

Department of Molecular Parasitology, Nagoya City University, Graduate School of Medical Sciences, Nagoya 467-8601, Japan. [yabu@med.nagoya-cu.ac.jp].

Ascofuranone, an antibiotic isolated from *Ascochyta visiae*, showed trypanocidal activity in *Trypanosoma vivax*-infected mice. A single dose of 50 mg/kg ascofuranone effectively cured the mice without the help of glycerol. Repeated administrations of this drug further enhanced its chemotherapeutic effect. After two, three, and four consecutive days treatment, the doses needed to cure the infection decreased to 25, 12, and 6 mg/kg, so that the total doses administered were 50, 36 and 24 mg/kg, respectively. Ascofuranone (50 mg/kg) also had a prophylactic effect against *T. vivax* infection within the first two days after administration. This prophylactic activity diminished to 80 percent by day 3 and completely disappeared four days after administration. Of particular interest in this study was that ascofuranone had trypanocidal activity in *T. vivax*-infected mice in the absence of glycerol, whereas co-administration of glycerol or repeated administrations of this drug are needed for *Trypanosoma brucei brucei* infection. Our present results strongly suggest that ascofuranone is also an effective tool in chemotherapy against African trypanosomiasis in domestic animals.

## 8. TRYPANOSOME RESEARCH

### (a) CULTIVATION OF TRYPANOSOMES

### (b) TAXONOMY, CHARACTERIZATION OF ISOLATES

[See also **29**: 13611, 13613, 13656, 13667, 13685]

13802. **Afework, Y., Maser, P., Etschmann, B., von Samson-Himmelstjerna, G., Zessin, K.H. & Clausen, P.H., 2006.** Rapid identification of isometamidium-resistant stocks of *Trypanosoma b. brucei* by PCR-RFLP. *Parasitology Research*, **99** (3): 253-261.

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Analyses were made on the adenosine transporter-1 gene in *Trypanosoma brucei* (TbAT1), encoding a P2-like nucleoside transporter, from *T. brucei brucei* field stocks to investigate a possible link between the presence of mutations in this gene and isometamidium resistance. We have analysed the gene from 11 isometamidium-sensitive field stocks isolated from cattle in Uganda, two sensitive reference clones and two resistant reference clones. A sequence alignment showed that the isometamidium-sensitive *T. b. brucei* contained the wild-type sequence patterns. In contrast, the isometamidium-resistant *T. b. brucei* stocks showed

the mutant-type sequence patterns with six point mutations that had previously been reported in a laboratory-derived arsenical-resistant *T. brucei* strain. To analyse the restriction fragment length polymorphism pattern of a fragment of TbAT1 (nucleotides 430-1108), the 677-bp polymerase chain reaction products from eight of the isometamidium-sensitive and two of the isometamidium-resistant *T. b. brucei* were subjected to digestion with Sfa NI. The results revealed two different banding patterns: the digest resulted in fragment sizes of 566 and 111 bp in the case of TbAT1 from isometamidium-sensitive stocks, whereas it produced fragment sizes of 435 and 242 bp in the case of TbAT1 from isometamidium-resistant stocks. Thus, the isometamidium-sensitive and isometamidium-resistant *T. b. brucei* could be successfully distinguished by digestion with the restriction endonuclease Sfa NI.

13803. **Balmer, O. & Tostado, C., 2006.** New fluorescence markers to distinguish co-infecting *Trypanosoma brucei* strains in experimental multiple infections. *Acta Tropica*, **97** (1): 94-101.

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Multiple-genotype infections are increasingly recognized as important factors in disease evolution, parasite transmission dynamics, and the evolution of drug resistance. However, the distinction of co-infecting parasite genotypes and the tracking of their dynamics have been difficult with traditional methods based on various genotyping techniques, leaving most questions unaddressed. Here we report new fluorescence markers of various colours that are inserted into the genome of *Trypanosoma brucei* to phenotypically label live parasites of all life cycle stages. If different parasite strains are labelled with different colours they can be easily distinguished from each other in experimental studies. A total of 10 *T. brucei* strains were successfully transfected with different fluorescence markers and were monitored in culture, tsetse flies and mice, to demonstrate stability of marker expression. The use of fluorescence activated cell sorting (FACS) allowed rapid and accurate identification of parasite strains labelled with different markers. Cell counts by FACS were virtually identical to counts by traditional microscopy (n=75, Spearman's rho: 0.91, p<0.0001) but were considerably faster and had a significantly lower sampling error (66 percent lower, d.f.=73, t=-17.1, p<0.0001). Co-infecting strains transfected with fluorescence genes of different colour were easily distinguished by eye and their relative and absolute densities were reliably counted by FACS in experimental multiple infections in mice. Since the FACS can simultaneously determine the population sizes of differently labelled *T. brucei* strains or subspecies it allows detailed and efficient tracking of multiple-genotype infections within a single host or vector individual, enabling more powerful studies on parasite dynamics. In addition, it also provides a simple way to separate genotypes after experimental mixed infections, to measure responses of the single strains to an applied treatment, thus eliminating the need for laborious cloning steps. The markers presented broaden the spectrum of tools available for experimental studies on multiple-genotype infections. They are fundamentally different from isoenzyme analysis and other genotyping approaches in that they allow the distinction of parasite genotypes based on an easily recognizable phenotypic trait. They will be of specific interest to researches addressing ecological, evolutionary and epidemiological questions using trypanosomes as an experimental system.

13804. **Cortez, A.P., Ventura, R.M., Rodrigues, A.C., Batista, J.S., Paiva, F., Anez, N., Machado, R.Z., Gibson, W.C. & Teixeira, M.M., 2006.** The taxonomic and phylogenetic relationships of *Trypanosoma vivax* from South America and Africa. *Parasitology*, **133** (2): 159-169.

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The taxonomic and phylogenetic relationships of *Trypanosoma vivax* are controversial. It is generally suggested that South American, and East and West African isolates could be classified as subspecies or species allied to *T. vivax*. This is the first phylogenetic study to compare South American isolates (Brazil and Venezuela) with West/East African *T. vivax* isolates. Phylogeny using ribosomal sequences positioned all *T. vivax* isolates tightly together on the periphery of the clade containing all Salivarian trypanosomes. The same branching of isolates within *T. vivax* clade was observed in all inferred phylogenies using different data sets of sequences (SSU, SSU plus 5.8S or whole ITS rDNA). *T. vivax* from Brazil, Venezuela and West Africa (Nigeria) were closely related corroborating the West African origin of South American *T. vivax*, whereas a large genetic distance separated these isolates from the East African isolate (Kenya) analysed. Brazilian isolates from cattle asymptomatic or showing distinct pathology were highly homogeneous. This study did not disclose significant polymorphism to separate West African and South American isolates into different species/subspecies and indicate that the complexity of *T. vivax* in Africa and of the whole subgenus *Trypanosoma* (Duttonella) might be higher than previously believed.

13805. **Delespaux, V., Chitanga, S., Geysen, D., Goethals, A., Van den Bossche, P. & Geerts, S., 2006.** SSCP analysis of the P2 purine transporter TcoAT1 gene of *Trypanosoma congolense* leads to a simple PCR-RFLP test allowing the rapid identification of diminazene resistant stocks. *Acta Tropica*. **In press; corrected proof.**

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Analyses were made on a *Trypanosoma congolense* contig coding a putative P2-like nucleoside transporter (the contig was named in this study TcoAT1). The sequence includes a start and stop codon and presents a high similarity with the gene TbAT1 of *T. brucei* (Smallest Sum Probability 2.8e-136). To investigate a possible link between point mutations and diminazene aceturate (DA) resistance in mice, the TcoAT1 putative genes of 26 *T. congolense* strains, characterised for DA sensitivity in the single dose mouse test, were screened by means of the Single Strand Conformation Polymorphism technique (SSCP). Results showed that the SSCP profiles of 23 out of 26 (88.5 percent) *T. congolense* strains were confirmed by the sensitivity test in mice with the commonly accepted criterion for sensitivity to diminazene being a CD80 of 20mg/kg in the mouse test. The remaining *T. congolense* strains showed a resistant SSCP profile and relapsed in mice after treatment at doses lower than 20mg/kg indicating that the SSCP is more sensitive than the single dose mouse test for the detection of resistance to diminazene. However, none of the strains used in this study showed a sensitive SSCP profile while they were resistant in the single dose mouse test. The sequencing of the TcoAT1 gene of two sensitive, two intermediate and two resistant

strains allowed the set up of a PCR-RFLP test for the discrimination between sensitive and resistant strains confirming the SSCP results for the 26 strains of this study.

13806. **El-Rayah, I.E., & El-Malik, K.H., 2006.** Characterization of quinapyramine (TrypacideReg.) drug-resistant *Trypanosoma evansi*. *African Journal of Biotechnology*, **5** (10): 951-955.

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Molecular karyotyping by pulsed field gel electrophoresis was used to characterize *Trypanosoma evansi* isolates. Ten *T. evansi* isolates from camels were collected in Eastern and Western Sudan. Isolates from Eastern Sudan which were kept under continuous prophylactic treatment with quinapyramine (TrypacideReg.), were found to bear a single pattern and belonged to one karyotype group. From Western Sudan where trypanosomosis management was done by individual treatment of proven parasitaemic cases, isolates with diverse karyotype patterns were obtained. This study concluded that the occurrence of karyotype homogeneity amongst *T. evansi* isolates from field situations where anti-trypanosomal compounds have been used may infer the existence of drug resistance.

13807. **Garcia, H., Garcia, M.E., Perez, H. & Mendoza-Leon, A., 2005.** The detection and PCR-based characterization of the parasites causing trypanosomiasis in water-buffalo herds in Venezuela. *Annals of Tropical Medicine and Parasitology*, **99**: 359-370.

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The usefulness of PCR-based assays for detecting trypanosomiasis in water buffaloes and other livestock was explored, under field conditions, in Venezuela. The sensitivity and specificity of the assays, which were based on established primer pairs (21-mer/22-mer and ILO1264/ILO1265), were evaluated, partly by comparison with the results of parasitological tests (stained bloodsmears and microhaematocrit centrifugation) and immunological assays (IFAT) run in parallel. The optimised PCR-based assays showed a sensitivity of 10 pg. DNA. The use of the 21-mer/22-mer primer pair gave a test that was specific for species in the subgenus *Trypanozoon* (including *Trypanosoma evansi*), whereas use of ILO1264/ILO1265 produced a test that was specific for *T. vivax*. The results of a hybridization assay using *T. evansi*-DNA and *T. vivax*-DNA probes indicated no cross-hybridization between the *T. evansi* and *T. vivax* PCR products. The results of the bloodsmear examinations, microhaematocrit centrifugations (MHC) and IFAT indicated that 23 (6.7 percent), 39 (11.4 percent) and 135 (39.5 percent) of the 342 blood samples investigated (including 316 from water buffaloes) contained trypanosomes, respectively. The results of the PCR-based assays indicated that 68 (19.9 percent) of the same blood samples contained *T. vivax* (or at least *T. vivax* DNA), and that none contained *T. evansi* or any other member of the subgenus *Trypanozoon*. For the detection of trypanosomes, the assay therefore appeared almost twice as sensitive as the MHC. These results are the first on the molecular characterization of the

trypanosomes infecting water buffaloes in Venezuela. When the results of the MHC (which is the most practical, and frequently used, alternative detection method) were used as the gold standard, the PCR-based assay for *T. vivax* was found to have 100 percent sensitivity, 90.4 percent specificity, a positive predictive value of 0.57, a positive likelihood ratio of 10.45, and a negative likelihood ratio of 0.00. The assay therefore appears a reasonable choice for detecting *T. vivax* in the mammalian livestock of Venezuela and elsewhere.

13808. **Gibson, W., Peacock, L., Ferris, V., Williams, K. & Bailey, M., 2006.** Analysis of a cross between green and red fluorescent trypanosomes. *Biochemical Society Transactions*, **34** (4): 557-559.

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*Trypanosoma brucei* undergoes genetic exchange in its insect vector, but the mechanism is unknown and no one has yet seen the process. By crossing genetically engineered red and green fluorescent trypanosomes, we have been able to pinpoint the location of genetic exchange in the fly and search for intermediate stages. In experimental crosses of red and green parental trypanosomes, yellow hybrid trypanosomes first appeared in the fly salivary glands as early as 13 days after infection and were observed only in flies with a mixture of red and green trypanosomes in one or both salivary glands. Despite high numbers of flies with mixed infections, yellow trypanosomes were not detected in the fly midgut or proventriculus. The hybrid nature of yellow trypanosomes was confirmed by analysis of molecular karyotypes and microsatellite alleles. As well as yellow hybrids, hybrid trypanosomes with red, green or no fluorescence were also recovered from fly salivary glands. Analysis of microsatellite alleles in parental and progeny clones showed Mendelian inheritance. Our findings are consistent with the hypothesis that mating takes place between trypanosomes in the salivary glands of the fly before they attach to the salivary gland epithelium.

13809. **Gonzalez, L.E., Garcia, J.A., Nunez, C., Perrone, T.M., Gonzalez-Baradat, B., Gonzatti, M.I. & Reyna-Bello, A., 2005.** *Trypanosoma vivax*: A novel method for purification from experimentally infected sheep blood. *Experimental Parasitology*, **111** (2): 126-129.

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*Trypanosoma vivax* is the principal etiological agent of bovine trypanosomiasis, a widely disseminated disease in tropical and subtropical regions. Here, we present a simple and reproducible method for the purification of *T. vivax* from experimentally infected and immunosuppressed sheep, using an isopycnic Percoll gradient, followed by DEAE-cellulose chromatography, with an estimated yield of 11-15 percent. This method could be used for the purification of *T. vivax* geographical isolates from various locations and from different natural hosts.

13810. **Hamilton, P.B., Stevens, J.R., Gidley, J., Holz, P. & Gibson, W.C., 2005.** A new lineage of trypanosomes from Australian vertebrates and terrestrial bloodsucking leeches (*Haemadipsidae*). *International Journal for Parasitology*, **35** (4): 431-443.

