Sleeping Sickness Rediscovered

S.C. Welburn, E. Févre and P. Coleman

Antwerp, Belgium
December 1998

This meeting focused on human sleeping sickness, which, despite improvements in diagnosis and control, has returned with a vengeance across central Africa.

Control Activities

National Programmes. Simon Van Niuewenhove (WHO, Geneva, Switzerland) outlined recent trends in the distribution of human sleeping sickness, and the current extent and scale of *Trypanosoma brucei gambiense* infection. The disease is often consigned as a problem of the past, but its resurgence was displayed through a series of brief reports of the situation in the worst affected countries [The Democratic Republic of Congo (RDC), Angola, Sudan and Uganda], where existing surveillance strategies are failing to capture the true extent of the current epidemic. For example, within the RDC, over 100000 new cases have been detected in the past six years – a quarter of these in 1997 alone. These cases were detected through an active surveillance system covering only 9% of the 13 million people considered to be at risk. War, civil unrest and human population movements are exacerbating the problem. The lack of sufficient funds and the logistical difficulties of working with deteriorating infrastructures in their respective countries were identified by Théophile Josenando (Angola) and Constantin Miaka Mia Bilenge (RDC) as critical constraints to the effective implementation of large-scale surveillance and early treatment activities.

International and Bilateral Agencies and Programmes. The response of international agencies to the resurgence of human sleeping sickness was also presented by representatives from WHO, European Union and Programme Against African Trypanosomiases (PAAT). A failure in the 'co-ordination' of national programmes with bilateral initiatives implemented through non-governmental organizations (NGOs) was considered, by Pierre Cattand (WHO, Geneva), to be detrimental to the efficiency of control operations. The new WHO Programme against Human Trypanosomiasis (established 1995) is seen as providing a cohesive and co-ordinated direction to public and private stakeholders, so optimizing the efficiency of disease control at the national level.

NGOs. The presentations of the various NGO activities perhaps highlighted the lack of centralized co-ordination. Although the field activities of NGOs such as Medische Missie Samenwerking (MEMISA), Médecins Sans Frontières (MSF) and Fond Medical Tropicale (FOMETRO) are invaluable in the control of human sleeping sickness, the epidemiological data generated from such work could be better used if it were made available through a centralized database. Regional initiatives [such as the Organisation de Coopération et de Coordination pour la Lutte Contre les Grandes Endémies en Afrique de l'Ouest (OCCGE)], and bilateral initiatives (including those funded by the French and Belgian governments) also play a role.

Control Tools

Diagnosis. Little has changed in the past decade regarding the technologies for trypanosomiasis control in the field. Philippe Büscher (ITM, Antwerp, Belgium) discussed improvements to existing diagnostic tools, emphasizing that efforts had concentrated on improving existing technologies such as blood smear, quantitative buffy coat technique (QBC), the card agglutination test for trypanosomosis (CATT), ELISA, microcentrifuge and *in vitro* inoculation kits. Veerle Lejon (ITM, Antwerp, Belgium) went on to discuss tools for stage determination in making a diagnosis. The two methods are the detection in the cerebro-spinal fluid (CSF) of: (1) trypanosome specific antibody; and (2) IgM. Laurent Penchenier [Organisation de Coopération et de Coordination pour la lutte contre les Grandes Endémies en Africa l'Ouest (OCEAC)] compared the CATT test with the latex agglutination test for *T. gambiense*, while Andrea Garcia [Office de Recherches Scientifiques Technquies Outre Mer (ORSTOM)] looked at the problem of aparasitaemic individuals (who remain seropositive) in longitudinal surveying: do these people contribute to focus persistence?

Vector control. The different methods of tsetse control in Southern Africa were summarized by Peter van den Bossche [Regional Tsetse and Trypanosomiosis Control Programme (RTTCP), Harare, Zimbabwe]. The objective must be to involve communities in small-scale projects with appropriate technology, where a social approach must accompany the technical and economic aspects of the programme. Joshua Okoth [Livestock Health Research Institute (LIRI), Tororo, Uganda] discussed human ecology and its relation to tsetse ecology, with reference to southern Uganda, suggesting that changing agricultural practices might result in peridomesticity in *Glossina fuscipes fuscipes*.

