



TRYLEIDIAG PRESS REVIEW

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EVENTS HIGHLIGHTS

Working with Pathogen Genomes

2–6 March 2009

Wellcome Trust Genome Campus, Hinxton, Cambridge, UK

Deadline for applications: 21 November 2008

This residential workshop will aim to give microbiologists who have a working knowledge of computational sequence analysis, a firm grounding in the use of the latest genome analysis software (Artemis and ACT) developed at the Wellcome Trust Sanger Institute Pathogen Sequencing Unit (PSU).

Artemis is a powerful annotation tool and DNA viewer that allows the user to analyse sequence data generated in-house as well as being able to upload and re-analyse data taken from databases such as EMBL or Genbank. ACT is a comparative genomic tool that allows direct, and interactive, comparisons of multiple genomes/sequences. This enables the user to exploit the growing number of genomes from closely related organisms to look at genome architecture and evolution. For more information regarding these analysis tools see [Artemis: a DNA sequence viewer and annotation tool](#) and [ACT: a DNA sequence comparison viewer](#).

The course will be taught by members of the PSU and will take the form of a series of modules covering most aspects of sequence analysis and exploitation. Each module will be introduced with a short talk followed by 'hands on' worked examples using many of the organisms sequenced by the PSU (bacterial pathogens, e.g. Salmonella and Chlamydia), as well as small eukaryotic pathogens (such as Plasmodium and Trypanosomes) to illustrate points in whole genome analysis.

2008 Global Ministerial Forum on Research for Health

November 2008

Bamako, Mali

Le Forum ministériel mondial sur la recherche pour la santé se tiendra à Bamako, au Mali, du 17 au 19 novembre 2008. Il réunira plus de 1000 décideurs politiques et chercheurs pour réfléchir sur les principaux liens à tisser entre le secteur de la santé et celui de la recherche, la science et la technologie, l'enseignement supérieur et le système d'innovation mondial.

Renforcer le leadership pour la santé, l'équité et le développement :

- donner des moyens d'action aux gouvernements pour qu'ils élaborent sur des bases structurées des politiques prioritaires de recherche en santé dans le cadre de leurs stratégies de recherche plus générales
- améliorer la capacité des systèmes à mettre en œuvre ces politiques
- intensifier la coopération internationale pour faire face aux enjeux nationaux et mondiaux en matière de recherche en santé



RESEARCH NEWS

The continuing problem of human African trypanosomiasis (sleeping sickness).

Kennedy PG.

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Ann Neurol. 2008 Aug;64(2):116-26.

Human African trypanosomiasis, also known as sleeping sickness, is a neglected disease, and it continues to pose a major threat to 60 million people in 36 countries in sub-Saharan Africa. Transmitted by the bite of the tsetse fly, the disease is caused by protozoan parasites of the genus *Trypanosoma* and comes in two types: East African human African trypanosomiasis caused by *Trypanosoma brucei rhodesiense* and the West African form caused by *Trypanosoma brucei gambiense*. There is an early or hemolymphatic stage and a late or encephalitic stage, when the parasites cross the blood-brain barrier to invade the central nervous system. Two critical current issues are disease staging and drug therapy, especially for late-stage disease. Lumbar puncture to analyze cerebrospinal fluid will remain the only method of disease staging until reliable noninvasive methods are developed, but there is no widespread consensus as to what exactly defines biologically central nervous system disease or what specific cerebrospinal fluid findings should justify drug therapy for late-stage involvement. All four main drugs used for human African trypanosomiasis are toxic, and melarsoprol, the only drug that is effective for both types of central nervous system disease, is so toxic that it kills 5% of patients who receive it. Eflornithine, alone or combined with nifurtimox, is being used increasingly as first-line therapy for gambiense disease. There is a pressing need for an effective, safe oral drug for both stages of the disease, but this will require a significant increase in investment for new drug discovery from Western governments and the pharmaceutical industry.

Efficacy of the diamidine DB75 and its prodrug DB289, against murine models of human African trypanosomiasis.

Thuita JK, Karanja SM, Wenzler T, Mdachi RE, Ngotho JM, Kagira JM, Tidwell R, Brun R.

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

Acta Trop. 2008 Aug 5.

The choice of drugs for the treatment of sleeping sickness is extremely limited. To redress this situation, the recently synthesised diamidine, 2,5-bis(4-amidinophenyl)-furan (DB75, furamidine) and its methamidoxime prodrug, 2,5-bis(4-amidinophenyl)-furan-bis-O-methylamidoxime (DB289, pafuramidine) were, together with pentamidine, evaluated for efficacy in acute rodent models. The activity was compared in three common mouse models that mimic the first stage of human African trypanosomiasis. The mice were infected with the pleomorphic *T. b. rhodesiense* strains KETRI2537 and STIB900 or with the monomorphic *T. b. brucei* strain STIB795. Importantly, DB75 showed activity



superior to that of pentamidine at comparable doses in all three mouse models. Complete cures were achieved with oral dosing of the prodrug DB289 in all three models without any overt toxicity. This shows that the prodrug strategy was successful in terms of reducing toxicity and increasing efficacy and oral bioavailability.

Isolation and analysis of the genetic diversity of repertoires of VSG expression site containing telomeres from *Trypanosoma brucei gambiense*, *T. b. brucei* and *T. equiperdum*.