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Little is known about the trypanosomes of indigenous Australian vertebrates and their vectors. We surveyed a range of vertebrates and blood-feeding invertebrates for trypanosomes by parasitological and PCR-based methods using primers specific to the small subunit ribosomal RNA (SSU rRNA) gene of genus *Trypanosoma*. Trypanosome isolates were obtained in culture from two common wombats, one swamp wallaby and an Australian bird (*Strepera* sp.). By PCR, blood samples from three wombats, one brush-tailed wallaby, three platypuses and a frog were positive for trypanosome DNA. All the blood-sucking invertebrates screened were negative for trypanosomes both by microscopy and PCR, except for specimens of terrestrial leeches (*Haemadipsidae*). Of the latter, two *Micobdella* sp. specimens from Victoria and 18 *Philaemon* sp. specimens from Queensland were positive by PCR. Four *Haemadipsa zeylanica* specimens from Sri Lanka and three *Leiobdella jawarerenis* specimens from Papua New Guinea were also PCR positive for trypanosome DNA. We sequenced the SSU rRNA and glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH) genes in order to determine the phylogenetic positions of the new vertebrate and terrestrial leech trypanosomes. In trees based on these genes, Australian vertebrate trypanosomes fell in several distinct clades, for the most part being more closely related to trypanosomes outside Australia than to each other. Two previously undescribed wallaby trypanosomes fell in a clade with *Trypanosoma theileri*, the cosmopolitan bovid trypanosome, and *Trypanosoma cyclops* from a Malaysian primate. The terrestrial leech trypanosomes were closely related to the wallaby trypanosomes, *T. cyclops* and a trypanosome from an Australian frog. We suggest that haemadipsid leeches may be significant and widespread vectors of trypanosomes in Australia and Asia.

13811. **Jamal, S., Sigauque, I., Macuamule, C., Neves, L., Penzhorn, B.L., Marcotty, T. & Bossche, P.v.d., 2005.** The susceptibility of *Trypanosoma congolense* isolated in Zambezia Province, Mozambique, to isometamidium chloride, diminazene aceturate and homidium chloride. *Onderstepoort Journal of Veterinary Research*, **72** (4): 333-8.

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Resistance to trypanocidal drugs has been detected in various African countries and is a serious impediment to the control of livestock trypanosomosis. To determine whether drug resistant trypanosome strains are present in the Zambezia Province of Mozambique a study was initiated. To assess the effect of the farming system and the drug-use regimen on the development of drug resistance, trypanosome isolates were collected from cattle from subsistence and commercial livestock production systems. The susceptibility of seven isolates against isometamidium chloride, diminazene aceturate and homidium chloride was tested in mice using a multiple-dose test. In four of the seven isolates high levels of drug resistance to diminazene aceturate and isometamidium chloride were detected. In most cases the observed

levels of drug resistance correlated with the drug-use practices in the particular livestock production system.

13812. **Jamnadass, R.H., Pelle, R., Pandit, P., Ricard, B. & Murphy, N.B., 2006.**  
*Trypanosoma brucei*: composition, organisation, plasticity, and differential transcription of NlaIII repeat elements in drug-resistant and sensitive isolates. *Experimental Parasitology*, **113** (4): 244-255.

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A *Trypanosoma brucei brucei* DNA repeat sequence termed NlaIII repeat (NR) was originally isolated from a multidrug-resistant field isolate CP547. Identification and characterisation of an extrachromosomal element from a multidrug-resistant isolate of *T. brucei brucei* was then carried out and subsequently studied in a laboratory strain. Circular extrachromosomal DNA was identified in the nuclear genome of *T. brucei* and NRs shown to be exclusively episomal. Here we show that NR sequences in CP547 are present on linear chromosomes as well as on episomal circular elements. Sequence analysis shows that NRs are composed of three classes of sub-repeat arranged in a specific order. Heterogeneity in size and sequence of an episomal 6.6kbp element was shown in successive passages of the original CP547 isolate and derived clones in mice. Its copy number was unstable and was affected by selective pressure with the trypanocide diminazene aceturate. Some of the extrachromosomal elements appear to be composed of RNA-DNA hybrids. NR sequences were transcribed in a developmentally regulated manner but transcripts did not contain the spliced-leader sequence found on all trypanosome mRNAs.

13813. **Li, F.G., Gasser, R.B., Zheng, J.Y., Claes, F., Zhu, X.Q. & Lun, Z.R., 2005.**  
Application of multiple DNA fingerprinting techniques to study the genetic relationships among three members of the subgenus *Trypanozoon* (Protozoa: Trypanosomatidae). *Molecular and Cellular Probes*, **19** (6): 400-407.

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Three different DNA fingerprinting techniques, the mobile genetic element (MGE)-PCR, simple sequence repeat (SSR)-PCR and random amplified polymorphic DNA (RAPD)-PCR, were used to define a large set of genetic markers to study genetic similarity within and among *Trypanosoma brucei*, *Trypanosoma equiperdum* and *Trypanosoma evansi* strains (n = 18) from China, Africa and South America and to investigate their genetic relationships. Using the three fingerprinting techniques, >890 bands (ranging in size from 0.2 to 2 kb) were defined for all 18 strains of *Trypanosoma*. Within each of the strains, 39-59 bands were defined. The similarity coefficients between strains ranged from 41 to 94 percent, with a mean of 65 percent. There was more genetic similarity among strains within *T. evansi* (mean 79 percent) compared with *T. equiperdum* (65 percent) and *T. brucei* (59 percent). The similarity coefficient data were used to construct the dendrogram, which revealed that (irrespective of species) the majority of strains from China and South America grouped together to the exclusion of those from Africa. The exceptions were a *T. brucei* strain from



Africa and a *T. equiperdum* strain of unknown origin. Hence, employing data sets generated using the three different fingerprinting methods, it was not possible to unequivocally distinguish among *T. brucei*, *T. evansi* and *T. equiperdum*, although there was a tendency for *T. evansi* strains to group together to the exclusion of *T. brucei*. The findings provide support for the hypothesis that *T. evansi* originated from a mutated form of *T. equiperdum* and stimulate further investigations of the genetic make-up and evolution of members of the subgenus *Trypanozoon*.

13814. **Li, F., Zheng, J., Jia, W. & Lun, Z., 2005.** Analysis of molecular profiles among *Trypanozoon* species and subspecies by MGE-PCR method. *Chinese Journal of Parasitology and Parasitic Diseases*, **23** (5):277-82.

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To analyse the relationship between genetic variability and evolution among *Trypanosoma brucei* (including *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense*, *T. evansi* and *T. equiperdum* isolates), genomic DNAs of 26 trypanosome isolates were amplified by a mobile genetic elements (MGE) -PCR technique and cluster analysis was performed based on the molecular profiles with Neighbor-Joining method. The genetic variability among trypanosome isolates examined was obvious with an average genetic distance of 41.2 percent (ranged from 0 to 100 percent). Similarity coefficient among *T. brucei* isolates was 41.15 percent which was lower than that between *T. evansi* and *T. equiperdum* isolates. The closest relationship was found between *T. evansi* and *T. brucei* isolates with a similarity coefficient of 62.94 percent. The genetic variability between *T. b. rhodesiense* and *T. b. brucei* isolates was higher than that among *T. b. gambiense* isolates. In conclusion, species and subspecies displayed a higher genetic variability; *T. equiperdum* isolates collected from China and from South America, and *T. evansi* isolates from China and from South America, should have a similar origin.

13815. **Likeufack, A.C., Brun, R., Fomena, A. & Truc, P., 2006.** Comparison of the *in vitro* drug sensitivity of *Trypanosoma brucei gambiense* strains from West and Central Africa isolated in the periods 1960–1995 and 1999–2004. *Acta Tropica*. **In press; corrected proof.**

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The situation of human African trypanosomiasis remains serious with one of the main threats being the increasing number of relapses or treatment failures after melarsoprol treatment. In order to investigate and to compare drug sensitivities of trypanosomes isolated at different time periods and in different locations, two sets of *Trypanosoma brucei gambiense* strains were used. One set was isolated in the time period 1960–1981 and the

other one in 1995–2004 from different locations of West and Central Africa. These isolates were not selected based on the treatment outcome but on availability. The drug sensitivity profile for all available drugs in use and the diamidine compound DB75 was established. IC<sub>50</sub> values were not significantly different between the “old” and “new” stocks. No indications for emerging drug resistance to any drug could be observed. The results indicate a relative stability of *in vitro* sensitivity of *T. b. gambiense* to trypanocidal drugs in space (West and Central Africa) and time (1960–2004).

13816. **MacLeod, A., 2004.** Minisatellites and MVR-PCR for the individual identification of parasite isolates. In: *Parasite Genomics Protocols*, Humana Press, Totowa, USA., pp.187-202.

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In recent years, a wide variety of biochemical and molecular typing systems have been employed in the study of parasite diversity aimed at investigating the level of genetic diversity and delineating the relationships among different species and subspecies. Parasite sequence-specific polymerase chain reaction (PCR)-based genotyping systems are among the most useful tools employed to date, because they can be applied to very small quantities of host-contaminated parasite material and, using repeated loci such as mini- and microsatellites, allow the identification and tracking of individual strains as well as the determination of allele and genotype frequencies in populations. Although minisatellites have been used very successfully to study parasite populations, in particular *Trypanosoma brucei* populations, there are some technical problems involved in the use of these markers. For example, minisatellite alleles tend to vary in a quasi-continuous fashion, making unambiguous allele identification difficult. The development of minisatellite variant repeat (MVR) mapping by the polymerase chain reaction (MVR-PCR) as a digital approach to DNA typing has overcome many of the drawbacks of minisatellite length analysis. The system assays the dispersion patterns of MVRs within minisatellite alleles, producing an easily interpretable code for each allele. This technique not only allows unequivocal allele identification but also reveals cladistic information that can be used to determine the possible genetic relationships among the different strains and subspecies. The MVR mapping technique has been applied successfully to minisatellites in the parasite *Plasmodium falciparum* to uniquely identify strains, and more extensively in *Trypanosoma brucei*, where it was used to determine population structure and to examine the relationships among *T. brucei* subspecies, providing evidence for multiple origins of human infectivity. In this chapter, the methods for genotyping of *T. brucei* parasites using both minisatellite allele length and MVR mapping are described in full and can be easily adapted to apply to minisatellites in other parasites.

13817. **Masiga, D.K., Ndung'u, K., Tweedie, A., Tait, A. & Turner, C.M., 2006.** *Trypanosoma evansi*: Genetic variability detected using amplified restriction fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) analysis of Kenyan isolates. *Experimental Parasitology*, **114** (3): 147-153.

*Tsetse and Trypanosomiasis Information*

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We compared two methods to generate polymorphic markers to investigate the population genetics of *Trypanosoma evansi*; random amplified polymorphic DNA (RAPD) and amplified restriction fragment length polymorphism (AFLP) analyses. AFLP accessed many more polymorphisms than RAPD. Cluster analysis of the AFLP data showed that 12 *T. evansi* isolates were very similar ('type A') whereas 2 isolates differed substantially ('type B'). Type A isolates have been generally regarded as genetically identical but AFLP analysis was able to identify multiple differences between them and split the type A *T. evansi* isolates into two distinct clades.

13818. **Masumu, J., Geysen, D., Vansnick, E., Geerts, S. & Van den Bossche, P., 2006.** A modified AFLP for *Trypanosoma congolense* isolate characterisation. *Journal of Biotechnology*, **125** (1): 22-26.

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The amplified fragment length polymorphism (AFLP) technique is a reliable and powerful DNA fingerprint tool for genetic characterisation and analysis. In this paper, we described a modified AFLP with high resolution for *Trypanosoma congolense* using one enzyme and agarose or Elchrom gel electrophoresis. Eleven allopatric and fourteen sympatric isolates of *T. congolense* savannah were used to assess the resolution of the method and its ability to characterise *T. congolense* isolates. Two enzymes (Eco RI or Bgl II) and corresponding non-selective and selective primers were used to identify the most appropriate combination. Patterns generated by Bgl II enzyme and a single selective primer A, C, G or T produced clear profiles. Each of the four selective primers produced different profiles for all the 25 *T. congolense* isolates. Due to the reduction in the number of bands, profiles could be analysed using agarose or Elchrom gels. Although comparison of a great number of samples could benefit from software help, this technique did not require fluorescence detection methods. The results of the present study demonstrated that this modified AFLP makes the characterisation of *T. congolense* easier while maintaining high resolution.

13819. **Masumu, J., Marcotty, T., Geysen, D., Geerts, S., Vercruyse, J., Dorny, P. & den Bossche, P.V., 2006.** Comparison of the virulence of *Trypanosoma congolense* strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. *International Journal of Parasitology*, **36** (4): 497-501.

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The virulence of 31 genetically different *Trypanosoma congolense* strains belonging to the Savannah subgroup and isolated from cattle at 11 sites in a trypanosomiasis endemic area of eastern Zambia was compared. Virulence testing, done in OF1 mice, revealed three

virulence categories. Strains were considered extremely virulent when the median survival time ranged between 5 and 9 days. Moderately virulent strains had a median survival time between 10 and 30 days and low virulence, more than 30 days. For each strain, the prepatent period was determined and the PCV of the infected animals was measured at regular intervals. A total of six (19.4 percent) strains belonged to the extremely virulent category with a short prepatent period (mean 2.3+/-0.3 days), high parasitaemia, decline in PCV of 15.6+/-1.1 percent during the first 7 days p.i. and a short median survival time (mean 6 days). The remainder of the strains belonged to the moderate (13 strains) or low (12 strains) virulence categories with median survival times of 13 and 60 days, respectively. They had longer prepatent periods (means 3.2+/-1.6 days and 3.5+/-1.6 days for moderately virulent and strains with low virulence, respectively) and the decline in PCV was less steep (decline of 14.2+/-0.6 and 9.7+/-0.6 percent during the first 7 days of infection with moderately virulent strains and strains with low virulence, respectively). Extremely virulent strains were isolated from cattle at four sampling sites with 60 percent of the cattle from one sampling site harbouring such extremely virulent strains. Results from this study demonstrated substantial differences in the virulence of *T. congolense* strains of the Savannah subgroup, isolated in one geographic area from a single host species. On the assumption that information on virulence obtained from tests in mice can be extrapolated to cattle, the high proportion of strains with low to moderate virulence is thought to be attributed to the important role of susceptible cattle as reservoirs of trypanosomes in the study area and the ensuing selection against extremely virulent strains.

