First International Seminar on Non Tsetse-Transmitted Animal Trypanosomoses *:
Conclusions and Recommendations

Annecy (France), 14-16 October 1992

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Summary: The author presents the conclusions and recommendations of the First International Seminar on Non Tsetse-Transmitted Animal Trypanosomoses (NTTAT) held in Annecy (France) on 14-16 October 1992, at which twenty-two countries and five international organisations were represented. Recommendations were made on the topics examined during the six seminar sessions, namely:

- general aspects of NTTAT
- epidemiology and diagnosis
- chemotherapy
- basic research
- the current situation of NTTAT in various countries
- final conclusions.

This seminar enabled participants to collate the information currently available on NTTAT and to discuss the present state of research on these infections.


INTRODUCTION

This seminar was planned at the Tenth Meeting of the Office International des Epizooties (OIE) Ad hoc Group on Non Tsetse-Transmitted Animal Trypanosomoses (NTTAT) – formerly “T. evansi Group” – (Paris, 24 May 1989) and announced at the Seventh International Congress of Parasitology (ICOPA VII; Paris, 20-24 August 1990). The purpose of the seminar was to present the current state of research on trypanosome infections and to gather as much information as possible on these diseases.

* The use of the suffix “-osis” is recommended by the World Association for Advances in Veterinary Parasitology. Full details of this nomenclature were published in 1988, in a paper entitled “Standardised Nomenclature of Animal Parasitic Diseases (SNOAPAD)” (Vet. Parasitol., 29, 299-326).

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Sponsored by the OIE, the United Nations Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO), the seminar received material support from the Marcel Mérieux and Alphonse Laveran Foundations. Active support was also provided by other organisations, such as the Ministry of Cooperation and Development in France, the Overseas Development Administration (ODA) of the United Kingdom and the International Foundation for Science (IFS) of Sweden.

Twenty-two countries and five international organisations were represented at this seminar.

The scientific committee was composed of Prof. Th. Baltz (Laboratory of Immunology and Molecular Parasitology, University of Bordeaux II, France), Dr A.G. Luckins (Centre for Tropical Veterinary Medicine [CTVM], Edinburgh, United Kingdom) and Dr L. Touratier (Secretary of the OIE Ad hoc Group on NTTAT, Bordeaux, France).

Under the honorary chairman, Dr C. Mérieux, the seminar consisted of six sessions on the following topics:

- general aspects of NTTAT
- epidemiology and diagnosis
- chemotherapy
- basic research
- the current situation of NTTAT in various countries
- final conclusions.

Published below are the conclusions and recommendations prepared by the chairman and co-chairman of each session (named at the end of each section) and incorporating amendments proposed by the participants.

CONCLUSIONS AND RECOMMENDATIONS OF THE SESSIONS

I. GENERAL ASPECTS

Outbreaks of disease due to infection with *Trypanosoma evansi* and *T. vivax* may be affected by immunosuppression or intercurrent infections.

The relative importance of mechanical transmission of *T. vivax* in Africa is not known.

Experimental research on *T. vivax* is constrained by the restricted range of susceptible host species, the fragility of the parasite and the absence of suitable culture systems. In addition, it is uncertain whether results from research on rat-adapted stocks of *T. vivax* are applicable to strains of the parasite which are restricted to ruminants.

Much less is known of the biochemistry and molecular biology of *T. vivax* than of the *Trypanozoon* sub-genus.

The importance of wild animal reservoirs in the epidemiology of both *T. evansi* and *T. vivax* needs to be determined.
It is difficult to distinguish between *T. evansi* (*T. equinum*) and *T. equiperdum*.

Infections in ruminants with South American stocks of *T. vivax* appear to be less pathogenic under field conditions, possibly as a consequence of mechanical transmission.

When livestock breeds are improved and production becomes more intensive, *T. evansi* can become a serious problem (e.g. in pigs). This may be an important general consideration in livestock production programmes involving intensification.

The relative contributions of disease and nutrition to cachexia have yet to be determined, although it was noted that the tumour necrosis factor during infection may be involved in weight loss.

Immunosuppression can rarely be ascribed to trypanosomosis alone, as animals in the field are usually subject to intercurrent infections.

Studies have been made of the seasonality of *T. vivax* infection and the preponderance of overt infection in adult animals, raising such matters as seasonality in vector numbers and efficiency, cyclical versus mechanical transmission and possible maternal transmission.

There is some indirect evidence that isometamidium chloride affords a shorter period of prophylactic protection against *T. vivax* than against other species of trypanosomes. However, conclusive experimental data are lacking.