Young R, Taylor JE, Kurioka A, Becker M, Louis EJ, Rudenko G.

BMC Genomics. 2008 Aug 12;9(1):385.

ABSTRACT: **BACKGROUND:** African trypanosomes (including *Trypanosoma brucei*) are unicellular parasites which multiply in the mammalian bloodstream. *T. brucei* has about twenty telomeric bloodstream form Variant Surface Glycoprotein (VSG) expression sites (BESs), of which one is expressed at a time in a mutually exclusive fashion. BESs are polycistronic transcription units, containing a variety of families of expression site associated genes (ESAGs) in addition to the telomeric VSG. These polymorphic ESAG families are thought to play a role in parasite-host adaptation, and it has been proposed that ESAG diversity might be related to host range. Analysis of the genetic diversity of these telomeric gene families has been confounded by the underrepresentation of telomeric sequences in standard libraries. We have previously developed a method to selectively isolate sets of trypanosome BES containing telomeres using Transformation associated recombination (TAR) cloning in yeast. **RESULTS:** Here we describe the isolation of repertoires of BES containing telomeres from three trypanosome subspecies: *Trypanosoma brucei gambiense* DAL 972 (causative agent of West-African trypanosomiasis), *T. b. brucei* EATRO 2340 (a nonhuman infective strain) and *T. equiperdum* STIB 818 (which causes a sexually transmitted disease in equines). We have sequenced and analysed the genetic diversity at four BES loci (BES promoter region, ESAG6, ESAG5 and ESAG2) from these three trypanosome BES repertoires. **CONCLUSIONS:** With the exception of ESAG2, the BES sequence repertoires derived from *T. b. gambiense* are both less diverse than and nearly reciprocally monophyletic relative to those from *T. b. brucei* and *T. equiperdum*. Furthermore, although we find evidence for adaptive evolution in all three ESAG repertoires in *T. b. brucei* and *T. equiperdum*, only ESAG2 appears to be under diversifying selection in *T. b. gambiense*. This low level of variation in the *T. b. gambiense* BES sequence repertoires is consistent both with the relatively narrow host range of this subspecies and its apparent long-term clonality. However, our data does not show a clear correlation between size of trypanosome host range and either number of BESs or extent of ESAG genetic diversity.

Comment on: Diagnosing central nervous system trypanosomiasis: two stage or not to stage.

Armour RH.

Lister Hospital, Stevenage, Hitchin, Hertfordshire, UK.

Trans R Soc Trop Med Hyg. 2008 Aug 8.



Reply to comment on: Diagnosing central nervous system trypanosomiasis: two stage or not to stage.

Kennedy PG.

Division of Clinical Neurosciences, Faculty of Medicine, University of Glasgow, Institute of Neurological Science, Southern General Hospital, Glasgow G51 4TF, UK.

Trans R Soc Trop Med Hyg. 2008 Aug 8. [Epub ahead of print]

The role of B-cells and IgM antibodies in parasitemia, anemia, and VSG switching in *Trypanosoma brucei*-infected mice.

Magez S, Schwegmann A, Atkinson R, Claes F, Drennan M, De Baetselier P, Brombacher F.

Division of Immunology, Institute for Infectious Diseases and Molecular Medicine (IIDMM), Health Science Faculty, University of Cape Town, and International Centre for Genetic Engineering and Biotechnology (ICGEB), Cape Town, South Africa.

PLoS Pathog. 2008 Aug 8;4(8):e1000122.

African trypanosomes are extracellular parasitic protozoa, predominantly transmitted by the bite of the haematophagous tsetse fly. The main mechanism considered to mediate parasitemia control in a mammalian host is the continuous interaction between antibodies and the parasite surface, covered by variant-specific surface glycoproteins. Early experimental studies have shown that B-cell responses can be strongly protective but are limited by their VSG-specificity. We have used B-cell (microMT) and IgM-deficient (IgM^{-/-}) mice to investigate the role of B-cells and IgM antibodies in parasitemia control and the *in vivo* induction of trypanosomiasis-associated anemia. These infection studies revealed that the initial setting of peak levels of parasitemia in *Trypanosoma brucei*-infected microMT and IgM^{-/-} mice occurred independent of the presence of B-cells. However, B-cells helped to periodically reduce circulating parasites levels and were required for long term survival, while IgM antibodies played only a limited role in this process. Infection-associated anemia, hypothesized to be mediated by B-cell responses, was induced during infection in microMT mice as well as in IgM^{-/-} mice, and as such occurred independently from the infection-induced host antibody response. Antigenic variation, the main immune evasion mechanism of African trypanosomes, occurred independently from host antibody responses against the parasite's ever-changing antigenic glycoprotein coat. Collectively, these results demonstrated that in murine experimental *T. brucei* trypanosomiasis, B-cells were crucial for periodic peak parasitemia clearance, whereas parasite-induced IgM antibodies played only a limited role in the outcome of the infection.

Trypanosomiasis vector control in Africa and Latin America.

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Parasit Vectors. 2008 Aug 1;1(1):24.