13820. **Masumu, J., Marcotty, T., Ndeledje, N., Kubi, C., Geerts, S., Vercruyse, J., Dorny, P. & van den Bossche, P., 2006.** Comparison of the transmissibility of *Trypanosoma congolense* strains isolated in a trypanosomiasis endemic area of eastern Zambia by *Glossina morsitans morsitans*. *Parasitology*, **133** (3): 331-334.

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Transmission experiments were conducted to compare the transmissibility of genetically different *Trypanosoma congolense* (Savannah subgroup) strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. A total of 17 strains were compared. Three strains were extremely virulent with a short pre-patent period, high parasitaemia and a short median survival time (between 5 and 9 days) in mice. The remainder of the strains belonged to the moderate (6 strains) or low (8 strains) virulence categories with median survival times between 10 and 30 days and >30 days, respectively. Batches of 40 teneral *Glossina morsitans morsitans* (Diptera: Glossinidae) were offered a single bloodmeal on mice infected with one of those strains. Flies were dissected to determine their infection status 21 days later. The proportion of flies with procyclic and metacyclic infections differed significantly between trypanosome strains and was significantly higher in flies infected with extremely virulent strains ( $P=0.033$  and  $P=0.016$  for the differences in the procyclic infection rate of strains with moderate and low virulence, respectively and  $P=0.005$  and  $P=0.019$  for the differences in the metacyclic infection rate of strains with moderate and low virulence, respectively). On the other hand, moderately virulent strains had, in general, higher procyclic and metacyclic infection rates compared to low virulent strains. But the differences were not significant ( $P>0.05$ ). The outcome of those experiments shows clear differences in

transmissibility of trypanosome strains associated with their virulence. This observation confirms the theory for the evolution and maintenance of virulence in a parasite population and may explain the persistence of virulent trypanosome strains in a susceptible host population.

13821. **Ngaira, J.M., Olemba, N.K., Njagi, E.N.M. & Ngeranwa, J.J.N., 2005.** The detection of non-RoTat 1.2 *Trypanosoma evansi*. *Experimental Parasitology*, **110** (1): 30-38.

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The majority of *Trypanosoma evansi* can be detected using diagnostic tests based on the variant surface glycoprotein (VSG) of *Trypanosoma evansi* Rode *Trypanozoon* antigen type (RoTat) 1.2. Exceptions are a number of *T. evansi* isolated in Kenya. To characterize *T. evansi* that are undetected by RoTat 1.2, we cloned and sequenced the VSG cDNA from *T. evansi* JN 2118Hu, an isolate devoid of the RoTat 1.2 VSG gene. A 273 bp DNA segment of the VSG gene was targeted in PCR amplification for the detection of non-RoTat 1.2 *T. evansi*. Genomic DNA samples from different trypanosomes were tested including 32 *T. evansi*, 10 *Trypanosoma brucei*, three *Trypanosoma congolense*, and one *Trypanosoma vivax*. Comparison was by PCR amplification of a 488 bp fragment of RoTat1.2 VSG gene. Results showed that the expected 273 bp amplification product was present in all five non-RoTat 1.2 *T. evansi* tested and was absent in all 27 RoTat 1.2-positive *T. evansi* tested. It was also absent in all other trypanosomes tested. The PCR test developed in this study is specific for non-RoTat 1.2 *T. evansi*.

13822. **Njiru, Z.K., Constantine, C.C., Masiga, D.K., Reid, S.A., Thompson, R.C.A. & Gibson, W.C., 2006.** Characterization of *Trypanosoma evansi* type B. *Infection, Genetics and Evolution*, **6** (4): 292-300.

Division of Health, School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, South Street, WA 6150, Australia; Trypanosomiasis Research Centre, Kenya Agricultural Research Institute, P.O. Box 362, Kikuyu, Kenya.

A distinctive feature of *Trypanosoma evansi* is the possession of a kinetoplast that contains homogeneous DNA minicircles, but lacks DNA maxicircles. Two major sequence variants of the minicircle have been described and here we have sequenced the type B variant and designed a specific PCR test to distinguish it from type A. Further a test based on maxicircles to distinguish *T. brucei brucei* from *T. evansi* was designed and evaluated. Using the designed PCR tests, we detected three type B isolates from camel blood samples collected in northern Kenya, more than 20 years after the first isolation of type B. Comparison of minicircle sequences from all four type B isolates shows >96 percent identity within the group, and 50-60 percent identity to type A minicircles. Phylogenetic analysis based on minicircle sequences reveals two clusters, one comprising isolates of type A and one of type B, while random amplification of polymorphic DNA show slight polymorphic bands within type B. Most *T. evansi* isolates analysed were heterozygous at a repetitive coding locus

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(MORF2). All type B isolates had one genotype designated 3/5 based on the alleles present. Three camel isolates, which had homogenous type A minicircles, lacked the RoTat 1.2 gene, while another five isolates were *T. b. brucei*, based on the heterogeneity of their minicircles and presence of maxicircles as demonstrated by PCR amplification of the gene for cytochrome oxidase subunit 1. Our results confirm the existence of *T. evansi* type B isolates, *T. b. brucei* and existence of *T. evansi* type A without RoTat 1.2 gene in Kenyan isolates.

13823. **Piontkivska, H.H. & Hughes, A. L., 2005.** Environmental kinetoplastid-like 18S rRNA sequences and phylogenetic relationships among Trypanosomatidae: paraphyly of the genus *Trypanosoma*. *Molecular and Biochemical Parasitology*, **144** (1): 94-99.

Department of Biological Sciences, 242 Cunningham Hall, Kent State University, Kent, OH 44242, USA.

Using kinetoplastid-like sequences from deep-sea environmental samples as an out-group, we applied phylogenetic analysis to 18S rRNA sequences of the families Trypanosomatidae and Bodonidae (Euglenozoa: Kinetoplastida). The monophyly of the genus *Trypanosoma* was not supported by a number of different methods. Rather, the results indicate that the American and African trypanosomes constitute distinct clades, therefore, implying that the major human disease agents *T. cruzi* (cause of Chagas' disease) and *T. brucei* (cause of African sleeping sickness) are not as closely related to each other as they were previously thought to be. Likewise, the results did not support monophyly of the genera *Leishmania*, *Leptomonas*, *Bodo* and *Cryptobia*.

13824. **Ravel, S., Patrel, D., Koffi, M., Jamonneau, V. & Cuny, G., 2006.** Cyclical transmission of *Trypanosoma brucei gambiense* in *Glossina palpalis gambiensis* displays great differences among field isolates. *Acta Tropica*. **In press; corrected proof.**

IRD, UR177, Laboratoire de Recherche et de Coordination sur les Trypanosomoses, IRD-CIRAD, TA 207/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France.

Six sets of teneral *Glossina palpalis gambiensis* (Diptera: Glossinidae) were fed on mice infected with six different isolates of *Trypanosoma brucei gambiense* (each mouse was infected with one of the isolates), previously isolated from patients in the sleeping sickness focus of Bonon, Côte d'Ivoire and in Makoua, Congo. All the tsetse flies were dissected 42 days post-infection and midgut and salivary glands were examined for trypanosomes by microscopical examination. No infection was observed with the reference stock whereas each of the five recently isolated trypanosome isolates was able to infect tsetse flies, with rates of infection varying between 9.7 and 18.2 percent depending on the isolate. Three isolates displayed only immature infections with 9.7, 17.3 and 18 percent of the flies showing trypanosomes in their midgut. One isolate gave both immature (12.1 percent) and mature infections (6.1 percent). Finally, the last isolate involved only mature infections in 9.7 percent of the *Glossina* species examined. These substantial differences in the cyclical transmission

of *T. b. gambiense* in the same fly species could have important implications for the epidemiology of the transmission of Human African Trypanosomiasis.

13825. **Truc, P., Gibson, W. & Herder, S., 2006.** Genetic characterization of *Trypanosoma evansi* isolated from a patient in India. *Infection, Genetics and Evolution*. **In press; corrected proof.**

Institut de Recherche pour le Développement, UR177, Instituto de Combate et Controlo das Tripanossomiasas (ICCT), CP 2657, Luanda, Angola.

The first human case of trypanosomiasis caused by *Trypanosoma evansi* was recently discovered in India. We have focused on the parasite to investigate whether this atypical infection was due to a particular genotype of *T. evansi*. The SRA gene was not detected by PCR in the Indian human *T. evansi* (TEVH) DNA sample. TEVH appears to be closely related to Vietnam WH, with identical alleles for TRBPA and MT30-33 AC/TC microsatellites. Furthermore, *T. evansi* has homogeneous kDNA minicircles and the minicircles of isolate TEVH were shown to be of Type A. Thus, the *T. evansi* isolated from an Indian patient appears to be a typical *T. evansi* as far as we can judge, suggesting that the explanation for this unusual infection may lie with the patient.

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[See also **29**: nos. 13466, 13478, 13494, 13502, 13505, 13510, 13541, 13544, 13545, 13558, 13584, 13588, 13597]

13826. **Ackers, J.P., Dhir, V. & Field, M.C., 2005.** A bioinformatic analysis of the RAB genes of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*, **141** (1): 89-97.

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK.

13827. **Alibu, V.P., Richter, C., Voncken, F., Marti, G., Sanjay, S., Renggli, C.K., Seebeck, T., Brun, R. & Clayton, C., 2006.** The role of *Trypanosoma brucei* MRPA in melarsoprol susceptibility. *Molecular and Biochemical Parasitology*, **146** (1): 38-44.

Universitat Heidelberg, Zentrum für Molekulare Biologie (ZMBH), Im Neuenheimer Feld 282, D69120 Heidelberg, Germany.

We previously showed that over-expression of *Trypanosoma brucei* MRPA, a member of the multidrug resistance protein family in *T. brucei*, reproducibly resulted in resistance to the anti-trypanosomal drug melarsoprol *in vitro*. MRPA is predicted to mediate efflux of melarsoprol as a conjugate with trypanothione, a glutathione-spermidine conjugate which is the major small thiol in trypanosomes. Here, we show that depletion of MRPA by RNA interference resulted in moderate hypersensitivity to both melarsoprol and melarsen oxide.

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Over-expression of MRPA alone is not sufficient to cause melarsoprol resistance *in vivo*, although it is sufficient *in vitro*. This discrepancy is not an effect of drug metabolism since over-expression of MRPA alone conferred resistance to melarsoprol and its principal metabolite, melarsen oxide, *in vitro*. Over-expression of MRPA was not detected in four melarsoprol-resistant trypanosome isolates from sleeping sickness patients.

13828. **Alphey, M.S., Burton, A., Urbaniak, M.D., Boons, G.J., Ferguson, M.A. & Hunter, W.N., 2006.** *Trypanosoma brucei* UDP-galactose-4'-epimerase in ternary complex with NAD<sup>+</sup> and the substrate analogue UDP-4-deoxy-4-fluoro- $\alpha$ -D-galactose. *Acta Crystallographica Section F Structural Biology and Crystallography Communications*, **62** (9): 829-834.

Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

13829. **Alphey, M.S. & Hunter, W.N., 2006.** High-resolution complex of papain with remnants of a cysteine protease inhibitor derived from *Trypanosoma brucei*. *Acta Crystallographica Section F Structural Biology and Crystallography Communications*, **62** (6): 504-508.

Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

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Department of Infections and Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK.

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Instituto de Quimica y Fisicoquimica Biologica IQUIFIB-CONICET, Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires, Junin 956, CP1113, Argentina.

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C Tschudi: Yale University, School of Medicine, Department of Epidemiology and Public Health, 295 Congress Ave., New Haven, CT 06536 USA.



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Department of Internal Medicine, Yale University Medical School, BCMM  
136D, 295 Congress Avenue, Box 9812, New Haven, CT 06536-8012, USA.

13834. **Arhin, G.K.S., Shen, S. Y., Ullu, E. & Tschudi, C., 2004.** A PCR-based method for gene deletion and protein tagging in *Trypanosoma brucei*. *Methods in Molecular Biology*, **270**: 277-86.

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut, USA.

Sequence information on the *Trypanosoma brucei* genome is rapidly accumulating. As a consequence, there is a need for techniques to analyse gene function systematically. Here, we describe a polymerase chain reaction (PCR)-based method for direct gene deletion and the generation of epitope-tagged fusion proteins. The approach is based on methodologies developed for *Saccharomyces cerevisiae* and involves PCR amplification of a reporter cassette using primers containing flanking sequences specific to the target gene. The PCR product is then transfected directly into procyclic *T. brucei* cells, and homologous recombinants that carry the deleted or tagged target gene are identified.

13835. **Balmer, O., Palma, C., Macleod, A. & Caccone, A., 2006.** Characterization of di-, tri- and tetranucleotide microsatellite markers with perfect repeats for *Trypanosoma brucei* and related species. *Molecular Ecology Notes* **6** (2): 508-510.

Yale University, Department of Ecology and Evolutionary Biology, 165  
Prospect St., New Haven, USA.[oliver.balmer@yale.edu,  
oliver.balmer@pronet.ch].

*Trypanosoma brucei*, a unicellular parasite causing human sleeping sickness and animal nagana, has a great impact on the socioeconomic environment of sub-Saharan Africa. The dynamics of the parasite are still poorly understood. We have characterized 14 polymorphic di-, tri- and tetranucleotide microsatellite loci with perfect repeats (only one motif) exhibiting between five and 16 alleles in *T. brucei* isolates from all over Africa and from all described subspecies. The microsatellites will be useful in addressing population genetic questions in *T. brucei* to better understand the population structure and spread of this important parasite.