Treatment and follow-up. Simon Van Nieuwenhove (WHO, Geneva) stressed that to be most effective, a treatment regime should cure the patient quickly and avoid disability. With no ideal drugs available, and with commercial development of new compounds unlikely, as the market is too small to justify heavy investment in new drugs by the private sector, we are left with the old, toxic drugs. Production of suramin, most used in *T. b. rhodesiense* treatment, was to be stopped by the manufacturer, but requests from WHO and others managed to maintain production for humanitarian reasons. Even when drugs are available at the community level, they are often unaffordable (eg. Nifurtimox costs US$ 20 for 21 days treatment, and Eflornithine US$ 1070 for 14 days intravenous treatment). Donors are needed, as local communities cannot afford these drug costs. Christian Burri (Swiss Tropical Institute, Basel) presented a possible method of reducing the treatment time of late stage trypanosomiasis, by changing the administration schedule of melarsoprol, to reduce hospitalization costs; and Sylvie Bisser, Instituut voor Tropische Geneeskunde, Antwerp (ITG) presented the results of a clinical trial in the RDC that showed that melarsoprol could be combined with nifurtimox. Another clinical trial, in northern Uganda [Dominique Legros, Groupe Européen d'Expertise en Epidémiologie Pratique (EPICENTRE)] found Pentamidine to be less effective than expected against *T. b. gambiense*, which raised the question as to whether drug efficacy is focus specific. Finally, Ron Kaminsky (Swiss Tropical Institute, Basel) discussed the tolerance of *T. b. rhodesiense* to Eflornithine (DFMO).

Public Health. Public heath aspects of human sleeping sickness in terms of community health care provision, were discussed by Claude Laveissiere (OCEAC) who emphasized that local knowledge is an essential part of disease

PII: S0169-4758(99)01490-8

control. Paul Coleman (University of Edinburgh, UK and International Livestock Research Institute, Nairobi) had modelled the human public health impact of controlling the *T. b. rhodesiense* reservoir in cattle. He suggested that targeting chemotherapy in reservoir populations can reduce significantly the number of cases of human sleeping sickness. Joseph Ndung'u [Kenya Trypanosomiaisis Reseach Institute, (KETRI), Kenya] highlighted the techniques that can be bought into play to prevent an outbreak of sleeping sickness caused by *T. b. rhodesiense*.

Research

Parasite and Vector Biology. The neuropathology of sleeping sickness was discussed by Krister Kristenson (Karolinska Institute, Sweden) in terms of interactions between trypanosome-released molecules and the host defence system, and the effect that these might have on clinical features of the disease. Moiz Bakheit (Karolinska Institute, Sweden) outlined the bi-directional signalling processes believed to be responsible for immune and neuronal dysfunction in the host. A trypanosome lymphocyte-triggering factor has been identified that stimulates production of IFN-γ, which then stimulates parasite growth. Stefan Magez examined the association between TNF-α induction and disease severity in mice. TNF-α was shown to be crucial for control of parasitaemia in infected animals but did not influence survival. TNF-α also appeared to be directly trypanocidal.

Alain Buguet (CRSSA, France) showed, using human patients, that as sleeping sickness progresses in severity, major disruptions occur in circadian rhythms, and that release of cortisol, growth hormone and prolactin are all affected possibly due to the release of active compounds by the host and/or parasite. Victor Pentreath (Salford University, UK) showed that the gut is profoundly affected showing altered permeability in a mouse model of experimental sleeping sickness. Jerry Sternberg (University of Aberdeen, UK) used a mouse model to show that NO is involved in suppression of splenic lymphocyte function and dyserthropieisis, which leads to anaemia. Philippe Vincendeau (Université de Bordeaux, France) showed that macrophages from infected mice produce NO, which is required for parasite killing. Little is known about the mechanics of parasite death. Sue Welburn (University of Edinburgh, UK) showed that, after stimulation with a lectin to induce parasite death, several novel genes are upregulated in dying parasites. Knowledge of the hitherto unknown pathways that lead to cell death of these parasites might lead us to novel mechanisms of parasite elimination.

Progress in the development of the first generation genetic linkage map for *T. brucei* was presented by Mike Turner (Glasgow University, UK). Genetic mapping is important in that: (1) it compliments physical mapping and thus underpins genomic sequencing; and (2) indicates functional (as opposed to structural) genomic organization, which allows the analysis of the inheritance of traits of medical and biological interest (eg. human infectivity, drug resistance). The methodological approach relies on a large number of polymorphic markers generated using amplified fragment length polymorphism (AFLP) analysis. In the F1 progeny resulting from a cross between clones of two stocks of *T. brucei*, 16 linkage groups containing 155 polymorphic markers were identified.