ABSTRACT: Vectors of trypanosomiasis - tsetse (Glossinidae) in Africa, kissing-bugs (Triatominae) in Latin America - are very different insects but share demographic characteristics that render them



highly vulnerable to available control methods. For both, the main operational problems relate to re-invasion of treated areas, and the solution seems to be in very large-scale interventions covering biologically-relevant areas rather than adhering to administrative boundaries. In this review we present the underlying rationale, operational background and progress of the various trypanosomiasis vector control initiatives active in both continents.

Factors influencing individual and community participation in the control of tsetse flies and human African trypanosomiasis in Urambo District, Tanzania.

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Tanzan J Health Res. 2008 Jan;10(1):20-7.

This study was carried out to assess the knowledge and level of individual and community participation in the control of Human African trypanosomiasis in Urambo District, western Tanzania. Semi structured questionnaires were used to collect information from individuals at house hold level. Retrospective data of HAT was sought from the medical officers in-charge of health facilities. The results indicate that, 191 (90.5%, n = 211) individuals knew tsetse flies and 187 (88.6%, n = 211) knew HAT. All nine key informants reported that, the communities were aware of HAT while seven key informants reported that, the communities were aware of health risks associated with tsetse bites in human. There was poor knowledge about the role played by animals in the transmission of HAT (26.7%, n = 187). Majority of those who knew HAT (n = 187) were willing to contribute labour (70.1%) and money (64.2%) to tsetse and HAT control whereas amongst those who knew tsetse flies, 66.5% and 60.7% were willing to contribute labour and money, respectively. Amongst those who knew any HAT control technique (n = 108), 78.7% and 82.4% were willing to contribute money and labour respectively. A total of 454 cases of HAT were reported in the area from 1999 to 2006. It is concluded that, the factors influencing individual and community participation include the knowledge of tsetse, HAT and control measures.

Blood smear analysis in babesiosis, ehrlichiosis, relapsing fever, malaria, and Chagas disease.

Blevins SM, Greenfield RA, Bronze MS.

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Cleve Clin J Med. 2008 Jul;75(7):521-30.

Blood smear analysis is especially useful for diagnosing five infectious diseases: babesiosis, ehrlichiosis, relapsing fever due to *Borrelia* infection, malaria, and American trypanosomiasis (Chagas disease). It should be performed in patients with persistent or recurring fever or in those who have traveled to the developing world or who have a history of tick exposure, especially if accompanied by hemolytic anemia, thrombocytopenia, or hepatosplenomegaly.



Glycogen Synthase Kinase 3 is a Potential Drug Target for African Trypanosomiasis Therapy.

Ojo KK, Gillespie JR, Riechers AJ, Napuli AJ, Verlinde CL, Buckner FS, Gelb MH, Domostoj MM, Wells SJ, Scheer A, Wells TN, Van Voorhis WC.

Division of Allergy and Infectious Diseases, Department of Medicine, Department of Biochemistry, Department of Chemistry, University of Washington Seattle WA 98195; Merck Serono Geneva Research Centre, Merck Serono International S.A., Chemin des Mines 9, CH-1202 Genève.

Antimicrob Agents Chemother. 2008 Jul 21.

Development of a safe, effective and inexpensive therapy for African trypanosomiasis is an urgent priority. In this study, we evaluate the validity of *T. brucei* glycogen synthase kinase 3 (GSK-3) as potential drug target. RNA interference of either of two GSK-3 homologues in bloodstream form *T. brucei* led to growth arrest and altered parasite morphology, demonstrating their requirement for cell survival. Since the growth arrest after RNAi appeared more profound for TbruGSK-3 "short" (Tb10.161.3140) vs. TbruGSK-3 "long" (Tb927.7.2420), we focused on TbruGSK-3 short for further studies. TbruGSK-3 short with an N-terminal maltose-binding protein fusion was cloned, expressed and purified in a functional form. The potency of a GSK-3-focused inhibitor library against recombinant enzyme of TbruGSK-3 short, as well as bloodstream form parasites was evaluated with the aim of determining if compounds inhibiting enzyme activity could also block parasites' growth and proliferation. Among the cell active compounds, there was an excellent correlation of activity inhibiting TbruGSK short enzyme and inhibition of *T. brucei* growth. Thus there is reasonable genetic and chemical validation of GSK-3 short as a drug target for *T. brucei*. Finally, selective inhibition may be required for therapy targeting the GSK-3 enzyme, and a molecular model of TbruGSK short enzyme suggests that compounds can be found that selectively inhibit TbruGSK-3 short over the human GSK-3 enzymes.

Isolation of *Leishmania tropica* from a Patient with Visceral Leishmaniasis and Disseminated Cutaneous Leishmaniasis, Southern Iran.

Alborzi A, Pouladfar GR, Fakhar M, Motazedian MH, Hatam GR, Kadivar MR.

Professor Alborzi Clinical Microbiology Research Center, and Department of Medical Parasitology and Mycology, Shiraz University of Medical Sciences, Shiraz, Iran; Department of Medical Parasitology and Mycology, Mazandaran University of Medical Sciences, Sari, Iran.

Am J Trop Med Hyg. 2008 Sep;79(3):435-437.

We report a case visceral leishmaniasis with disseminated cutaneous leishmaniasis caused by *Leishmania tropica* in southern Iran. Typing of this parasite was performed by a species-specific polymerase chain reaction and isoenzyme electrophoresis.