13836. **Bell, A., Monaghan, P. & Page, A.P., 2006.** Peptidyl-prolyl cis-trans isomerases (immunophilins) and their roles in parasite biochemistry, host-parasite interaction and antiparasitic drug action. *International Journal for Parasitology*, **36** (3): 261-276.

Department of Microbiology, Moyne Institute of Preventive Medicine,  
University of Dublin, Trinity College, Dublin 2, Ireland. [abell@tcd.ie].

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Wellcome Centre for Molecular Parasitology, The Anderson College, University of Glasgow, Glasgow G11 6NU, Scotland, UK.

The generation of energy in African trypanosomes is a subject of undoubted importance. In bloodstream-form organisms, substrate-level phosphorylation of glucose is sufficient to provide the energy needs of the parasite. The situation in procyclic-form trypanosomes is more complex. For many years, it was accepted that glucose metabolism followed a conventional scheme involving glycolysis, the tricarboxylic acid cycle and ATP-producing oxidative phosphorylation linked to the electron-transport chain. However, progress in sequencing the *Trypanosoma brucei* genome and the development of gene-knockout and RNA interference technology has provided novel insight. Coupling these new technologies with classical approaches, including NMR and mass spectrometry to analyse glycolytic intermediates and end products, have yielded several surprises. In this article, we summarize how these recent data have helped to change the view of metabolism in procyclic-form *T. brucei*.

13838. **Biton, M., Mandelboim, M., Arvatz, G. & Michaeli, S., 2006.** RNAi interference of XPO1 and Sm genes and their effect on the spliced leader RNA in *Trypanosoma brucei*. *Molecular Biochemistry and Parasitology*, **150** (2): 132-143.

The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel.

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INSERM U565 and CNRS UMR5153 and MNHN USM0503, Museum National d'Histoire Naturelle, 43 rue Cuvier, 75231 Paris Cedex 05, France.

13840. **Bringaud, F., Riviere, L. & Coustou, V., 2006.** Energy metabolism of trypanosomatids: Adaptation to available carbon sources. *Molecular and Biochemical Parasitology*, **149** (1):1-9.

Laboratoire de Génomique Fonctionnelle des Trypanosomatides, Université Victor Segalen Bordeaux 2, UMR-5162 CNRS, 146 rue Leo Saignat, 33076 Bordeaux Cedex, France.

13841. **Broadhead, R.D., Daw, H.R., Farr, H., Griffiths, S., Hart, S.R., Portman, N., Shaw, M.K., Ginger, M.L., Gaskell, S.J., McKean, P.G. & Gull, K., 2006.** Flagellar motility is required for the viability of the bloodstream trypanosome. *Nature*, **440** (7081): 224-227.

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K. Gull: University of Oxford, Sir William Dunn School of Pathology, S. Parks Rd, Oxford OX1 3RE, UK.

Here we present a detailed proteomic analysis of the trypanosome flagellum. RNA interference (RNAi)-based interrogation of this proteome provides functional insights into human ciliary diseases and establishes that flagellar function is essential to the bloodstream-form trypanosome. We show that RNAi-mediated ablation of various proteins identified in the trypanosome flagellar proteome leads to a rapid and marked failure of cytokinesis in bloodstream-form (but not procyclic insect-form) trypanosomes, suggesting that impairment of flagellar function may provide a method of disease control. A postgenomic meta-analysis, comparing the evolutionarily ancient trypanosome with other eukaryotes including humans, identifies numerous trypanosome-specific flagellar proteins, suggesting new avenues for selective intervention.

13842. **Brown, S.V., Hosking, P., Li, J.L. & Williams, N., 2006.** ATP synthase is responsible for maintaining mitochondrial membrane potential in bloodstream form *Trypanosoma brucei*. *Eukaryotic Cell* **5** (1): 45-53

Department of Microbiology and Immunology, 253 Biomedical Research Building, University at Buffalo, Buffalo, New York 14214, USA.

13843. **Callejas, S., Leech, V., Reitter, C. & Melville, S., 2006.** Hemizygous subtelomeres of an African trypanosome chromosome may account for over 75 percent of chromosome length. *Genome Research*, **16** (9): 1109-1118.

Department of Pathology, University of Cambridge, CB2 1QP, Cambridge, UK.

We have developed a Tb927 high-resolution DNA microarray to study DNA content variation along chromosome I, one of the most size-variable chromosomes, in different strains and subspecies of *T. brucei*. Results show considerable copy number polymorphism, especially at subtelomeres, but are insufficient to explain the observed size difference. Additional sequencing reveals that >50 percent of a larger chromosome I consists of arrays of variant surface glycoprotein genes (VSGs), involved in avoidance of acquired immunity. In total, the subtelomeres appear to be three times larger than the diploid core. These results reveal that trypanosomes can utilize subtelomeres for amplification and divergence of gene families to such a remarkable extent that they may constitute most of a chromosome, and that the VSG repertoire may be even larger than reported to date. Further experimentation is required to determine if these results are applicable to all size-variable chromosomes.

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K. Stuart: Seattle Biomedical Research Institute, 307 Westlake Ave N., Suite 500, Seattle, WA 98109 USA.

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Laboratorio de Biología Molecular de la Enfermedad de Chagas-INGEBI-CONICET, Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, University of Buenos Aires, Buenos Aires, Argentina.

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Laboratory of Glycobiology, Fundación Instituto Leloir, Avenida Patricias Argentinas, 435 C1405 BWE Buenos Aires, Argentina.

13847. **Cestari, I.S., Haver, N.J., Barbosa-Silva, A. & Ramirez, M.I., 2006.** PROTOGIM: a novel tool to search motifs and domains in hypothetical proteins of protozoan genomes. *Parasitology Research*, **98** (4):375-7.

Departamento de Bioquímica e Biología Molecular, Instituto Oswaldo Cruz (IOC/FIOCRUZ), Av. Brasil, 4365, 21045-900, Rio de Janeiro, Brazil. [marcelr@fiocruz.br].

Whole sequencing of protozoan trypanosomatid genomes revealed the presence of several predicted unknown genes coding for hypothetical proteins. Pairwise, alignment-based, computational methods available online are unable to identify the function of these sequences. To detect clues to identify the function of hypothetical proteins, a user-friendly, bioinformatic tool named PROTOzoan Gene Identification Motifs (PROTOGIM, available on <http://www.biowebdb.org/protogim>) was developed, which allows the user to search functional patterns of hypothetical proteins through the screening of regular expression in the sequences. The analysis of 1,194 trypanosomatid hypothetical proteins through PROTOGIM resulted in an identification of motifs and domains in 98 percent of the cases, demonstrating the reliability and accuracy of the employed method. The added value of this tool is the possibility to modify or insert new regular expressions to perform an analysis against either one or several sequences at the same time. An *in silico* strategy along with biochemical and molecular characterizations creates new possibilities to find the functions of hypothetical proteins at the postgenome era.

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Department of Biology/Cell and Developmental Biology, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland.

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Department of Biology/Zoology, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland.

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Division of Microbial Pathogenesis and Immune Response, Department of Biomedical Sciences, Meharry Medical College, Nashville, TN 37208, USA. [mchaudhuri@mmc.edu]

Trypanosome alternative oxidase (TAO) is the cytochrome-independent terminal oxidase of the mitochondrial electron transport chain. TAO is a diiron protein that transfers electrons from ubiquinol to oxygen, reducing the oxygen to water. The mammalian bloodstream forms of *Trypanosoma brucei* depend solely on TAO for respiration. The inhibition of TAO by salicylhydroxamic acid (SHAM) or ascofuranone is trypanocidal. TAO is present at a reduced level in the procyclic form of *T. brucei*, where it is engaged in respiration and is also needed for developmental processes. Alternative oxidases similar to TAO have been found in a wide variety of organisms but not in mammals, thus rendering TAO an important chemotherapeutic target for African trypanosomiasis

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Division of Infectious Diseases, Department of Medicine, University of British Columbia, Faculty of Medicine, Vancouver Coastal Health Research Institute, Vancouver, British Columbia, Canada. [artc@interchange.ubc.ca].

13853. **Cifuentes-Rojas, C., Halbig, K., Sacharidou, A., Nova-Ocampo, M.d. & Cruz-Reyes, J., 2005.** Minimal pre-mRNA substrates with natural and converted sites for full-round U insertion and U deletion RNA editing in trypanosomes. *Nucleic Acids Research*, **33**(20): 6610-20.

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Zentrum für Molekulare Biologie (ZMBH), Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany.

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Department of Microbiology, University of Illinois, Urbana, 61801, USA.[cronan@life.uiuc.edu]

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*Tsetse and Trypanosomiasis Information*

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In *Trypanosoma brucei*, transcription by RNA polymerase II accounts for the expression of the spliced leader (SL) RNA and most protein coding mRNAs. To understand the regulation of RNA polymerase II transcription in these parasites, we have purified a transcriptionally active enzyme through affinity chromatography of its essential subunit, RPB4. The enzyme preparation is active in both promoter-independent and promoter-dependent *in vitro* transcription assays. Importantly, the enzyme is sensitive to alpha-amanitin inhibition, a hallmark of eukaryotic RNA polymerase II enzymes. Using mass spectrometric analysis we have identified the previously unobserved RPB12 subunit of *T. brucei* RNA polymerase II. TbRPB12 contains a conserved CX(2)CX(10-15)CX(2)C zinc binding motif that is characteristic of other eukaryotic RPB12 polypeptides. We also identified seven proteins that associate with *T. brucei* RNA polymerase II. While both bioinformatics and biochemical analysis have focused on the subunit structure of trypanosome RNA polymerases, this is the first study that reveals a functional RNA polymerase II enzyme.

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*Trypanosoma vivax* is a haemoparasite affecting the livestock industry in South America and Africa. Despite the high economic relevance of the disease caused by *T. vivax*, little work has been done on its molecular characterization, in contrast with human trypanosomes, such as *T. brucei* and *T. cruzi*. The present study reports the construction of a semi-normalized genomic library and the sequencing of 160 Genome Sequence Survey (GSS) ends of *T. vivax*. The analyses of this preliminary data show that this simple and rapid approach worked well to generate some potential new markers for this species.

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Departamento de Microbiologia Geral, Instituto de Microbiologia Prof. Paulo de Goes, Centro de Ciencias da Saude, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

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Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

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LSPCMIB, UMR-CNRS 5068, Groupe de Chimie Organique Biologique, Université Paul Sabatier, Bat. IIR1, 118 Route de Narbonne 31062, Toulouse Cedex 9, France.

13864. **Deng, J.E., Ernst, N.L., Turley, S., Stuart, K.D. & Hol, W.G.J., 2005.** Structural basis for UTP specificity of RNA editing TUTases from *Trypanosoma brucei*. *The EMBO Journal*, **24** (3): 4007-4017.

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Trypanosomatids are pathogenic protozoa that undergo a unique form of post-transcriptional RNA editing that inserts or deletes uridine nucleotides in many mitochondrial pre-mRNAs. Editing is catalyzed by a large multiprotein complex, the editosome. A key editosome enzyme, RNA editing terminal uridylyl transferase 2 (TUTase 2; RET2) catalyzes the uridylylate addition reaction. Here, we report the 1.8 angstrom crystal structure of the *Trypanosoma brucei* RET2 apoenzyme and its complexes with uridine nucleotides. This structure reveals that the specificity of the TUTase for UTP is determined by a crucial water molecule that is exquisitely positioned by the conserved carboxylates D421 and E424 to sense a hydrogen atom on the N3 position of the uridine base. The three-domain structure also unveils a unique domain arrangement not seen before in the nucleotidyltransferase superfamily, with a large domain insertion between the catalytic aspartates. This insertion is present in all trypanosomatid TUTases. We also show that TbRET2 is essential for survival of the bloodstream form of the parasite and therefore is a potential target for drug therapy

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Macrophages are crucial in immunity to infection. They possess potent antimicrobial function, and efficiently process and present peptide antigens for T-cell activation. Despite this, the intracellular protozoan parasites *Toxoplasma gondii*, *Trypanosoma cruzi* and *Leishmania* spp. target macrophages for infection. Each has adopted unique strategies to subvert macrophage antimicrobial functions. The parasites sabotage killing activities through



sophisticated manipulation of intracellular macrophage signaling pathways. These subversive activities are probably dictated by the need to evade microbicidal effector function, as well as to avoid proinflammatory pathology that can destabilize the host-parasite interaction. The molecular details of how intracellular protozoans manipulate macrophage signal transduction pathways for their own ends are beginning to emerge.

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Laboratory of Molecular Parasitology, Institute for Molecular Biology and Medicine (IBMM), Université Libre de Bruxelles, Gosselies, Belgium.

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Department of Microbiology and Immunology, University of Maryland School of Medicine, 20 Penn Street, Baltimore, MD 21201, USA.

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Glycolysis and gluconeogenesis are, in part, driven by the interconversion of 3- and 2-phosphoglycerate (3-PG and 2-PG) which is performed by phosphoglycerate mutases (PGAMs) which can be cofactor dependant (dPGAM) or cofactor independent (iPGAM). The African trypanosome, *Trypanosoma brucei*, possesses the iPGAM form which is thought to play an important role in glycolysis. Here, we report on the use of RNA interference to down-regulate the *T. brucei* iPGAM in procyclic form *T. brucei* and evaluation of the resulting phenotype. We first demonstrated biochemically that depletion of the steady state levels of iPGAM mRNA correlates with a marked reduction of enzyme activity. We further show that iPGAM is required for cell growth in procyclic *T. brucei*.

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*Trypanosoma brucei* has become one of the model systems for unicellular pathogens to study fundamentally important biological phenomena. The method of choice today to examine gene function in these organisms is RNA interference (RNAi). Messenger RNA (mRNA) degradation is triggered by double-stranded RNA (dsRNA) produced *in vivo* from transgenes transcribed from opposing tetracycline (tet)-inducible T7 RNA polymerase promoters, or hairpin RNA transcribed from the tet-inducible procyclic acidic repetitive protein promoter. This chapter describes some of the methods we employ for ablation of gene expression by RNAi in *T. brucei* with particular emphasis on transfection and cloning of procyclic cells, induction of dsRNA expression, isolation of RNA, and analysis of dsRNA and target mRNA.