Perhaps the most exciting new work presented was by Luc Vanhamme [Instituut voor Tropische Geneeskunde (ITG), Brussels], who described a series of elegant experiments, spanning a decade, which have led to the identification of the *SRA* gene that appears to confer resistance to human serum lysis. This gene for human serum resistance can be transferred to a previously susceptible parasite conferring the human resistance phenotype.

Phillipe Truc (ORSTOM, France) described how new methods of diagnosis for *T. b. gambiense* sleeping sickness have been applied. It appears that parasites present during an infection might not be clonal and that patients might be carrying more than one clone of *T. b. gambiense* at any given time.

Much interest has been shown in the composition of surface coats of trypanosomes, both bloodstream forms and procyclic forms. Mark Carrington (University of Cambridge, UK) gave an overview of the role of the VSG, the predominant surface protein on bloodstream form trypanosomes, as a protective molecule. Similarly, Isabel Roditi (Universität Bern, Switzerland) described the surface composition of procyclics (insect form parasites) and showed that this parasite has two types of coat protein, one of which ceases to be expressed after parasite establishment. Jan Van den Abbeele (ITM, Antwerp, Belgium) revisited differentiation in tsetse, and looked at the processes involved during maturation to mammalian infective forms in the salivary glands.

Drugs and Vaccines

C.C. Wang (University of California, USA) suggested that advances in our knowledge on the African trypanosomes in recent years provide greatly improved opportunities for discovering more efficacious and safer new anti-sleeping sickness drugs in the 21st century. Felix Kuzoe discussed DFMO and its use for treatment of *T. b. gambiense*. The drug is costly, and the regime unpleasant, but DFMO remains the only drug that can be used to treat melarsoprol-resistant strains, and a lower-cost oral formulation of the drug is needed. Jorge Atouguia (Instituto de Higiene e Medicina Tropical, Lisboa, Portugal) described the successful treatment of infected mice with topical applications of melarsoprol. Bernard Bouteille (IENT, France) described experimental cure of mice with Megazol. Louis Maes (Tibotec, Belgium) highlighted the pros and cons of screening for 'new' anti-trypanosomal drugs. Reto Brun (STI, Switzerland) discussed novel compounds that have been tested for anti-trypanocidal activity. KETRI are looking at a Chinese compound, SIPI 1029, using green monkey populations as models, but relapse has occurred in all treated animals. Novartis (Switzerland) is looking at CGP 40215, but have so far reported problems with stage II disease treatment, as the drug does not seem to cross the blood–brain barrier. Aciel Haemers [Universitaire Instelling Antwerpen (UIA), Belgium] took a different perspective, and, rather than screening drugs for activity, is attempting to synthesize a custom-designed compound with a specific biochemical target, and test it both *in vivo* and *in vitro*. Mike Barrett (University of Glasgow, UK) discussed drug uptake via nutrient transporters, where it should be possible to transpose the part of the active compounds that enable novel compounds to cross the parasite membrane. Curtis Powell (Applied Immunology Research and Development Corporation, USA) discussed the potential for using components of the flagellar pocket of trypanosomes as the basis of development of a vaccine for trypanosomiasis. Edith Authie [Centre de Coopération Internationale en Recherche Agronomique pour le Development (CIRAD), France] described a series of elegant experiments that show that immunization against cysteine proteases in cattle might assist the host in establishing resistance to the pathology normally associated with trypanosome infection.

WHO/TDR/CTD Round Table

Where do we go from here? A special session was chaired by Alvaro Moncayo (TDR, WHO, Geneva). The need for integration of trypanosomiasis control with the rest of the public health care system was emphasized by Constantin Miaka (RDC) and by Dawson Mbulamberi (Uganda). For this, cheap and reliable diagnostic tools, as well as a proper evaluation of the relative merits of the existing tests, were considered essential. The question of integrating trypanosomiasis control with other diseases for surveillance and treatment purposes, making full use of the public health care system, was raised by David Molyneux (Liverpool School of Tropical Medicine, Liverpool, UK); such integration would result in sustainability. The already important role of NGOs should be extended, and integrated to maximize limited resources. Until country representatives ask their governments to make trypanosomiasis control a priority, the international community will not take more interest.