Isolation and identification of *Leishmania donovani* from *Phlebotomus orientalis*, in an area of eastern Sudan with endemic visceral leishmaniasis.



Hassan MM, Elamin EM, Mukhtar MM.

Department of Epidemiology, Tropical Medicine Research Institute, National Centre for Research, Ministry of Science and Technology, P.O. Box 1304, Khartoum, Sudan.

Ann Trop Med Parasitol. 2008 Sep;102(6):553-5.PMID: 18782494 [PubMed - in process]

New Treatment Approach in Indian Visceral Leishmaniasis: Single-Dose Liposomal Amphotericin B Followed by Short-Course Oral Miltefosine.

Sundar S, Rai M, Chakravarty J, Agarwal D, Agrawal N, Vaillant M, Olliaro P, Murray HW.

1Kala-Azar Medical Research Center, Department of Medicine, Banaras Hindu University, Institute of Medical Sciences, Varanasi, and 2MLN Medical College, Allahabad, India; 3Clinical Epidemiology and Public Health Unit, Center for Health Studies, CRP-Santé, Luxembourg; 4UNICEF/UNDP/WB/WHO Special Programme for Research and Training in Tropical Diseases, Geneva, Switzerland; 5The Centre for Tropical Medicine, Centre for Tropical Medicine and Vaccinology, Nuffield Department of Medicine, University of Oxford, Churchill Hospital, Oxford, United Kingdom; and 6Department of Medicine, Weill Cornell Medical College, New York, New York.

Clin Infect Dis. 2008 Sep 9.

Background. In Bihar, India, home to nearly one-half of the world's burden of visceral leishmaniasis, drug resistance has ended the usefulness of pentavalent antimony, which is the traditional first-line treatment. Although monotherapy with other agents is available, the use of 2 drugs with different modes of action might increase efficacy, shorten treatment duration, enhance compliance, and/or reduce the risk of parasite resistance. To test the feasibility of a new approach to combination therapy in visceral leishmaniasis (also known as kala-azar), we treated Indian patients with a single infusion of liposomal amphotericin B (L-AmB), followed 1 day later by short-course oral miltefosine. **Methods.** We used a randomized, noncomparative, group-sequential, triangular design and assigned 181 subjects to treatment with 5 mg/kg of L-AmB alone (group A; 45 subjects), 5 mg/kg of L-AmB followed by miltefosine for 10 days (group B; 46 subjects) or 14 days (group C; 45 subjects), or 3.75 mg/kg of L-AmB followed by miltefosine for 14 days (group D; 45 subjects). When it became apparent that all regimens were effective, 45 additional, nonrandomized patients were assigned to receive 5 mg/kg of L-AmB followed by miltefosine for 7 days (group E). **Results.** Each regimen was satisfactorily tolerated, and all 226 subjects showed initial apparent cure responses. Nine months after treatment, final cure rates were similar: group A, 91% (95% confidence interval [CI], 78%-97%); group B, 98% (95% CI, 87%-100%); group C, 96% (95% CI, 84%-99%); group D, 96% (95% CI, 84%-99%); and group E, 98% (95% CI, 87%-100%). **Conclusions.** These results suggest that treatment with single-dose L-AmB followed by 7-14 days of miltefosine is active against Indian kala-azar. This short-course, sequential regimen warrants additional testing in India and in those regions of endemicity where visceral leishmaniasis may be more difficult to treat. **Trial registration.** ClinicalTrials.gov identifier: NCT00370825.

Leishmania donovani infection down-regulates TLR2-stimulated IL-12p40 and activates IL-10 in cells of macrophage/monocytic lineage by modulating MAPK pathways through a contact-dependent mechanism.



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Clin Exp Immunol. 2008 Sep 5.

The failure of *Leishmania*, an intracellular pathogen, to stimulate a pro-inflammatory response following entry into macrophages has been well reported. This occurs in spite of the fact that ligands for the toll-like receptors (TLR) have been recently shown on the parasite surface and their role in disease protection well documented. The outcome of infection in leishmaniasis is determined by the Th1 versus Th2 nature of the effector response and the generation of IL-12 and IL-10 by the infected macrophages is important for this decision. We evaluated the effect of *L. donovani* infection of monocytes (cell line THP-1, and monocytes derived from human peripheral blood) on Pam3cys (TLR2 ligand) and lipopolysaccharide (TLR4 ligand) stimulated production of IL-12p40 and IL-10. *L. donovani* infection caused suppression of TLR2 and TLR4-stimulated IL-12p40, with an increase in IL-10 production. Parasites also modulated the TLR2-stimulated mitogen-activated protein kinase (MAPK) pathway by suppressing MAPK P(38) phosphorylation and activating extracellular regulated kinase (ERK)1/2 phosphorylation. These effects could be reversed either by using a MAPK P(38) activator, anisomycin, or ERK1/2 inhibitor, U0126. *L. donovani* caused modulation of TLR2-stimulated MAPK pathways in a contact-dependent mechanism. In addition parasite structural integrity but not viability was required for suppression of TLR2-stimulated IL-12p40 and activation of IL-10. These observations suggest that *L. donovani* has evolved survival strategies that subvert the pro-inflammatory response generated through TLRs.

Oxidation of hemoglobin and redistribution of band 3 promote erythrophagocytosis in visceral leishmaniasis.