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African trypanosomes are protozoan parasites, most species of which are transmitted by tsetse flies. They reside in the mammalian bloodstream and evade the immune system by periodically switching the major protein on their surface—a phenomenon called antigenic variation, mediated by gene rearrangements in the trypanosome genome. The trypanosomes eventually enter the central nervous system and cause a fatal disease, commonly called nagana in domestic cattle and sleeping sickness in humans. Two sub-species of *Trypanosoma brucei* infect humans (*T. b. rhodesiense* and *T. b. gambiense*) and one sub-species does not survive in humans (*T. b. brucei*) because it is lysed by the human-specific serum protein, apolipoprotein L-I. Wild animals in Africa have other (less well understood) molecular mechanisms of suppressing the number of African trypanosomes in the blood, and some indigenous breeds of African cattle also display a partial "trypanotolerance" whose genetic loci have recently been mapped.

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Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.

13872. **Dreesen, O. & Cross, G.A., 2006.** Consequences of telomere shortening of an active VSG expression site in telomerase-deficient *Trypanosoma brucei*. *Eukaryotic Cell*. **In press; corrected proof.**

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*Trypanosoma brucei* evades the host immune response by sequential expression of a large family of variant surface glycoproteins (VSG) from one of approximately 20

subtelomeric expression sites (ES). VSG transcription is monoallelic, and little is known about the regulation of antigenic switching. To explore whether telomere length could affect antigenic switching, we created a telomerase-deficient cell line, in which telomeres shortened at a rate of 3-6 bp at each cell division. Upon reaching a critical length, short silent ES telomeres were stabilized by a telomerase-independent mechanism. The active ES telomere progressively shortened and frequently broke. Upon reaching a critical length, the short active ES telomere stabilized, but the transcribed VSG was gradually lost from the population and replaced by a new VSG through duplicative gene conversion. We propose a model in which subtelomeric break induced replication mediated repair at a short ES telomere leads to duplicative gene conversion and expression of a new VSG.

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Protein farnesylation is a form of posttranslational modification that occurs in most, if not all, eukaryotic cells. Inhibitors of protein farnesyltransferase (PFTIs) have been developed as anticancer chemotherapeutic agents. Using the knowledge gained from the development of PFTIs for the treatment of cancer, researchers are currently investigating the use of PFTIs for the treatment of eukaryotic pathogens. This "piggy-back" approach not only accelerates the development of a chemotherapeutic agent for protozoan pathogens, but it is also a means of mitigating the costs associated with *de novo* drug design. PFTIs have already been shown to be efficacious in the treatment of eukaryotic pathogens in animal models, including both *Trypanosoma brucei*, the causative agent of African sleeping sickness, and *Plasmodium falciparum*, one of the causative agents of malaria. In this paper, current evidence and progress that support the targeting of protein farnesyltransferase [farnesyl-diphosphate farnesyltransferase] for the treatment of protozoal infections are summarized.

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Johns Hopkins Medical School, Department of Biological Chemistry, 725 N. Wolfe St., Baltimore, MD 21205 USA.

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Department of Biological Sciences, University of Hull, Hull, HU6 7RX, UK.  
[k.ersfeld@hull.ac.uk].

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The host range of African trypanosomes is influenced by innate protective molecules in the blood of primates. A subfraction of human high-density lipoprotein (HDL) containing apolipoprotein A-I, apolipoprotein L-I, and haptoglobin-related protein is toxic to *Trypanosoma brucei brucei* but not the human sleeping sickness parasite *Trypanosoma brucei rhodesiense*. It is thought that *T. b. rhodesiense* evolved from a *T. b. brucei*-like ancestor and expresses a defense protein that ablates the antitrypanosomal activity of human HDL. To directly investigate this possibility, we developed an *in vitro* selection to generate human HDL-resistant *T. b. brucei*. Here we show that conversion of *T. b. brucei* from human HDL sensitive to resistant correlates with changes in the expression of the variant surface glycoprotein (VSG) and abolished uptake of the cytotoxic human HDLs. Complete transcriptome analysis of the HDL-susceptible and -resistant trypanosomes confirmed that VSG switching had occurred but failed to reveal the expression of other genes specifically associated with human HDL resistance, including the serum resistance-associated gene (SRA) of *T. b. rhodesiense*. In addition, we found that while the original active expression site was still utilized, expression of three expression site-associated genes (ESAG) was altered in the HDL-resistant trypanosomes. These findings demonstrate that resistance to human HDLs can be acquired by *T. b. brucei*.

13878. **Figarella, K., Uzcategui, N.L., Beck, A., Schoenfeld, C., Kubata, B.K., Lang, F. & Duzsenko, M., 2006.** Prostaglandin-induced programmed cell death in *Trypanosoma brucei* involves oxidative stress. *Cell Death and Differentiation*, **13** (10): 1802-1814.

Interfaculty Institute of Biochemistry, University of Tuebingen, Germany.

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We have undertaken 2-DE and MS to identify proteins associated with arsenical drug resistance in *Trypanosoma brucei*. This parasite causes sleeping sickness in humans, and arsenical drug resistance is a significant potential problem. Comparative analysis of approximately 2,000 spots resolved by 2-DE in the soluble proteomes of drug-sensitive and drug-resistant isogenic lines of *T. brucei* identified a protein spot whose absence associated with resistance to the arsenical drug, Cymelarsan. MS matched this protein to an identical pair of tandem genes Tb09.211.0120 and 0130 that encode a putative nascent polypeptide associated complex subunit. This protein also occurs as an isoform located in both resistant and sensitive lines at a similar molecular weight, but different pI. The difference between isogenic lines was confirmed by Western blot using an antibody against recombinant protein. Both genes were identical in sequence between drug-sensitive and drug-resistant lines and both were transcribed as determined by RT-PCR. We postulate that the missing protein isoform arose due to the lack of a PTM.

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Genomes of some parasites contain dozens of alternative and highly diverged surface antigens, of which only a single one is expressed in any cell. Individual cells occasionally change expression of their surface antigen, allowing them to escape immune surveillance. These switches appear to occur in a partly random way, creating a diverse set of antigenic variants. In spite of this diversity, the parasitaemia develops as a series of outbreaks, in which each outbreak is dominated by relatively few antigenic types. Host-specific immunity eventually clears the dominant antigenic types, and a new outbreak follows from antigenic types that have apparently been present all along at low frequency. This pattern of sequential dominance by different antigenic types remains unexplained. We review the five most prominent theories, which have developed mainly from studies of the protozoans *Trypanosoma* and *Plasmodium*, and the bacterial spirochete *Borrelia*. The most promising theories depend on some combination of mechanisms to create favoured connectivity pathways through the matrix of transitions between variants. Favoured pathways may arise from biased switches at the molecular level of gene expression or from biases imposed by immune selection. We illustrate the concept of connectivity pathways by reanalysis of data on transitions between variants from *Borrelia hermsii*.

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Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK.

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Complete or partial genome sequences have recently become available for several medically and evolutionarily important parasitic protozoa, viz. *Trypanosoma cruzi*, *T. brucei*, *Leishmania* spp., *Plasmodium falciparum*, *Toxoplasma gondii*, *Giardia* sp. and *Entamoeba histolytica*. Through the application of bioinformatics complete metabolic repertoires for these parasites can be predicted. For experimentally intractable parasites insight provided by metabolic maps generated *in silico* has been startling. At its more extreme end, such bioinformatics reckoning facilitated the discovery in some parasites of mitochondria remodelled beyond previous recognition, and the identification of a non-photosynthetic chloroplast relic in malarial parasites. However, for experimentally tractable parasites, mapping of the general metabolic terrain is only a first step in understanding how the parasite modulates its streamlined, yet still often puzzlingly complex, metabolism in order to complete life cycles within host, vector, or environment. This review provides a comparative overview and discussion of metabolic strategies used by several different parasitic protozoa in order to subvert and survive host defences, and illustrates how genomic data contribute to the elucidation of parasite metabolism.

13884. **Girard, M., Giraud, S., Courtioux, B., Jauberteau-Marchan, M.O. & Bouteille, B., 2005.** Endothelial cell activation in the presence of African trypanosomes. *Molecular and Biochemical Parasitology*, **139** (1): 41-49.

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During human African trypanosomiasis, trypanosomes (*Trypanosoma brucei gambiense* or *T. b. rhodesiense*) invade the central nervous system (CNS). Mechanisms of blood-brain barrier and blood-cerebrospinal fluid barrier leakage remain unknown. To better understand the relationships between trypanosomes and endothelial cells, the principal cell population of those barriers, we cultured a human bone marrow endothelial cell (HBMEC) line in the presence or absence of *T. b. gambiense*, to study cell activation. As indicated by NF-kappaB translocation to the nucleus, cells were activated in the presence of trypanosomes. The expression of the adhesion molecules ICAM-1, E-selectin and VCAM-1 increased in co-culture. The parasites induced the synthesis of the pro-inflammatory cytokines TNF-alpha, IL-6 and IL-8, and of nitric oxide (NO) by HBMEC. Cells were also cultured in the presence of parasite variant surface glycoproteins (VSGs), and an increase in TNF-alpha, IL-6, IL-8, and NO synthesis was also observed. Soluble VSGs induced NF-

kappaB translocation, and the expression of adhesion molecules, indicating that they could possibly be the molecular soluble factor responsible for endothelial cell activation. The permeability coefficient of HBMEC layer increased when cells were cultured in the presence of trypanosomes, parasite culture supernatant, or VSGs. Thus, *T. b. gambiense* can activate endothelial cells *in vitro*, through the release of soluble activating factors. Consequences of endothelial cell activation by parasite products may include a potentiation of the inflammatory reaction, leukocyte recruitment, passage of trypanosomes into the CNS, and barrier dysfunction observed during CNS involvement of HAT.

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Department of Microbiology and Immunology and Witebsky Center for Microbial Pathogenesis and Immunology, SUNY Buffalo School of Medicine, Buffalo, NY 14214, USA.

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African trypanosomes (*Trypanosoma brucei*) have a digenetic lifecycle that alternates between the mammalian bloodstream and the tsetse fly vector. In the bloodstream, replicating long slender parasites transform into non-dividing short stumpy forms. Upon transmission into the fly midgut, short stumpy cells differentiate into actively dividing procyclics. A hallmark of this process is the replacement of the bloodstream-stage surface coat composed of variant surface glycoprotein (VSG) with a new coat composed of procyclin. Pre-existing VSG is shed by a zinc metalloprotease activity (MSP-B) and glycosylphosphatidylinositol-specific phospholipase C (GPI-PLC). We now provide a detailed analysis of the coordinate and inverse regulation of these activities during synchronous differentiation. MSP-B mRNA and protein levels are upregulated during differentiation at the same time as proteolysis whereas GPI-PLC levels decrease. When transcription or translation is inhibited, VSG release is incomplete and a substantial amount of protein stays cell-associated. Both modes of release are still evident under these conditions, but GPI hydrolysis plays a quantitatively minor role during normal differentiation. Nevertheless, GPI biosynthesis shifts early in differentiation from a GPI-PLC sensitive structure to a resistant procyclic-type anchor. Translation inhibition also results in a marked increase in the mRNA levels of both MSP-B and GPI-PLC, consistent with negative regulation by labile protein factors. The relegation of short stumpy surface GPI-PLC to a secondary role in differentiation suggests that it may play a more important role as a virulence factor within the mammalian host.

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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  $K_m$  value of 0.048  $\mu$ M. We also confirmed the presence of the previously reported U1 uracil transporter and found it capable of mediating uridine uptake as well, with a  $K_m$  of 33  $\mu$ M. A higher-affinity U2 uridine transporter ( $K_m = 4.1 \mu$ M) 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*.

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Research Unit for Tropical Diseases, Christian de Duve Institute of Cellular Pathology and Laboratory of Biochemistry, Université catholique de Louvain, ICP-TROP 74.39, Avenue Hippocrate 74, B-1200 Brussels, Belgium.

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Department of Medical Microbiology and Immunology, University of Wisconsin Medical School of Medicine and Public Health, 1300 University Avenue, Madison, Wisconsin 53706-1532, USA.

Activation of a type I cytokine response is important for early resistance to infection with *Trypanosoma brucei rhodesiense*, the extracellular protozoan parasite that causes African sleeping sickness. The work presented here demonstrates that trypanosome DNA activates macrophages to produce factors that may contribute to this response. Initial results demonstrated that *T. brucei rhodesiense* DNA was present in the plasma of C57BL/6 and C57BL/6-scid mice following infection. Subsequently, the effect of trypanosome DNA on macrophages was investigated; parasite DNA was found to be less stimulatory than *Escherichia coli* DNA but more stimulatory than murine DNA, as predicted by the CG dinucleotide content. Trypanosome DNA stimulated the induction of a signal transduction cascade associated with Toll-like receptor signalling in RAW 264.7 macrophage cells. The signalling cascade led to expression of mRNAs, including interleukin-12 (IL-12) p40, IL-6, IL-10, cyclooxygenase-2, and beta interferon. The treatment of RAW 264.7 cells and bone

marrow-derived macrophages with trypanosome DNA induced the production of NO, prostaglandin E2, and the cytokines IL-6, IL-10, IL-12, and tumour necrosis factor alpha. In all cases, DNase I treatment of *T. brucei rhodesiense* DNA abolished the activation. These results suggest that *T. brucei rhodesiense* DNA serves as a ligand for innate immune cells and may play an important contributory role in early stimulation of the host immune response during trypanosomiasis.