*T. equiperdum* may be more widely distributed (in Africa and elsewhere) than is currently recognised (potentially in donkeys and other Equidae), due to the difficulty in distinguishing between the trypanosome species and between the clinical signs of surra and dourine.

**Future research**

Research is needed on mechanically-transmitted trypanosomes in order to provide:

* a) A means of clearly differentiating between parasite species (together with a determination of disease distributions), including isolation of more *bona fide* field strains of *T. equiperdum*.

* b) Details on the economic importance of trypanosomoses in relation to productivity.

* c) Better knowledge of the epidemiology of these diseases:
  - extent of phenotypic (serological) differences between parasites
  - seasonality and the nature of vectors
  - determination of the existence of (wild) animal reservoirs
  - the role of waning (or impaired) immunity.

* d) Strategies for control:
  - coordinated basic research on the biology and molecular biology of the parasites
  - improvement and standardisation of diagnostic techniques
  - local management
  - chemotherapy
  - vector biology and control
  - vaccines (pan-trypanosome or species-specific solutions?).
Session I was chaired by A.G. Luckins (CTVM, Scotland, United Kingdom) with the assistance of P. Gardiner (International Laboratory for Research on Animal Diseases [ILRAD], Kenya).

II. EPIDEMIOLOGY AND DIAGNOSIS

Three papers were presented on disease diagnosis and four on parasite characterisation. The papers on diagnosis contained a comprehensive overview of methods currently in use, as well as details of those being developed. Subjects discussed included clinical and biochemical parameters, parasite detection, antibody detection, antigen detection and deoxyribonucleic acid (DNA) detection through amplification by polymerase chain reaction (PCR). The development of a simple field-oriented latex agglutination slide test for trypanosome antigen detection was described as a basis for specific diagnosis of \textit{T. evansi}, \textit{T. equiperdum}, \textit{T. vivax} and \textit{T. congolense}, and as a means of screening for all the major groups of pathogenic trypanosomes. PCR technology for diagnosis of \textit{T. evansi} in whole blood appeared to be both specific and sensitive and showed good correlation with serological results. The results could be read directly from ethidium bromide-stained agarose gels.

The papers on trypanosome characterisation included a presentation on the phenotypic and serological diversity among \textit{T. vivax} isolates from South America and Africa based on isoenzyme analysis, repetitive DNA sequence probe analysis, serological analysis and cross-protection studies. The data showed that stocks of \textit{T. vivax} from South America bore a phenotypic resemblance to West African \textit{T. vivax} but not to Kenyan isolates. Stocks of \textit{T. vivax} from Colombia appeared to belong to one major serodeme.

Predominant variable antigen types (VATs) have been used to provide serological characterisation of stocks of \textit{T. evansi} from Indonesia and to determine their significance in the endemic stability of \textit{T. evansi} in field infections. Based on a comparison of chromosome profiles of \textit{T. evansi} isolates, there was greater genetic diversity in stocks from Indonesia than in those from the Sudan. Studies on capybara (\textit{Hydrochoerus hydrochaeris}) in Venezuela revealed a high prevalence of \textit{T. evansi} infection in these animals as measured by both parasitological and serological diagnostic techniques; the capybara may thus serve as a potential reservoir of \textit{T. evansi} infection for livestock.

Diagnosis

The Woo technique has been shown to be a most suitable technique for parasitological diagnosis in terms of practicality, cost and sensitivity.

Enzyme-linked immunosorbent assay (ELISA) has proved to be a reliable laboratory test for antibody detection, and standardisation of the test procedure and antigen preparation will lead to further improvements. However, for field use, there is an urgent need for simple direct or indirect agglutination tests.

Antigen detection by ELISA has proven successful in the field, in an evaluation conducted under the auspices of the Joint FAO/International Atomic Energy Agency (IAEA) Division of Animal Health and Production. The applicable trypanosome species are \textit{Trypanozoon} spp., \textit{T. vivax} and \textit{T. congolense}. 
The latex agglutination versions of the tests for circulating antigens are being evaluated. These have a potential for field application due to their simplicity.

PCR technology is producing very promising results in the detection of parasite DNA, but this technique needs to be further simplified and standardised before it can be considered for field use.

Recommendations

The following recommendations were made:

a) Pilot versions of the new tests should be made available for evaluation via a network to be coordinated by the Secretariat of the OIE Ad hoc Group on NTTAT.

b) The standardisation and evaluation of diagnostic techniques would be greatly aided by the establishment of a central reference serum bank or a network of serum banks.