Saha Roy S, Chowdhury KD, Sen G, Biswas T.

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Mol Cell Biochem. 2008 Sep 6.

In visceral leishmaniasis (VL), oxidative assault on erythrocytes perturbs their cellular environment and makes them vulnerable to premature hemolysis. In this study, we assessed the contribution of oxidation-induced modifications of hemoglobin and membrane protein band 3 in the reduced survival of red cells in VL. Oxidative transformation of oxyhemoglobin to hemichrome enhanced its interaction with erythrocyte membrane in the infected animals. Association between denatured globin and band 3 contributed to the formation of insoluble copolymer of macromolecular dimension. Disulfide bonding appeared to be necessary in the making of high molecular weight aggregates during copolymerization. Hemichrome induced clustering of band 3 promoted generation of epitopes on erythrocyte cell surface. This provided a signal favoring immunologic recognition of redistributed band 3 by autologous IgG followed by erythrophagocytosis. An eventual outcome of the sequence of events pointed to early removal of affected red cells from circulation during the disease.

Hemophagocytic lymphohistiocytosis associated with Epstein



Barr virus and Leishmania donovani coinfection in a child from Cyprus.

Koliou MG, Soteriades ES, Ephros M, Mazeris A, Antoniou M, Elia A, Novelli V.

Department of Pediatrics, Infectious Diseases and Immunology Unit, Archbishop Makarios Hospital, Nicosia, Cyprus. mkoliou@spidernet.com.cy

J Pediatr Hematol Oncol. 2008 Sep;30(9):704-7.

We present a case of a 9-month-old girl from Cyprus with hemophagocytic lymphohistiocytosis associated with Epstein Barr virus and Leishmania donovani coinfection. Treatment with liposomal amphotericin B resulted in a dramatic resolution of clinical and laboratory abnormalities. To our knowledge, this is the first reported case of a coinfection-associated hemophagocytic lymphohistiocytosis and the first clinical report of visceral leishmaniasis infection in Europe by L. donovani.

Induction of apoptosis in host cells: a survival mechanism for Leishmania parasites?

Getti GT, Cheke RA, Humber DP.

School of Health and Bioscience, University of East London, Stratford Campus, Romford Road, London E15 4LZ, UK.

Parasitology. 2008 Sep 8:1-9.

SUMMARYLeishmania parasites invade host macrophages, causing infections that are either limited to skin or spread to internal organs. In this study, 3 species causing cutaneous leishmaniasis, L. major, L. aethiopica and L. tropica, were tested for their ability to interfere with apoptosis in host macrophages in 2 different lines of human monocyte-derived macrophages (cell lines THP-1 and U937) and the results confirmed in peripheral blood mononuclear cells (PBMC). All 3 species induced early apoptosis 48h after infection. Moreover, the percentage of infected THP-1 and U937 macrophages increased significantly (up to 100%) following treatment with an apoptosis inducer. Since phosphatidyl serine externalization on apoptosing cells acts as a signal for engulfment by macrophages, induction of apoptosis in the parasitized cells could actively participate in spreading the infection. In summary, parasite-containing apoptotic bodies with intact membranes could be released and phagocytosed by uninfected macrophages.

Detection of Leishmania kDNA in human serum samples for the diagnosis of visceral leishmaniasis.

de Assis TS, Caligiorne RB, Romero GA, Rabello A.

Laboratory of Clinical Research, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz (Fiocruz), Av. Augusto de Lima 1715, Belo Horizonte, Minas Gerais 30190-002, Brazil.

Trans R Soc Trop Med Hyg. 2008 Sep 4.



The performance of PCR to detect *Leishmania* kDNA in serum for the diagnosis of visceral leishmaniasis (VL) was assessed in serum samples from 65 patients with VL, 17 non-infected individuals and 17 patients with other febrile hepatosplenic diseases. Serum PCR showed a sensitivity of 85%, specificity of 100% and efficiency of 90%. The sensitivity values obtained for blood PCR (97%) and rK39 ELISA (95%) were significantly higher ($P=0.01$) than the values observed for *L. chagasi* ELISA (88%) and serum PCR (85%), whilst no difference was observed among the specificity rates obtained with rK39 ELISA (94%; $P=0.47$) and *L. chagasi* ELISA (85%; $P=0.06$). This work suggests that the use of serum samples may be an alternative for the diagnosis of VL when peripheral blood samples are not available or require significant operational efforts.

Leishmanicidal activity of Yucatecan medicinal plants on *Leishmania* species responsible for cutaneous leishmaniasis.

Getti G, Durgadoss P, Domínguez-Carmona D, Martín-Quintal Z, Peraza-Sánchez S, Peña-Rodríguez LM, Humber D.

J Parasitol. 2008 Sep 4:1.