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African trypanosomes are parasitic protozoa that cause sleeping sickness and nagana. Trypanosomes are not only of scientific interest because of their clinical importance, but also because these protozoa contain several very unusual biological features, such as their specially adapted mitochondrion and the compartmentalization of glycolytic enzymes in glycosomes. The energy metabolism of *Trypanosoma brucei* differs significantly from that of their hosts and changes drastically during the life cycle. Despite the presence of all citric acid cycle enzymes in procyclic insect-stage *T. brucei*, citric acid cycle activity is not used for energy generation. Recent investigations on the influence of substrate availability on the type of energy metabolism showed that absence of glycolytic substrates did not induce a shift from a fermentative metabolism to complete oxidation of substrates. Apparently, insect-stage *T. brucei* use parts of the citric acid cycle for other purposes than for complete degradation of mitochondrial substrates. Parts of the cycle are suggested to be used for (i) transport of acetyl-CoA units from the mitochondrion to the cytosol for the biosynthesis of fatty acids, (ii) degradation of proline and glutamate to succinate, (iii) generation of malate, which can then be used for gluconeogenesis. Therefore the citric acid cycle in trypanosomes does not function as a cycle.

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The regulation of differentiation is particularly important in microbial eukaryotes that inhabit multiple environments. The parasite *Trypanosoma brucei* is an extreme example of this, requiring exquisite gene regulation during transmission from mammals to the tsetse fly vector. Unusually, trypanosomes rely almost exclusively on post-transcriptional mechanisms for regulated gene expression. Hence, RNA binding proteins are potentially of great significance in controlling stage-regulated processes. We have previously identified TbZFP1 as a trypanosome molecule transiently enriched during differentiation to tsetse midgut

procyclic forms. This small protein (101 amino acids) contains the unusual CCCH zinc finger, an RNA binding motif. Here, we show that genetic ablation of TbZFP1 compromises repositioning of the mitochondrial genome, a specific event in the strictly regulated differentiation programme. Despite this, other events that occur both before and after this remain intact. Significantly, this phenotype correlates with the TbZFP1 expression profile during differentiation. This is the first genetic disruption of a developmental regulator in *T. brucei*. It demonstrates that programmed events in parasite development can be uncoupled at the molecular level. It also further supports the importance of CCCH proteins in key aspects of trypanosome cell function.

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African trypanosomes are extracellular blood parasites that cause sleeping sickness in humans and nagana in cattle. The therapeutics used to control and treat these diseases are very ineffective and thus, the development of new drugs is urgently needed. We have previously suggested to use trypanosome-specific RNA aptamers as tools for the development of novel trypanocidal compounds. Here, we report the selection of a 2'-NH<sub>2</sub>-modified RNA aptamer that binds to live trypanosomes with an affinity of 70 +/- 15 nM. The aptamer adopts a stable G-quartet structure and has a half-life in human serum of > 30 h. RNA binding is restricted to the flagellar attachment zone, located between the cell body and the flagellum of the parasite. We demonstrate that antigen-tagged preparations of the aptamer can bind to live trypanosomes and that they can be used to re-direct immunoglobulins to the parasite surface.

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Seattle Biomedical Research Institute, 307 Westlake Avenue North, Suite 500, Seattle, WA 98108-5219, USA; Department of Pathobiology, University of Washington, Seattle, WA 98195, USA; Department of Genetics and Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, USA.

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We have undertaken a large scale study of the proteins expressed in the procyclic form of the parasite *Trypanosoma brucei*, which causes African sleeping sickness, using 2-DE and MS. The complete data set encompasses over 2,000 identifications, of which 770 are distinct proteins. We have discovered that multiple protein isoforms appear to be common in *T. brucei*, as most proteins have been matched to more than one gel spot. We have developed visualisation software to investigate the differences between isoforms, based on the information from the results of database searches with MS data. We are able to highlight instances where PTMs are the most likely cause of variant forms. In other cases, spots that appear reproducibly across replicates contain fragments of proteins, arising either as experimental artefacts or as part of protein degradation. We are also able to classify clusters of gel spots into different groups based on the pattern of peptides that have been matched from MS data. The entire data set is stored within a relational database system that allows complex queries (<http://www.gla.ac.uk/functionalgenomics>). Using specific proteins as examples, we demonstrate how the visualisation software and the database query facilities can be used.

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This study aimed to provide the foundation for an integrative approach to the identification of the mechanisms underlying the response to infection with *Trypanosoma congolense*, and to identify pathways that have previously been overlooked. We undertook a large-scale gene expression analysis study comparing susceptible A/J and more tolerant C57BL/6 mice. In an initial time course experiment, we monitored the development of parasitaemia and anaemia in every individual. Based on the kinetics of disease progression, we extracted total RNA from liver at days 0, 4, 7, 10 and 17 post infection and performed a microarray analysis. We identified 64 genes that were differentially expressed in the two strains in non-infected animals, of which nine genes remained largely unaffected by the disease. Gene expression profiling at stages of low, peak, clearance and recurrence of parasitaemia suggest that susceptibility is associated with high expression of genes coding for chemokines (e.g. Ccl24, Ccl27 and Cxcl13), complement components (C1q and C3) and interferon receptor alpha (Ifnar1). Additionally, susceptible A/J mice expressed higher levels of some potassium channel genes. In contrast, messenger RNA levels of a few immune response, metabolism and protease genes (e.g. Prss7 and Mmp13) were higher in the tolerant C57BL/6 strain as compared to A/J.

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INSERM U565, CNRS UMR5153, and MNHN USM 0503, Museum National d'Histoire Naturelle, 75231 Paris, France.

Eukaryotic cilia and flagella are cytoskeletal organelles that are remarkably conserved from protists to mammals. Their basic unit is the axoneme, a well-defined cylindrical structure composed of microtubules and up to 250 associated proteins. These complex organelles are assembled by a dynamic process called intraflagellar transport. Flagella and cilia perform diverse motility and sensitivity functions in many different organisms. Trypanosomes are flagellated protozoa, responsible for various tropical diseases such as sleeping sickness and Chagas disease. In this review, we first describe general knowledge on the flagellum: its occurrence in the living world, its molecular composition, and its mode of assembly, with special emphasis on the exciting developments that followed the discovery of intraflagellar transport. We then present recent progress regarding the characteristics of the trypanosome flagellum, highlighting the original contributions brought by this organism. The

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Trypanosomes have an unusual mitochondrial genome, called kinetoplast DNA, that is a giant network containing thousands of interlocked minicircles. During kinetoplast DNA synthesis, minicircles are released from the network for replication as theta-structures, and then the free minicircle progeny reattach to the network. We report that a mitochondrial protein, which we term p38, functions in kinetoplast DNA replication. RNA interference (RNAi) of p38 resulted in loss of kinetoplast DNA and accumulation of a novel free minicircle species named fraction S. Fraction S minicircles are so underwound that on isolation they become highly negatively super twisted and develop a region of Z-DNA. p38 binds to minicircle sequences within the replication origin. We conclude that cells with RNAi-induced loss of p38 cannot initiate minicircle replication, although they can extensively unwind free minicircles.

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Trypanosomes are protozoan parasites that cause major diseases in humans and other animals. *Trypanosoma brucei* and *Trypanosoma cruzi* are the etiologic agents of African and American Trypanosomiasis, respectively. In spite of large amounts of information regarding various aspects of their biology, including the essentially complete sequences of their genomes, studies directed towards an understanding of mechanisms related to DNA metabolism have been very limited. Recent reports, however, describing genes involved with DNA recombination and repair in *T. brucei* and *T. cruzi*, indicated the importance of these processes in the generation of genetic variability, which is crucial to the success of these parasites. Here, we review these data and discuss how the DNA repair and recombination machineries may contribute to strikingly different strategies evolved by the two trypanosomes to create genetic variability that is needed for survival in their hosts. In *T. brucei*, two genetic components are critical to the success of antigenic variation, a strategy that allows the parasite to evade the host immune system by periodically changing the expression of a group of variant surface glycoproteins (VSGs). One component is a mechanism that provides for the exclusive expression of a single VSG at any one time, and the second is a large repository of antigenically distinct VSGs. Work from various groups showing the importance of recombination reactions in *T. brucei*, primarily to move a silent VSG into an active VSG expression site, is discussed. *T. cruzi* does not use the strategy of antigenic variation for host immune evasion but counts on the extreme heterogeneity of their population for parasite adaptation to different hosts. We discuss recent evidence indicating the existence of major differences in the levels of genomic heterogeneity among *T. cruzi* strains, and suggest that metabolic changes in the mismatch repair pathway could be an important source of antigenic diversity found within the *T. cruzi* population.

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*Trypanosoma brucei* is the causative agent of African sleeping sickness in humans and contributes to the debilitating disease "nagana" in cattle. To date we know little about the genes that determine drug resistance, host specificity, pathogenesis and virulence in these parasites. The availability of the complete genome sequence and the ability of the parasite to undergo genetic exchange have allowed genetic investigations into this parasite and here we report the first genetic map of *T. brucei* for the genome reference stock TREU 927, comprising of 182 markers and 11 major linkage groups, that correspond to the 11 previously identified chromosomes. The genetic map provides 90 percent probability of a marker being

11 cM from any given locus. Its comparison to the available physical map has revealed the average physical size of a recombination unit to be 15.6 Kb/cM. The genetic map coupled with the genome sequence and the ability to undertake crosses presents a new approach to identifying genes relevant to the disease and its prevention in this important pathogen through forward genetic analysis and positional cloning.

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*Trypanosoma brucei* provides an excellent system for studies of many aspects of cell biology, including cell structure and morphology, organelle positioning, cell division and protein trafficking. However, the trypanosome has a complex life cycle in which it must adapt either to the mammalian bloodstream or to different compartments within the tsetse fly. These differentiation events require stage-specific changes to basic cell biological processes and reflect responses to environmental stimuli and programmed differentiation events that must occur within a single cell. The organization of cell structure is fundamental to the trypanosome throughout its life cycle. Modulations of the overall cell morphology and positioning of the specialized mitochondrial genome, flagellum and associated basal body provide the classical descriptions of the different life cycle stages of the parasite. The dependency relationships that govern these morphological changes are now beginning to be understood and their molecular basis identified. The overall picture emerging is of a highly organized cell in which the rules established for cell division and morphogenesis in organisms such as yeast and mammalian cells do not necessarily apply. Therefore, understanding the developmental cell biology of the African trypanosome is providing insight into both fundamentally conserved and fundamentally different aspects of the organization of the eukaryotic cell.

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Department of Genetics and Biochemistry, Clemson University Clemson, South Carolina 29634, USA and Department of Parasitology, Kunming Medical College Kunming Yunnan, P.R. China 650031.

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Department of Internal Medicine, University Hospital Giessen and Marburg, Aulweg 123, D-35392 Giessen, Germany. [rory.morty@innere.med.uni-giessen.de].

Peptidases of parasitic protozoans are emerging as novel virulence factors and therapeutic targets in parasitic infections. A trypanosome-derived aminopeptidase that exclusively hydrolysed substrates with Glp (pyroglutamic acid) in P1 was purified 9,248-fold from the plasma of rats infected with *Trypanosoma brucei brucei*. The enzyme responsible was cloned from a *T. brucei brucei* genomic DNA library and identified as type I PGP (pyroglutamyl peptidase), belonging to the C15 family of cysteine peptidases. We showed that PGP is expressed in all life cycle stages of *T. brucei brucei* and is expressed in four other blood-stream-form African trypanosomes. Trypanosome PGP was optimally active and stable at bloodstream pH, and was insensitive to host plasma cysteine peptidase inhibitors. Native purified and recombinant hyper-expressed trypanosome PGP removed the N-terminal Glp blocking groups from TRH (thyrotrophin-releasing hormone) and GnRH (gonadotropin-releasing hormone) with a  $k_{cat}/K_m$  value of 0.5 and 0.1 s<sup>-1</sup>. uM<sup>-1</sup> respectively. The half-life of TRH and GnRH was dramatically reduced in the plasma of trypanosome-infected rats, both *in vitro* and *in vivo*. Employing an activity-neutralizing anti-trypanosome PGP antibody, and pyroglutamyl diazomethyl ketone, a specific inhibitor of type I PGP, we demonstrated that trypanosome PGP is entirely responsible for the reduced plasma half-life of TRH, and partially responsible for the reduced plasma half-life of GnRH in a rodent model of African trypanosomiasis. The abnormal degradation of TRH and GnRH, and perhaps other neuropeptides N-terminally blocked with a pyroglutamyl moiety by trypanosome PGP may contribute to some of the endocrine lesions observed in African trypanosomiasis.

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Department of Internal Medicine, University of Giessen School of Medicine, Aulweg 123 (Room 6-11), D-35392 Giessen, Germany. [rory.morty@innere.med.uni-giessen.de].

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Oligopeptidases are emerging as important pathogenic factors and therapeutic targets in trypanosome infections. We describe here the purification, cloning, and biochemical analysis of a new oligopeptidase from two pathogenic African trypanosomes. This oligopeptidase, which we have called tropolysin (encoded by the *trn* gene), represents an evolutionarily distant member of the M3A subfamily of metallopeptidases, ancestral to thimet oligopeptidase, neurolysin, and saccharolysin. The *trn* gene was present as a single copy per haploid genome, was expressed in both the mammalian and insect stages of the parasite life cycle, and encoded an 84 kDa protein. Both purified and hyperexpressed tropolysin hydrolyzed bradykinin-derived fluorogenic peptide substrates at restricted sites, with an alkaline pH optimum, and were activated by dithiothreitol and reduced glutathione and by divalent metal cations, in the order  $Zn^{2+} > Co^{2+} > Mn^{2+}$ . Under oxidizing conditions, tropolysin reversibly formed inactive multimers. Tropolysin exhibited a preference for acidic amino acid side chains in P(4), hydrophobic side chains in P(3), and hydrophobic or large uncharged side chains in P(1), P(1)', and P(3)', while the S(2)' site was unselective. Highly charged residues were not tolerated in P(1)'. Tropolysin was responsible for the bulk of the kinin-degrading activity in trypanosome lysates, potently (k(cat) approximately 119 s<sup>-1</sup>) inactivated the vasoactive kinins bradykinin and kallidin, and generated angiotensin(1-7) from angiotensin I. This hydrolysis both abolished the capacity of bradykinin to stimulate the bradykinin B(2) receptor and abrogated bradykinin prohypotensive properties *in vivo*, raising the possibility that tropolysin may play a role in the dysregulated kinin metabolism observed in the plasma of trypanosome-infected hosts.