Characterisation of trypanosomes

It is now clear that *T. evansi* and *T. vivax* are genetically diverse with regard to antigen type repertoires (serodemes), karyotype (chromosome electrophoresis patterns) and isoenzyme patterns (zymodemes). They also have different levels of drug sensitivity. All these characteristics are rapidly changing, perhaps largely due to genetic recombination events.

Recommendation

Cryopreservation of reference strains and clones is essential. Documented cryostabilates should be available free of charge and upon request.

Session II was chaired by N. Van Meirvenne (Antwerp, Belgium) with the assistance of V.M. Nantulya (Brentec Laboratory, Kenya).

III. CHEMOTHERAPY

At present, the following drugs are available for the treatment of non tsetse-transmitted animal trypanosomoses: suramin (Naganol®), homidium (Ethidium® [bromide] and Novidium® [chloride]), isometamidium (Trypamidium®, Samorin®), diminazene aceturate (Berenil®), quinapyramine (Antrycide®) and melarsomine (Cymelarsan®). The last of these drugs (melarsomine MelCy) has recently been introduced and is very effective in camels (0.25 mg/kg i.m.). This compound may replace suramin, the production of which has been discontinued.

There has been no indication of significant residual effects in the tissues of cattle treated with isometamidium; the meat of such animals is devoid of toxicity, carcinogenicity and teratogenicity. The use of isometamidium for the control of *T. vivax* infections of cattle in South America can be successful only in combination with other measures, such as the use of insecticides and the isolation with chemoprophylaxis of new animals joining the herd. Resistance against all available drugs remains a problem. It seems that drug resistance may easily develop in immunosuppressed animals. This could also be the field situation with multiple *Trypanosoma* spp. infections.

Several accurate tests have been developed for the measurement of drug sensitivity. Among these are the pyruvate production test, the minimal inhibitory concentration test and the automated fluorescence determination test. Naturally-isolated strains of
T. evansi exhibit a wide variation in sensitivity to isometamidium. Cross-resistance between diminazene aceturate, melarsomine and pentamidine (but not suramin) has been described. The myristate analogue is a promising new compound which is believed to interfere with the biosynthesis of the glycolipid anchor of the trypanosome variant surface glycoprotein (VSG). Detailed knowledge of the three-dimensional structure and reaction mechanisms of target enzymes in the glycolytic pathway of the trypanosomes has led to the design and synthesis of a series of interesting new compounds which may lead to the development of new drugs.

Recommendations

The following recommendations were made:

a) The production of drugs which have been of vital importance in the past should not be discontinued.

b) Major priority should be given to the study of drug resistance and the modes of action of existing drugs. Research is required on the possible use of combination therapies to reduce the risks of developing drug resistance.

c) In view of the ease with which drug resistance may arise there remains an urgent need for research which may lead to the development of new drugs.

d) The appropriateness of melarsomine in the treatment of both human trypanosomosis and trypanosome infections in animals other than camels should be investigated.

Session III was chaired by F. Opperdoes (International Cooperation Programme, Research Unit for Tropical Diseases, Brussels, Belgium) with the assistance of J. Itard (Centre de coopération internationale en recherche agronomique pour le développement – Département d'élevage et de médecine vétérinaire [CIRAD-EMVT], France).

IV. BASIC RESEARCH

As all salivarian trypanosomes, including the non tsetse-transmitted trypanosomes, escape the defence mechanisms of the immune system of the host through antigenic variation, the identification of common non-polymorphic surface antigens represents a major goal in vaccine development. Basic research once again highlights the contribution of basic biology in identifying such target antigens. Indeed, the study of glucose metabolism and VSG expression sites has led to the discovery of two potential candidates: the glucose transporter and a flagellum-associated adenylcyclase.

Precise new tools have thus been developed for epidemiological surveillance and for the typing of trypanosomes, allowing detection even in insects. Due to the low number of isolates available, research on T. vivax and particularly T. equiperdum is falling behind that on T. evansi. This last species shows kinetoplastic DNA homogeneity, and strain differentiation depends on pulsed field gradient (PFG) karyotyping and repetitive sequence patterns.

Major differences in host immune systems may be responsible for interspecies differences in the outcome of disease. In this regard, the Camelidae merit more attention, as they generate antibodies devoid of light chains.
Ultimately, control of disease will depend not only on advances in basic research but also on methods used in livestock management. Better knowledge of the ecology and ethology of biting flies will contribute to low-cost measures for control of the insect vectors of \textit{T. evansi} and \textit{T. vivax}.

Session IV was chaired by Th. Baltz (University of Bordeaux II, France) with the assistance of R. Hamers (Free University of Brussels, Belgium)

\textbf{V. CURRENT DISEASE SITUATION}

Several topics discussed at this session, especially epidemiology, were also dealt with elsewhere. The conclusions reached were similar to those of the other sessions.