The leishmanicidal activity of 15 extracts and 4 pure metabolites obtained from *Urechites andrieuxii*, *Colubrina greggii*, *Dorstenia contrajerva*, and *Tridax procumbens* was evaluated using the newly developed MTS ($\{3-(4,5\text{-dimethylthiazol-2-yl})-5-(3\text{-carboxymethoxyphenyl})-2-(4\text{-sulfophenyl})-2\text{H-tetrazolium, inner salt}\}$) assay, optimized for promastigotes of *Leishmania major*, *L. tropica*, and *L. aethiopica*, as well as for *L. aethiopica* axenic amastigotes. The assay was then used for calculating the percentage of viable stationary phase parasites after a 24 hr treatment with each plant extract or pure metabolite. The 3 most active samples, 2 from *C. greggii* (NCG-5C and DCG-3A) and 1 from *T. procumbens* (TPZ-2A) showed LD50 values of 18.5, 7.2, and 62.4 microg/ml, respectively, on stationary promastigotes, and of 95.2, 27.1, and 94.2 microg/ml, on amastigotes of *L. aethiopica*. Moreover, TPZ-2A and DCG-3A also significantly reduced the percentage of infected monocyte-derived macrophages (THP-1). The percentage of infected cells went from 69.9 % (± 2.5), to 20.8 % (± 2) when treated with the DCG-3A fraction and to 14.9 % (± 0.5) when treated with TPZ-2A, without significantly decreasing the number of human cells. These findings indicate the presence of potentially bioactive metabolites in the roots of *C. greggii* and in *T. procumbens* and reflect the importance of pursuing the bioassay-guided purification of these metabolites.

Sand flies, *Leishmania*, and transcriptome-borne solutions.

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Sand fly-parasite and sand fly-host interactions play an important role in the transmission of leishmaniasis. Vector molecules relevant for such interactions include midgut and salivary proteins. These potential targets for interruption of propagation of *Leishmania* parasites have been poorly characterized. Transcriptomic analysis has proven to be an effective tool for identification of new sand fly molecules, providing exciting new insights into vector-based control strategies against leishmaniasis.



Comparison of treatment regimens of kala-azar based on culture & sensitivity of amastigotes to sodium antimony gluconate.

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Indian J Med Res. 2008 Jun;127(6):582-8.

BACKGROUND & OBJECTIVE: Present treatment strategies for kala-azar (visceral leishmaniasis, VL) include use of first line drug sodium antimony gluconate (SAG) to all patients but a large number of patients do not get relief with this drug. If a patient does not respond to a full course of SAG, a second or third line drug is given. We undertook this study to test whether an improved outcome can be achieved by employing a strategy of treatment based on culture and sensitivity of amastigotes to SAG compared with conventional empirical treatment. **METHODS:** In a double-blind, randomized, controlled trial done in Balaji Utthan Sansthan, Patna, of the 181 patients screened, 140 were finally randomly allocated to two groups A and B; group A patients were treated with SAG if their amastigotes were sensitive to SAG, and all patients in group B were treated with SAG to start with. Primary outcome measured was as no relapse within 6 months of follow up after cure and other outcomes measured were period of stay of patients in hospital, expenditure involved in the treatment, and infectivity periods of two groups, two-third of treatment period and whole of untreated period were taken as infectivity period. SAG was used at a dosage of 20 mg/kg given deep intramuscular injections in buttock for 28 days, amphotericin B (AMB) given at a dose of 1 mg/kg body wt daily for 20 days as a slow intravenous infusion in 5 per cent dextrose. **RESULTS:** Of the 70 patients in group A, 29 patients whose amastigotes were sensitive to SAG were treated with SAG, 2 patients were withdrawn due to drug toxicity; and 2 relapsed within 6 months of follow up and ultimate cure occurred in 25 (86.2%) patients only. Of the 70 patients in group B treated with SAG, 5 (7.1%) patients withdrew due to drug toxicity, 35 patients (50%) did not respond to treatment, 5 (7.1%) relapsed during 6 months of follow up and thus only 25 patients (35.7%) were ultimately cured. The difference between the two groups was significant ($P < 0.001$). No patient died during treatment due to any toxicity because of early withdrawal of patients from treatment apprehending toxicity. Patients whose amastigotes were resistant to SAG, withdrawn from the study due to SAG toxicity, relapsed after cure with SAG, and who did not respond to SAG in both the groups were treated with AMB and all were cured. Groups B and A patients spent 3065 and 2340 days respectively in hospital, group B 1.3 times more than group A. The likely period of spread of parasites in society was 1965 days in group B and 1644 days in group A, group B 1.4 times more than group A. The total expenditure on treatment in groups B and A was \$ 65,575 and \$ 50,590 respectively; group B patient had to spend 1.3 times more than group A. **INTERPRETATION & CONCLUSION:** A new strategy for treatment of kala-azar based on culture and sensitivity of amastigotes improved the cure rate, saved expenditure on the patient's treatment, patients had to stay for shorter periods in hospital and reduced the chance of spread of SAG resistant disease in society. Till the government opts for better drugs, the treatment based on culture and sensitivity of the parasites to SAG may be a better method.

Drug regimens for visceral leishmaniasis in Mediterranean countries.

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Trop Med Int Health. 2008 Aug 24.