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Wellcome Centre for Molecular Parasitology, The Anderson College, 56 Dumbarton Road, University of Glasgow, Glasgow G11 6NU, UK.

Protein kinases represent promising drug targets for a number of human and animal diseases. The recent completion of the sequenced genomes of three human-infective trypanosomatid protozoa, *Leishmania major*, *Trypanosoma brucei* and *Trypanosoma cruzi*, has allowed the kinome for each parasite to be defined as 179, 156 and 171 eukaryotic

protein kinases respectively, that is about one third of the human complement. The analysis revealed that the trypanosomatids lack members of the receptor-linked or cytosolic tyrosine kinase families, but have an abundance of STE and CMGC family protein kinases likely to be involved in regulating cell cycle control, differentiation and response to stress during their complex life cycles. In this review, we examine the prospects for exploiting differences between parasite and mammalian protein kinases to develop novel anti-parasitic chemotherapeutic agents.

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Department of Genetics and Developmental Biology and Department of  
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Department of Microbiology and Molecular Genetics, University of Medicine  
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Wellcome Trust Laboratories for Molecular Parasitology, Imperial College London, London SW7 2AZ, UK.

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Institute of Immunology and Infection Research, University of Edinburgh, Edinburgh EH13 0BL., UK.

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Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, 12, rue des Profs. Jeener et Brachet, B6041 Gosselies, Belgium.

The trypanosome genome is organized into long polycistronic units that seem to be permanently transcribed in proliferative stages of the parasite. Cellular differentiation is controlled primarily at the level of individual mRNA maturation and stability. The transcription units of the two major stage-specific antigens, the variant surface glycoprotein (VSG) of the bloodstream form and procyclin of the procyclic form, are subject to an



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

We are studying early events in the establishment of *Trypanosoma brucei* in the tsetse midgut using fluorescent trypanosomes to increase visibility. Feeding flies with the lectin-inhibitory sugars D-glucosamine (GlcN) or N-acetyl-glucosamine (GlcNAc) has previously been shown to enhance fly susceptibility to infection with trypanosomes and, as expected, we found that both sugars increased midgut infection rates of *Glossina morsitans morsitans* with *T. brucei*. However, GlcNAc did not show the inhibitory effect on salivary gland infection rate reported previously for GlcN. Both sugars significantly slowed the movement of the bloodmeal along the midgut. GlcN also significantly increased the size of the bloodmeal taken and fly mortality. The most surprising finding was that GlcNAc stimulated trypanosome growth not only in the midgut, but also *in vitro* in the absence of any factor derived from the fly. Thus our direct comparison of the effects of GlcN and GlcNAc on the trypanosome-tsetse interaction has shown that these sugars impact on trypanosome growth and tsetse physiology in different ways and are not interchangeable as suggested in the literature. The sugars cause multiple effects, not restricted solely to the inhibition of midgut lectins. These findings have implications for current models of tsetse susceptibility to trypanosome infection.

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Department of Microbiology and Immunology, Witebsky Center for Microbial Pathogenesis and Immunology, SUNY Buffalo School of Medicine, Buffalo, NY 14214, USA.

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Institut für Zellbiologie, Universität Bern, Murtenstrasse 31, CH-3010 Bern, Switzerland.

African trypanosomes undergo differentiation in order to adapt to the mammalian host and the tsetse fly vector. To characterize the role of a mitogen-activated protein (MAP) kinase homologue, TbMAPK5, in the differentiation of *Trypanosoma brucei*, we constructed a knockout in procyclic (insect) forms from a differentiation-competent (pleomorphic) stock.

Two independent knockout clones proliferated normally in culture and were not essential for other life cycle stages in the fly. They were also able to infect immunosuppressed mice, but the peak parasitemia was 16-fold lower than that of the wild type. Differentiation of the proliferating long slender to the nonproliferating short stumpy bloodstream form is triggered by an autocrine factor, stumpy induction factor (SIF). The knockout differentiated prematurely in mice and in culture, suggestive of increased sensitivity to SIF. In contrast, a null mutant of a cell line refractory to SIF was able to proliferate normally. The differentiation phenotype was partially rescued by complementation with wild-type TbMAPK5 but exacerbated by introduction of a nonactivatable mutant form. Our results indicate a regulatory function for TbMAPK5 in the differentiation of bloodstream forms of *T. brucei* that might be exploitable as a target for chemotherapy against human sleeping sickness.

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L'EA 37-32, Praticien, Virulence et résistance des Plasmodium, Faculté de Médecine, Université Claude Bernard, Lyon, France. [picot@sante.univ-lyon1.fr]

Apoptosis and programmed cell death are amongst the most fascinating new concepts for understanding the host-parasite relationship. A growing body data is raising questions about the impact of the death of an individual parasite on the survival of the parasite population as a whole. Does the parasite induce the death of the host cell as an expression of virulence or does it inhibit that death as a factor for transmissibility? Current evidence that these effects are mediated through specific highly regulated mechanisms suggests that deciphering programmed cell death could provide new tools for control of parasitic diseases.

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R McCulloch, University of Glasgow, Anderson College, Wellcome Centre for Molecular Parasitology, 56 Dumbarton Rd, Glasgow G11 6NU, Lanark, UK.

In *Trypanosoma brucei*, DNA recombination is crucial in antigenic variation, a strategy for evading the mammalian host immune system found in a wide variety of pathogens. *T. brucei* has the capacity to encode > 1,000 antigenically distinct variant surface glycoproteins (VSGs). By ensuring that only one VSG is expressed on the cell surface at one time, and by periodically switching the VSG gene that is expressed, *T. brucei* can evade immune killing for prolonged periods. Much of VSG switching appears to rely on a widely conserved DNA repair pathway called homologous recombination, driven by RAD51. Here, we demonstrate that *T. brucei* encodes a further five RAD51-related proteins, more than has been identified in other single-celled eukaryotes to date. We have investigated the roles of two of the RAD51-related proteins in *T. brucei*, and show that they contribute to DNA repair, homologous recombination and RAD51 function in the cell. Surprisingly, however, only one of the two proteins contributes to VSG switching, suggesting that the family of diverged RAD51 proteins present in *T. brucei* have assumed specialized functions in homologous recombination, analogous to related proteins in metazoan eukaryotes.

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Department of Pathology, New York University School of Medicine, 550 First Ave., New York, NY 10016, USA.

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The *Trypanosoma brucei* flagellum is a multifunctional organelle with critical roles in motility, cellular morphogenesis, and cell division. Although motility is thought to be important throughout the trypanosome lifecycle, most studies of flagellum structure and function have been restricted to the procyclic lifecycle stage, and our knowledge of the bloodstream form flagellum is limited. We have previously shown that trypanin functions as part of a flagellar dynein regulatory system that transmits regulatory signals from the central

pair apparatus and radial spokes to axonemal dyneins. Here we investigate the requirement for this dynein regulatory system in bloodstream form trypanosomes. We demonstrate that trypanin is localized to the flagellum of bloodstream form trypanosomes, in a pattern identical to that seen in procyclic cells. Surprisingly, trypanin RNA interference is lethal in the bloodstream form. These knockdown mutants fail to initiate cytokinesis, but undergo multiple rounds of organelle replication, accumulating multiple flagella, nuclei, kinetoplasts, mitochondria, and flagellum attachment zone structures. These findings suggest that normal flagellar beat is essential in bloodstream form trypanosomes and underscore the emerging concept that there is a dichotomy between trypanosome lifecycle stages with respect to factors that contribute to cell division and cell morphogenesis. This is the first time that a defined dynein regulatory complex has been shown to be essential in any organism and implicates the dynein regulatory complex and other enzymatic regulators of flagellar motility as candidate drug targets for the treatment of African sleeping sickness.

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The flagellum of *Trypanosoma brucei* is a multifunctional organelle with critical roles in motility and other aspects of the trypanosome life cycle. Trypanin is a flagellar protein required for directional cell motility, but its molecular function is unknown. Recently, a trypanin homologue in *Chlamydomonas reinhardtii* was reported to be part of a dynein regulatory complex (DRC) that transmits regulatory signals from central pair microtubules and radial spokes to axonemal dynein. DRC genes were identified as extragenic suppressors of central pair and/or radial spoke mutations. We used RNA interference to ablate expression of radial spoke (RSP3) and central pair (PF16) components individually or in combination with trypanin. Both *rsp3* and *pf16* single knockdown mutants are immotile, with severely defective flagellar beat. In the case of *rsp3*, this loss of motility is correlated with the loss of radial spokes, while in the case of *pf16* the loss of motility correlates with an aberrant orientation of the central pair microtubules within the axoneme. Genetic interaction between trypanin and PF16 is demonstrated by the finding that loss of trypanin suppresses the *pf16* beat defect, indicating that the DRC represents an evolutionarily conserved strategy for dynein regulation. Surprisingly, we discovered that four independent mutants with an impaired flagellar beat all fail in the final stage of cytokinesis, indicating that flagellar motility is necessary for normal cell division in *T. brucei*. These findings present the first evidence that flagellar beating is important for cell division and open the opportunity to exploit enzymatic activities that drive flagellar beat as drug targets for the treatment of African sleeping sickness.

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Laboratoire Hospitalier universitaire de Parasitologie et Mycologie Médicale, Équipe EA 3593, UFR de Médecine de l'Université des Antilles et de la Guyane, Cayenne, French Guiana.[ufrmedag2@wanadoo.fr].

A non-toxic and versatile protein salting-out DNA extraction method is here described for convenient and rapid extraction of nuclear DNA molecules from trypanosomatids. The procedure just involves four manipulations, does not require any organic solvent, and is performed in less than 1 h in a single tube. DNA yields obtained were similar to those from commercial kits and phenol-chloroform procedures. Samples extracted by this method were suitable for PCR and subsequent analyses. The reduced manual labour involved was perceived as an important benefit in medical diagnosis routine use as well as for large-scale taxonomic and eco-epidemiological studies of trypanosomatids.

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Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, USA.

Trypanosome U insertion and U deletion RNA editing of mitochondrial pre-mRNAs is catalyzed by multisubunit editing complexes as directed by partially complementary guide RNAs. The basic enzymatic activities and protein composition of these high-molecular mass complexes have been under intense study, but their specific protein interactions with functional pre-mRNA/gRNA substrates remains unknown. We show that editing complexes purified through extensive ion-exchange chromatography and immunoprecipitation make specific cross-linking interactions with A6 pre-mRNA containing a single 32P and photoreactive 4-thioU at the scissile bond of a functional site for full-round U deletion. At least four direct protein-RNA contacts are detected at this site by cross-linking. All four interactions are stimulated by unpaired residues just 5' of the pre-mRNA/gRNA anchor duplex, but strongly inhibited by pairing of the editing site region. Furthermore, competition analysis with homologous and heterologous transcripts suggests preferential contacts of the editing complex with the mRNA/gRNA duplex substrate. This apparent structural selectivity suggests that the RNA-protein interactions we observe may be involved in recognition of editing sites and/or catalysis in assembled complexes.

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Department of Internal Medicine, Yale University Medical School, New Haven, Connecticut 06536, USA.

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Canadian Institute for Advanced Research and Department of Biology, Dalhousie University, Halifax, Canada, B3H 4J1.

Five years ago, little was known about kinetoplastid evolution. Recent improvements in the taxon sampling for nuclear rRNA genes and several protein markers have transformed this understanding. Parasitism evolved at least four times in kinetoplastids. Obligate parasitic trypanosomatids are a relatively “derived” group within kinetoplastids; their closest relative is likely to be the free-living *Bodo saltans*, and the ancestral trypanosomatids were probably parasites of insects. Although subject to recent controversy, trypanosomes (genus *Trypanosoma*) probably constitute a monophyletic group. Several unusual features of trypanosomatid genomes (e.g. trans-splicing, mitochondrial RNA editing and intron poverty) are common in kinetoplastids and pre-date the adoption of parasitism. The framework of relationships is becoming robust enough for real comparative approaches to be used to understand kinetoplastid biology.

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Department of Biology, Slippery Rock University, Slippery Rock, PA 16057, USA. [carolyn.steglich@sru.edu].

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Abteilung Parasitologie, Hygiene-Institut der Ruprecht-Karls-Universität, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany. [dsteverding@hotmail.com].

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School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK; Department of Parasitology, Ruprecht-Karls-Universität, Heidelberg, Germany.

The transferrin (Tf) receptor of *Trypanosoma brucei* (TbTfR) is encoded by two expression-site-associated genes, ESAG6 and ESAG7. There are around 20 different expression sites containing different copies of these genes that encode TbTfRs with quite distinct affinities for Tf of various hosts. It was proposed that *T. brucei* has developed multiple expression sites encoding different TbTfRs to ensure sufficient iron uptake in the presence of antibodies competing for binding to Tf. Here it is shown that anti-TbTfR antibody titres produced during chronic murine trypanosomiasis are only one-tenth of those achieved by immunisation of mice using recombinant TbTfR. Calculations indicate that the concentrations of competing anti-TbTfR antibodies present during chronic *T. brucei* infection are too low to deprive the parasite of iron. In addition, during human African trypanosomiasis the antibody response to the TbTfR seems to be poor and transient. Altogether, the results suggest that the host antibody response to the TbTfR during chronic infection with *T. brucei* is too low, if present at all, to prevent sufficient iron uptake by bloodstream forms to promote their growth.