Without good epidemiological data on the extent of the problem of sleeping sickness within a country, control measures cannot be effective. Claude Laveissière took an entomological viewpoint, suggesting that actual transmission sites, the relative importance of domestic and wild animals as reservoirs of infection, and the best risk indicators for disease contraction should all be investigated further. Mickey Richer [International Medical Corp (IMC), Los Angeles, USA] was concerned with the animal reservoir, in that diagnostic techniques for field testing animal infections are needed to determine the importance of this reservoir. Christophe Paquet expressed concern at the lack of knowledge of risk factors for disease development in seropositive patients. Simon Van Meirvenne concluded by emphasizing that sustainability must be kept in mind in drug, diagnostic test and vector control research.

Acknowledgements
Sleeping Sickness Rediscovered, organized by the Prince Leopold Institute of Tropical Medicine (ITM), Antwerp, Belgium, was held at Conference Center 't Elzenveld, Lang Gasthuisstraat 45, Antwerp, Belgium, 14–18 December 1998.

Susan C. Welburn *and Eric Févre are at the Centre for Tropical Veterinary Medicine (CTVM), The University of Edinburgh, Easter Bush, Roslin, Midlothian, UK EH25 9RG. Paul Coleman holds a joint appointment with CTVM and the International Livestock Research Institute, Nairobi, Kenya.* ***Tel: +44 131 650 6261, Fax: +44 131 650 6216, e-mail: sue.welburn@ed.ac.uk***

New Molecular Targets for Filariasis Drug Discovery

J. Lazdins and M. Kron

Geneva, Switzerland November 1998

The challenge of this meeting was to identify new chemotherapeutic targets from the myriad of parasite enzymes, receptors, genome data and metabolic pathways that have recently been identified. *Onchocerca volvulus, Wuchereria bancrofti* and *Brugia malayi* afflict more than 100 million people, worldwide. Filarial drug discovery research at WHO has three specific goals: (1) discovery of new macrofilaricides; (2) discovery of anthelmintics that effect sterilization of adult female worms; and (3) identification of new microfilaricides, to combat any emergence of ivermectin resistance. Clinically, new drugs must be safe, curative, effective in few doses, cheap and chemically stable! (K. Awadzi, Honhoe Hospital, Ghana).

Second Generation Drugs

Two groups of anthelmintics in common use today act at ligand-gated ion channels in the nematode nervous system. Levamasole exerts its effects at the nicotinic acetylcholine receptor (nAChR), and avermectins act as irreversible agonists at glutamate-gated chloride channels (Glu-CL). Nematodes possess a variety of receptors, receptor subunits and channels, and their expression can be developmentally regulated. Using model organisms, it may be possible to determine which genes encoding nAChR, Glu-CL and their subunits are expressed at specific life cycle stages. Functional expression of such ligand-gated ion channels can serve as assays to screen chemically modified avermectins for desirable pharmacokinetic properties. In *Caenorhabditis elegans*, co-expression of the β-subunit of Glu-CL with the α-subunit can produce ivermectin-potentiated glutamate-gated channels[2] (A. Wolstenhome, University of Bath, UK). The genetics of ivermectin resistance in *C. elegans* and veterinary parasites can lead to identification of resistance mechanisms and responsible genes, which have homologues in human filarial parasites[3] (W. Grant, Flinders University, Australia). Transgenic strains of *C. elegans* expressing filarial targets will help to identify crucial genes. Imidazoles exert their antiparasitic effects by targeting tubulin (G. Lubega, Makere University, Uganda). Benzimidazole (BZ) binds filarial tubulin and binding studies of tubulin of *Haemonchus contortus* show that binding constants of several new fluorinated derivatives of BZ correlate with anthelmintic potency at recommended therapeutic doses[4]. However, mutation in few amino acids is correlated with drug resistance to BZ and clinical development of one of the most promising new macrofilaricidal imidazole derivatives, UMB078, was terminated because of potential carcinogenicity in animals.

Novel Targets

Intracellular bacteria have been detected in most filarial worms. The phylogeny of these organisms, *Wolbachia* spp, has been shown to be congruent with the host phylogeny[5]. Since co-divergence of hosts and symbionts can result in evolutionary co-adaptations, as in the case of arthropods and their endosymbionts, several research groups suggest that by eradicating filarial endobacteria, parasites may also die (C. Bandi and C. Genchi, Universita degli Studia, Italy; and A. Bianco, Liverpool School of Tropical Medicine, UK). Evidence supporting this view is the observation that tetracycline therapy reduces the number of *Wolbachia* in filarial oocytes and embryos[6] while inhibiting filarial embryogenesis.

PII: S0169-4758(99)01483-0