Until the early 1990s, pentavalent antimony was the only documented first-line drug employed for the treatment of zoonotic visceral leishmaniasis (VL) in the Mediterranean, with reported cure rates exceeding 95% in immunocompetent patients. The emergence of antimony resistance in other endemic settings and the increase in drug options have stimulated re-evaluation of the current therapeutic approaches and outcomes in Mediterranean countries. A scientific consortium ('LeishMed' network) collected updated information from collaborating clinical health centres of 11 endemic countries of Southern Europe, Northern Africa and the Middle East. In contrast with the previous situation, VL is now treated differently in the region, basically through three approaches: (1) In Northern Africa and in part of the Middle East, pentavalent antimony is still the mainstay for therapy, with no alternative drug options for treating relapses; (2) In some European countries and Israel, both pentavalent antimony and lipid-associated amphotericin B (AmB) formulations are used as first-line drugs, although in different patients' categories; (3) In other countries of Europe, mainly liposomal AmB is employed. Importantly, cure rates exhibited by different drugs, including antimonials in areas where they are still in routine use, are similarly high ($\geq 95\%$) in immunocompetent patients. Our findings show that antimony resistance is not an emerging problem in the Mediterranean. A country's wealth affects the treatment choice, which represents a balance between drug efficacy, toxicity and cost, and costs associated with patient's care.

Clinical and epidemiological features of visceral Leishmaniasis and HIV co-infection in 15 patients from Brazil

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J Parasitol. 2008 Sep 2:1.

Cases of Visceral Leishmaniasis (VL) during HIV infection have regularly been recorded in various foci in the world, mainly in southern Europe. HIV infection can increase the risk of developing VL by 10-100 times in endemic areas. We describe the occurrence of this co-infection in 15 patients from Brazil. The mean age was 38 \pm 8.8 years, with 86.6% males. The mean time between HIV diagnosis and the onset of visceral leishmaniasis was 44 \pm 39 months. The main signs and symptoms presented at admission were splenomegaly (73%), weight loss (73%), cough (67%), fever (67%), asthenia and diarrhea (60%). The mean T CD4+ lymphocytes count was 173.7 \pm 225.6 cells/mm³, and viral load was 51,030 \pm 133,737/mm³. Treatment consisted in pentavalent antimonials in the majority of cases (67%). The majority of patients (87%) recovered from VL infection. Death occurred in one case, and the causa mortis was septic shock. VL is an important opportunistic infection in HIV patients, which is potentially fatal, even when correct treatment is done. Treatment should be done with pentavalent antimonials or amphotericin B in the case of relapses. Although there is no consensus, secondary prophylaxis should be considered in severe cases.

Th1-stimulatory polyproteins of soluble Leishmania donovani promastigotes ranging from 89.9 to 97.1kDa offers long-lasting protection against experimental visceral leishmaniasis.

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Vaccine. 2008 Aug 29.

Our earlier studies identified a fraction (F2) of *Leishmania donovani* soluble promastigote antigen belonging to 97.4-68kDa for its ability to stimulate Th1-type cellular responses in cured visceral leishmaniasis (VL) patients as well as in cured hamsters. A further fractionation of F2-fraction into seven subfractions (F2.1-F2.7) and re-assessment for their immunostimulatory responses revealed that out of these, only four (F2.4-F2.7) belonging to 89.9-97.1kDa, stimulated remarkable Th1-type cellular responses either individually or in a pooled form (P4-7). In this study these potential subfractions were further assessed for their prophylactic potential in combination with BCG against *L. donovani* challenge in hamsters. Optimum parasite inhibition (approximately 99%) was obtained in hamsters vaccinated with pooled subfractions and they survived for 1 year. The protection was further supported by remarkable lymphoproliferative, IFN-gamma and IL-12 responses along with profound delayed type hypersensitivity and increased levels of *Leishmania*-specific IgG2 antibody as observed on days 45, 90 and 120 post-challenge suggesting that a successful subunit vaccine against VL may require multiple Th1-immunostimulatory proteins. MALDI-TOF-MS/MS analysis of these subfractions further revealed that of the 19 identified immunostimulatory proteins, Elongation factor-2, p45, Heat shock protein-70/83, Aldolase, Enolase, Triosephosphate isomerase, Disulfideisomerase and Calreticulin were the major ones in these subfractions.

Extranodal gammadelta-T-cell lymphoma in a dog with leishmaniasis.

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Vet Clin Pathol. 2008 Sep;37(3):298-301.

An 8-year-old intact male mongrel dog with alopecia and weight loss was referred to the Veterinary Faculty of Naples. The dog had pale mucous membranes, enlarged prescapular lymph nodes, and splenomegaly. Laboratory abnormalities included anemia, thrombocytopenia, and hyperglobulinemia. Bone marrow aspirate smears contained numerous *Leishmania* amastigotes and an immunofluorescent antibody titer was strongly positive (1:1280) for leishmaniasis. The dog was treated with a combination of meglumine antimoniate and allopurinol for 60 days and showed clinical improvement. Two months after the end of treatment the dog was again referred because of relapse of leishmaniasis and the presence of a firm subcutaneous mass on the medial right thigh. Based on cytologic examination of fine needle aspirates of the mass, a diagnosis of large-cell lymphoma was made. Flow cytometry of tumor cells revealed gammadelta-T-cell lymphoma with a CD5+, CD3+, TCRgammadelta+, CD4-, CD8-, CD45RA+ immunophenotype. Using nested PCR, amastigotes were not detected in the neoplastic tissue. An association between leishmaniasis and hematopoietic tumors has been described rarely. gammadelta-T cells may be involved in the host response to this parasite, and prolonged antigenic stimulation and chronic immunosuppression (typical of leishmaniasis) play a crucial role in the etiopathogenesis of T-cell lymphoma.