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Bloodstream forms of *Trypanosoma brucei brucei* were cultivated in the presence and absence of thiamine (vitamin B-1) and pyridoxine (vitamin B-6). The vitamins do not change growth behaviour, indicating that *Trypanosoma brucei* is prototrophic for the two vitamins even though *in silico* no bona-fide thiamine-biosynthetic genes could be identified in the *T. brucei* genome. Intracellularly, thiamine is mainly present in its diphosphate form. We were unable to detect significant uptake of [<sup>3</sup>H] thiamine and structural thiamine analogues such as pyrithiamine, oxithiamine and amprolium were not toxic for the bloodstream forms of *T. brucei*, indicating that the organism does not have an efficient uptake system for thiamine and its analogues. We have previously shown that, in the fission yeast *Saccharomyces pombe*, the toxicity of melarsen oxide, the pharmacologically active derivative of the frontline sleeping sickness drug melarsoprol, is abolished by thiamine and the drug is taken up by a thiamine-regulated membrane protein which is responsible for the utilization of thiamine. We show here that thiamine also has weak effects on melarsen oxide-induced growth inhibition and lysis in *T. brucei*. These effects were consistent with a low affinity of thiamine for the P2 adenosine transporter that is responsible for uptake of melaminophenyl arsenicals in African trypanosomes.

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Trypanosomatids of the order Kinetoplastida are major contributors to global disease and morbidity, and understanding their basic biology coupled with the development of new drug targets represents a critical need. Additionally, trypanosomes are among the more accessible divergent eukaryote experimental systems. The genome of *Trypanosoma brucei* contains 8,131 predicted open reading frames (ORFs), of which over half have no known homologues beyond the Kinetoplastida and a substantial number of others are poorly defined by *in silico* analysis. Thus, a major challenge following completion of the *T. brucei* genome sequence is to obtain functional data for all trypanosome ORFs. As *T. brucei* is more experimentally tractable than the related *Trypanosoma cruzi* and *Leishmania* spp. and shares >75 percent of their genes, functional analysis of *T. brucei* has the potential to inform a range of parasite biology. Here, we report methods for systematic mRNA ablation by RNA interference (RNAi) and for phenotypic analysis, together with online data dissemination. This represents the first systematic analysis of gene function in a parasitic organism. In total,

210 genes have been targeted in the bloodstream form parasite, representing an essentially complete phenotypic catalogue of chromosome I together with a validation set. Over 30 percent of the chromosome I genes generated a phenotype when targeted by RNAi; most commonly, this affected cell growth, viability, and/or cell cycle progression. RNAi against approximately 12 percent of ORFs was lethal, and an additional 11 percent had growth defects but retained short-term viability in culture. Although we found no evidence for clustering or a bias towards widely evolutionarily conserved genes within the essential ORF cohort, the putative chromosome I centromere is adjacent to a domain containing genes with no associated phenotype. Involvement of such a large proportion of genes in robust growth *in vitro* indicates that a high proportion of the expressed trypanosome genome is required for efficient propagation; many of these gene products represent potential drug targets.

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Differentiation in African trypanosomes (*Trypanosoma brucei*) entails passage between a mammalian host, where parasites exist as a proliferative slender form or a G0-arrested stumpy form, and the tsetse fly. Stumpy forms arise at the peak of each parasitaemia and are committed to differentiation to procyclic forms that inhabit the tsetse midgut. We have identified a protein tyrosine phosphatase (TbPTP1) that inhibits trypanosome differentiation. Consistent with a tyrosine phosphatase, recombinant TbPTP1 exhibits the anticipated substrate and inhibitor profile, and its activity is impaired by reversible oxidation. TbPTP1 inactivation in monomorphic bloodstream trypanosomes by RNA interference or pharmacological inhibition triggers spontaneous differentiation to procyclic forms in a subset of committed cells. Consistent with this observation, homogeneous populations of stumpy forms synchronously differentiate to procyclic forms when tyrosine phosphatase activity is inhibited. Our data invoke a new model for trypanosome development in which differentiation to procyclic forms is prevented in the bloodstream by tyrosine dephosphorylation. It may be possible to use PTP1B inhibitors to block trypanosomatid transmission.

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*Tsetse and Trypanosomiasis Information*

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*Sodalis glossinidius* is a maternally transmitted endosymbiont of tsetse flies (*Glossina* spp.), an insect of medical and veterinary significance. Analysis of the complete sequence of *Sodalis'* chromosome (4,171,146 bp, encoding 2,432 protein coding sequences) indicates a reduced coding capacity of 51 percent. Furthermore, the chromosome contains 972 pseudogenes, an inordinately high number compared with that of other bacterial species. A high proportion of these pseudogenes are homologues of known proteins that function either in defence or in the transport and metabolism of carbohydrates and inorganic ions, suggesting *Sodalis'* degenerative adaptations to the immunity and restricted nutritional status of the host. *Sodalis* possesses three chromosomal symbiosis regions (SSR): SSR-1, SSR-2, and SSR-3, with gene inventories similar to the Type-III secretion system (TTSS) *ysa* from *Yersinia enterocolitica* and SPI-1 and SPI-2 from *Salmonella*, respectively. While core components of the needle structure have been conserved, some of the effectors and regulators typically associated with these systems in pathogenic microbes are modified or eliminated in *Sodalis*. Analysis of SSR-specific *invA* transcript abundance in *Sodalis* during host development indicates that the individual symbiosis regions may exhibit different temporal expression profiles. In addition, the *Sodalis* chromosome encodes a complete flagella structure, key components of which are expressed in immature host developmental stages. These features may be important for the transmission and establishment of symbiont infections in the intra-uterine progeny. The data suggest that *Sodalis* represents an evolutionary intermediate transitioning from a free-living to a mutualistic lifestyle.

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Institute of Tropical Medicine, Department of Parasitology, Nationalestraat 155, B-2000 Antwerp, Belgium; Vrije Universiteit Brussel, Department of Biotechnology, Pleinlaan 2, B-1050 Brussels, Belgium.

In *Trypanosoma brucei brucei*, an invariant surface glycoprotein of molecular weight 75 kDa (ISG75) is uniformly distributed over the surface of a trypanosome and is specific for bloodstream-form parasites. For the other taxa of the *Trypanozoon* subgenus no data about this surface molecule are available. Therefore, we investigated the ISG75 in the genomes of several pathogenic *Trypanozoon* by Southern blot, PCR and RT-PCR and sequence analysis. Nucleotide sequence data reported in this paper are available in the GeneBank™, EMBL and DDBJ databases under the Accession numbers DQ200175-DQ200256. This study reveals that (i) all members of the *Trypanozoon* subgenus, i.e. *T. b. brucei*, *T. b. gambiense*, *T. b. rhodesiense*, *T. evansi* and *T. equiperdum*, harbour ISG75 as multiple gene copies with at least 4-16 copies per genome; (ii) ISG75 gDNA and cDNA sequences are distributed in 2 groups that share at least 75 percent and 77 percent identity respectively; (iii) sequences from both groups are transcribed in all species and subspecies of the *Trypanozoon* subgenus; (iv) the main differences between group I and group II are located in the variable region at the amino-terminus of the putative proteins; (v) however, all the sequences in both groups have some well-conserved features, such as the cysteine residues, an amino-terminal cleavable

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Laboratory of Infectious Disease, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.

Drug resistance in *Trypanosoma brucei* causes severe problems for people and domestic animals, but molecular mechanisms of the resistance are not well known. Programmed cell death (PCD) is a fundamental process in both multicellular and unicellular organisms, and it is speculated to be one of the important factors contributing to the emergence of drug resistance. We have previously reported that the expression of TAO appears to play a role in the inhibition of the PCD-like phenomenon development in *T. brucei*. In this study, to ascertain the correlation between the development of the PCD-like phenomenon and the expression of TAO in *T. brucei*, we genetically engineered *T. brucei* for conditional over-expression of the TAO gene. TAO over-expressing transgenic *T. brucei* was refractory to the development of the PCD-like phenomenon compared to the wild-type, indicating that expression of TAO might have a regulatory role on PCD development. Furthermore, the transgenic cells showed resistance to suramin and anticycide. We postulated that intracellular reactive oxygen species (ROS) may be involved in the mechanism of resistance to anticycide because augmentation of ROS in transgenic cells was lower than that in the wild-type cells following treatment with anticycide. These results suggest a possible correlation of PCD to drug resistance in *T. brucei*.

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Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, United Kingdom. [m.a.j.ferguson@dundee.ac.uk].

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Laboratorio de Química Biológica, Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, Apartado 21827, Caracas 1020A, Venezuela.

Lysophospholipid analogues (LPAs) comprise a class of metabolically stable compounds that have been developed as anticancer agents for over two decades, but which have also potent and selective antiparasitic activity, particularly against trypanosomatid parasites such as *Leishmania* and *Trypanosoma cruzi*, both *in vitro* and *in vivo*. The *in vivo* activities of LPAs result from direct effects on their target cells and are not dependent on a functional immune system. Because of their chemical nature, LPAs have a potential for interaction with a variety of subcellular structures and biochemical pathways. However, in mammalian cells LPA-induced growth inhibition and programmed cell death are usually associated with a blockade of phosphatidylcholine (PC) biosynthesis at the level of CTP: phosphocholine citidyltransferase, probably through an increase of cellular ceramide levels due to depressed sphingomyelin synthesis. Although in trypanosomatid parasites much less information is available, inhibition of PC biosynthesis by LPA has also been documented but at the level of phosphatidylethanolamine N-methyl-transferase, as well as LPA-induced classical apoptotic phenomena. The higher activity of LPAs as inhibitors of PC biosynthesis in parasites than in mammalian cells, probably due to different biochemical pathways involved in the two types of cells, could explain their selective antiparasitic action *in vivo*.

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African trypanosomes are parasitic protozoa of the order of Kinetoplastida, which cause sleeping sickness and nagana. Trypanosomes are not only of scientific interest because of their clinical importance, but also because these protozoa contain several very unusual biological features, such as their special energy metabolism. The energy metabolism of *Trypanosoma brucei* differs significantly from that of its host, not only because it comprises distinct enzymes and metabolic pathways, but also because some of the glycolytic enzymes are localized in organelles called glycosomes. Furthermore, the energy metabolism changes drastically during the complex life cycle of this parasite. This review focuses on the recent advances made in understanding the process of ATP production in *T brucei* during its life cycle and the consequences of the special subcellular compartmentation

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Trypanosomes are unicellular parasites and like all decent parasites, they try to obtain from the host as much material as possible, including lipids. However, the needs of a parasite are not always the same as those of the host, and therefore, mostly, some biosynthetic work still has to be done by the parasite itself. Very often at least modifications of the lipid components that are acquired from the host have to be made. Furthermore, next to the lipids *Trypanosoma brucei* indeed obtains from the host, some other lipid components have to be synthesized *de novo*. Especially the processes where the metabolism of *T. brucei* differs from that of the host will be discussed as at least some of them are excellent targets for the development of urgently needed new chemotherapeutics.

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Laboratorium voor Ultrastructuur, Vrije Universiteit Brussel and Department of Molecular and Cellular Interactions, Vlaams Instituut voor Biotechnologie, Pleinlaan 2, B-1050 Brussel, Belgium.

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Superoxide dismutases (SOD) are a family of antioxidant enzymes that function by removing superoxide anions from the cellular environment. Here, we show that the African trypanosome, *Trypanosoma brucei*, expresses four SOD isoforms, three of which we have validated biochemically as iron dependent, a feature normally associated with prokaryotic SODs. Localisation studies reveal that two of the enzymes are found predominantly in a

parasite-specific organelle, the glycosome (TbSODB1 and TbSODB2), while the other two are targeted to the mitochondrion (TbSODA and TbSODC). Functional analysis of the SOD repertoire in bloodstream form parasites was performed using an inducible RNA interference (RNAi) approach. Down-regulation of the glycosomal SOD transcripts corresponded with a significant reduction in the corresponding proteins and a dramatic level of cell death within the population. The importance of one of the mitochondrial enzymes (TbSODA) only became apparent when parasites were exposed to the superoxide-generating agent paraquat following induction of RNAi. These experiments therefore identify essential components of the superoxide metabolising arm of the *T. brucei* oxidative defence system and validate these enzymes as parasite-specific targets for drug design.

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Bloodstream trypanosomes take up iron needed for their propagation through the transferrin receptor that, in *Trypanosoma brucei*, is encoded by expression-site-associated genes (ESAGs), ESAG6 and 7 genes located in variant surface glycoprotein expression sites. ESAG6 and 7 genes in different expression sites have been shown to encode transferrin receptors with varying affinities for polymorphic transferrins. *T. brucei* could cope with the different host transferrins by switching between expression sites. ESAG6- and 7-encoded transferrin receptor appears to be present in *Trypanosoma evansi* but the genes have not yet been characterized. In this study, we cloned and sequenced different members of ESAG6 genes in seven isolates of *T. evansi* from geographically distinct localities in Thailand. We assessed the intra- and inter-species genetic variability in the transferrin receptor gene regions involved in transferrin binding and established that *T. evansi*, like *T. brucei*, has widely diverse ESAG6 genes. In addition, *T. evansi* possess a clade of ESAG6 variants not observed in *T. brucei* and different *T. evansi* strains share at least two conserved variants. We further noted that *T. evansi* possesses all the reported *T. equiperdum* ESAG6 variants as a subset. Our findings depict a correlation between the genetic diversity in the transferrin-binding regions of ESAG6 genes with the broad host range of *T. evansi* and *T. brucei* compared to the narrow host range of *Trypanosoma equiperdum*.

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Drug resistance is now a severe and increasing problem in trypanosomes, but molecular details of mechanisms of resistance are only beginning to unveil. There is urgent need to clearly elucidate the different mechanisms of drug resistance in trypanosomes in order to circumvent existing resistance problems and avoid emergence of resistance to the next



generation drugs. In this study, we cloned and characterized a novel gene, TeDR40, whose expression is associated with resistance to berenil in *Trypanosoma evansi*. Expression analysis showed that the gene was at least 1,000-fold upregulated in resistant parasites and the encoded protein appeared to have a ubiquitous cellular localization. To investigate the association of TeDR40 with berenil-resistance, we genetically modified wild-type berenil-sensitive *T. evansi* for inducible overexpression of the TeDR40 gene. Induction of over-expression of TeDR40 in *T. evansi* led to decreased ( $P < 0.01$ ) sensitivity to berenil. Our findings indicate a possible correlation between over-expression of a novel gene, TeDR40, and reduced sensitivity to berenil in an *in vitro*-cultured clonal line of *T. evansi*.

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