Infections with \textit{T. evansi} are of overriding importance in considering the health and well-being of camels.

Participants stressed the major economic impact of \textit{T. vivax} on cattle in Latin America, of \textit{T. evansi} on buffalo and cattle in Asia and on horses in Asia and Latin America. Although information was available on the disease situation in a number of Asian, American and African countries, information was generally scarce from countries where NTTAT are found.

It is necessary to define much more precisely the importance and economic impact of NTTAT, in order to be able to defend funding requests for research in this field and especially for the application of research results.

Several methods are suitable for the diagnosis of NTTAT. At present a clear distinction should be made between those methods which can be used in the field and those which will remain a laboratory research tool. The value of developing “pen-side” tests should not be overestimated; if field workers lack the resources necessary to send samples to a diagnostic laboratory, they will encounter similar problems in transporting test-kits to the pen.

More information is required on the mode, probability and risk of mechanical transmission of \textit{T. evansi} and \textit{T. vivax} under given circumstances.

Studies are needed to identify possible wild reservoirs of infection, such as the capybara in South America.

A better assessment is needed of the risk that mechanical transmission of trypanosomosis may persist in areas where tsetse flies have been eradicated.

Little information is currently available on the epidemiology and relative importance of \textit{T. equiperdum} and dourine.

Session V was chaired by G. Uilenberg (CIRAD-EMVT, France) with the assistance of P.H. Clausen (\textit{Gesellschaft für technische Zusammenarbeit} [GTZ]: Federal Agency for Technical Cooperation, Germany).
VI. CHEMOTHERAPY AND CONTROL METHODS: BRIDGING THE GAP BETWEEN RESEARCH AND DEVELOPMENT AND THE USER

Research and development must be targeted to meet the real needs of the livestock owner. The primary objective of applied research is to provide better tools with which to improve livestock productivity.

**Channels of communication**

There are two basic components to such communication:

a) Communication from the livestock owner to research and development personnel:
- collection and recording of practical field data
- dissemination of field data to research and development areas.

b) Communication from research and development personnel to the livestock owner:
- translation of research and development data into practical applications
- encouraging the user to appreciate the economic benefits of new treatment regimes.

**Systems**

Packages should be created for the livestock owner which comprise the following elements:
- chemotherapy (treatment methods and expertise)
- appropriate equipment
- guidance on animal husbandry.

**Resources**

There is a need for an increase in the following resources:

a) Financial resources
- from external sources, particularly donor institutions
- from internal sources, e.g. revolving funds.

b) Physical resources – an adequate level of physical resources must be ensured to provide the necessary veterinary services and appropriate support, e.g. diagnostic laboratories.

Session VI was chaired by E.W.G. Crouch (Independent consultant, United Kingdom).
CONCLUSIONS ET RECOMMANDATIONS DU PREMIER SÉMINAIRE INTERNATIONAL SUR LES TRYPANOSOMOSES ANIMALES NON TRANSMISES PAR LES GLOSSINES, ANNECY (FRANCE), 14-16 OCTOBRE 1992. – L. Touratier.

Résumé : L'auteur présente les conclusions et recommandations du Premier Séminaire international sur les trypanosomoses animales non transmises par les glossines (TANTG), tenu à Annecy (France) du 14 au 16 octobre 1992 et auquel ont participé vingt-deux pays et cinq organisations internationales. Ces recommandations concernent les thèmes étudiés lors des six sessions du Séminaire, à savoir :

- généralités sur les TANTG ;
- épidémiologie et diagnostic ;
- chimiothérapie ;
- recherche fondamentale ;
- situation actuelle des TANTG dans les différents pays du monde ;
- conclusions finales.

Ce Séminaire a permis de rassembler les informations existantes et de faire le point sur les recherches en cours sur ces infections.


Resumen: El autor presenta las conclusiones y recomendaciones del Primer Seminario internacional sobre tripanosomosis animales no transmitidas por glosinas (TANTG), celebrado en Annecy (Francia) del 14 al 16 de octubre de 1992 y en el cual participaron veintidós países y cinco organizaciones internacionales. Las recomendaciones están relacionadas con los temas estudiados durante las seis sesiones del seminario, a saber:

- generalidades sobre las TANTG;
- epidemiología y diagnóstico;
- quimioterapia;
- investigación fundamental;
- situación actual de las TANTG en los distintos países del mundo;
- conclusiones finales.

Este Seminario permitió reunir los datos existentes y hacer el balance de las investigaciones que se llevan actualmente a cabo sobre estas infecciones.