Cutaneous leishmaniasis caused by *Leishmania infantum* transmitted by *Phlebotomus tobbi*.



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Transmission of cutaneous leishmaniasis (CL) caused by *Leishmania infantum* was studied in South Anatolia, Turkey. Small, non-ulcerating lesions prevailed and patients were negative in rK39 tests for antibody detection for human visceral leishmaniasis (VL). The most abundant sand fly species, *Phlebotomus tobbi*, was found positive for *Leishmania* promastigotes with a prevalence of 1.4% (13 out of 898 dissected females). The isolated strains were identical with those obtained from patients with CL and were typed as *L. infantum*. Phylogenetic analysis revealed similarity to MON-188 and a clear difference from the MON-1 clade. Blood-meal identification showed that *P. tobbi* feeds preferentially on cattle and humans. This finding, the high number of CL patients and relative scarcity of dogs in the focus, suggests that the transmission cycle could be anthroponotic.



Political and regulatory

31 July 2008

ASM and FIND to Partner on Strengthening Infectious Disease Diagnosis in Resource-Poor and Transitional Nations

The American Society for Microbiology (ASM) and FIND, Foundation for Innovative New Diagnostics signed a Memorandum of Understanding (MOU) on 31 July 2008 confirming their agreement to work in partnership for projects aimed at strengthening infectious disease diagnosis and service integration in resource-poor and transitional countries.

“The collaboration between the ASM and FIND will focus on strengthening the foundation for infectious disease diagnosis in resource-poor nations, providing novel diagnostics and laboratory expertise for tuberculosis (TB) and other infectious diseases that are appropriate to the unique circumstances found in developing nations,” says Steven Specter, Chair of the ASM’s International Laboratory Capacity Building Committee.

The MOU signed by the ASM and FIND arises from a pilot project that the two organizations have been conducting in Cote d’Ivoire since April 2008. In the pilot collaboration, FIND is developing and providing rapid, accurate and affordable TB diagnostic tests that can be effectively deployed in resource poor areas while the ASM is sending experts in clinical microbiology to provide training and technical assistance in the implementation of these new tests.

“We expect our partnership to reinforce the expansion and further development of quality-assured laboratory services as part of a larger framework of health system strengthening within resource-poor settings. Combating poverty-related infectious diseases with the development and rapid introduction of new diagnostic tests where they are most needed is why partnerships like the one between FIND and ASM are extremely important,” says Giorgio Roscigno, Chief Executive Officer of FIND.

The World Health Organization estimates that two billion people, or approximately one third of the world’s population, are infected with the bacteria that cause TB. Africa represents 28% of all TB cases and has the highest infection rate per capita. Tuberculosis is a leading cause of death among people living with HIV/AIDS and most of the world’s 200,000 HIV/TB deaths occur in Africa.

One of the primary challenges is that modern diagnostic tools for tuberculosis in industrialized countries are not suited to the infrastructure of resource poor nations where in some cases just getting reliable electricity is a problem. New, reliable diagnostics that can be effectively implemented in developing nations, as well as the expertise to use them, are urgently needed.

FIND is prioritizing tests that can be adopted at the lowest level of the health system, where a large number of patients first seek care. The technologies targeted for each level are intended to match the human resources available and the degree of complexity of the diagnostic question. Some of the diagnostic tools expected to be introduced into control programs will be incremental improvements on existing technologies while others will be radically new.



ASM, through its International Laboratory Capacity Building program, is ensuring the quality-assured implementation of new and existing diagnostic tools in resource-limited countries through onsite training and technical assistance. For this purpose, ASM is strengthening clinical microbiology laboratories by mobilizing its members to build human resource capacity for diagnosis of TB and other infectious diseases.

The MOU is expected to serve as the beginning of a close collaboration between the ASM and FIND towards maximizing their complementary strengths and contributions to global health efforts.

Spread of Vector-borne Diseases and Neglect of Leishmaniasis, Europe

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**Authors' institutions are national reference laboratories for leishmaniasis diagnosis and surveillance and rely on consolidated countrywide networks of collaborating clinical health centers. Diagnosis records are cross-checked with case notifications to provide more realistic figures and estimates. VL, visceral leishmaniasis; CL, cutaneous Leishmaniasis; WHO, World Health Organization.*

†WHO-EURO, WHO Europe, 1996–2005; <http://data.euro.who.int/CISID>.

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#Source: retrospective canine leishmaniasis database, Centre National de Référence des Leishmania.

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The risk for reintroduction of some exotic vector-borne diseases in Europe has become a hot topic, while the reality of others is neglected at the public health policy level. Leishmaniasis is endemic in all southern countries of Europe, with ≈700 autochthonous human cases reported each year (3,950 if Turkey is included). Asymptomatic cases have been estimated at 30–100/1 symptomatic case, and leishmaniasis has up to 25% seroprevalence in domestic dogs. Even though leishmaniasis is essentially associated with *Leishmania infantum* and visceral leishmaniasis, new species, such as *L. donovani* and *L. tropica*, might colonize European sand fly vectors. Drug-resistant *L. infantum* strains might be exported outside Europe through dogs. Despite this possibility, no coordinated surveillance of the disease exists at the European level. In this review of leishmaniasis importance in Europe, we would like to bridge the gap between research and surveillance and control.