Report of the Twelfth International Meeting of the OIE Ad hoc Group on Non Tsetse-Transmitted Animal Trypanosomoses *

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L. TOURATIER **

Summary: Studies of Trypanosoma evansi strains of widely varied origin (Africa, Asia, South America) have continued, leading to two groups of diagnostic procedures, tested first in the laboratory and then in the field: the card agglutination trypanosomosis test and the enzyme linked immunosorbent assay to supplement microscopic examination of smears. A microscopy procedure for differentiating Trypanosoma species in smears was proposed. Diagnostic kits have again been made available free of charge to interested and suitably qualified research workers.

Several research workers have studied the lymphokines induced by T. evansi, and the question of the potential therapeutic use of these substances was raised.

Research workers in Asia were increasingly involved in various problems associated with T. evansi, including fundamental research and potential applications in the field. The epidemiology of T. evansi infections has been the subject of numerous research projects in Asia and South America.

Research on trypanocides has confirmed the activity of Cymelarsan®, which has since been marketed, the properties of the new derivative T46 (pharmacokinetic studies) and the value of in vitro and in vivo comparisons of currently available trypanocides.

The sphere of interest of the Group was extended to cover all forms of animal trypanosomosis not transmitted by tsetse flies.

KEYWORDS: Animal diseases - Conferences - Cymelarsan® - Diagnostic techniques - Lymphokines - Research - Trypanocides - Trypanosoma evansi Trypanosomosis.

* Enlargement of the Working Group on Trypanosoma evansi Infections.

** 228, boulevard du Président Wilson, 33000 Bordeaux, France.
INTRODUCTION

The Twelfth International Meeting on Non Tsetse-Transmitted Animal Trypanosomoses (an expanded version of the Group on Trypanosoma evansi Infections) was attended by twelve persons from eight countries and chaired by Dr W.N. Masiga. A brief review of the discussions was made available to all delegates attending the 59th General Session of the Office International des Epizooties (OIE), drawing attention to the following points:

- fundamental studies on strains of *T. evansi* from South-East Asia, West Africa, East Africa and South America have led to the introduction of DNA probes, a study of gene regulation in *T. evansi*, and a study of host-parasite interactions, particularly with respect to the tumour necrosis factor (TNF)
- evaluation of diagnostic techniques currently recommended by the OIE has been extended to detection of *T. evansi* antigen in dromedaries in Africa and in buffaloes in South-East Asia by means of enzyme-linked immunosorbent assay (ELISA); *T. evansi* has been identified in blood smears using a kinetoplastic DNA (kDNA) probe; a card agglutination trypanosomosis test (CATT) has been made available free of charge; the isoenzymes of *T. evansi* strains have been examined
- new epidemiological data on *T. evansi* in Asia and on *T. minasense* in South America
- information on new trypanocides included melarsomine (MelCy, Cymelarsan®) which is now being marketed and T₄₆ which is currently under development
- extension of the working field of the *T. evansi* Group to cover other animal trypanosomoses not transmitted by tsetse flies
- participation by members of the Group in international meetings on trypanosomosis: Yamoussoukro, Côte d'Ivoire (October 1991); Antwerp, Belgium (December 1991); Dubai, United Arab Emirates (February 1992) and in organising the First International Seminar on Non Tsetse-Transmitted Animal Trypanosomoses (Annecy, France, 14-16 October 1992).

INTERIM REPORT OF THE SECRETARY GENERAL

The report of the Eleventh Meeting of the Group, held on 17 May 1990, was published in the OIE Scientific and Technical Review (20) after incorporating corrections received from the participants.

Information derived from the scientific literature

The main events concerning *T. evansi* recorded between May 1990 and May 1991 referred to work carried out in Asia:

- in the People's Republic of China, trypanosomes were propagated in cell cultures (8) and a rapid sequencing technique developed using an appropriate probe (17)
- in India, on infection in camels (18) and experimental infection of buffalo (21)
  in Iraq, on the epidemiology of trypanosomoses (7)
- in Japan, on the demonstration of intracellular structure (5)
- in Malaysia, on experimental infection of rabbits (23)
- in Thailand, on the use of a DNA probe specific for *T. evansi* (22).

Some collaborative research was undertaken by laboratories in Asia and Europe:
  - collaboration between China and France on analysis of the kDNA of many Chinese strains (15, 16) and *in vitro* and *in vivo* comparisons of the activity of existing trypanocides (including recent compounds) (24, 25)
  - collaboration between China and Switzerland on the culture of *T. evansi* in cells and axenic media (9).

In Africa, the sensitivity of ELISA was examined in Burkina Faso (2) and Kenya (11) on various trypanosomes of animals, including *T. evansi*.

Collaborative research between research workers in Africa and Europe comprised:
  - collaboration between Algeria and France in an epidemiological survey which proved the absence of *T. evansi* in Algerian dromedaries (3)
  - collaboration between Tunisia and Italy in an investigation of goats and dromedaries in southern Tunisia, which revealed some positive reactions of dromedaries to the complement fixation test with *T. equiperdum* antigen (4).

In Kenya, the trypanocide MelCy (Cymelarsan®) was examined for *in vitro* and *in vivo* activity against strains of *T. evansi* and *T. brucei brucei* sensitive and resistant to other trypanocides (collaboration between the German Agency for Technical Cooperation [GTZ], and the laboratories KETRI and ILRAD) (26).

In South America, experimental infection of guinea pigs was studied from the immunological aspect in Brazil (14) while infections of pigs and horses were studied in Argentina (10).

In Europe, the formation of interleukin in ponies inoculated with a soluble fraction of *T. evansi* (1) and combined chemotherapy (6) were studied.

In the United States of America research was carried out on trypanocides, using molecular biology techniques (19).

Other information

Various technical information was acquired through correspondence, including:
  - use of ELISA in Venezuela to detect *T. evansi* in horses, revealing a prevalence rate of 70-80% among wild horses in southern Venezuela
  - comparisons in Vietnam between examination of blood smears, mouse inoculation and ELISA for the diagnosis of *T. evansi* in cows and dairy buffalo in southern Vietnam (resulting in a preference for ELISA)
  - attempted immunisation of rabbits against *T. evansi* in the Jilin Province of northern China
- chemotherapy trials on equine surra in the Philippines
- detection of *T. evansi* epimastigotes in larvae of *Cephalopina titillator* infesting dromedaries affected by surra in Egypt, and attempts to characterise the corresponding antigens.

Other information was obtained at the VIIth International Congress for Parasitology (ICOPA VII), held in Paris on 20-24 August 1990. Over 2,000 communications and poster displays on all aspects of parasitology were provided by approximately the same number of participants. A “Round Table” on trypanosomes of animals in Africa was held on 24 August, with Dr S.M. Touré as chairman, bringing together many research workers interested in *T. evansi*.

Other meetings included an international meeting on diseases of Camelidae, in Tobruk (Libya) in December 1990, and a refresher course on the diagnosis of *T. evansi* infection, in Bogor (Indonesia) in November-December 1990 (supported by Bhutan, India, Indonesia, Malaysia, Myanmar, Nepal and Vietnam).

**COURSE OF THE MEETING**

Research on the nucleic acids of *T. evansi* and practical applications

Prof. N. Van Meirvenne (Antwerp Institute of Tropical Medicine) reported two research projects undertaken by his colleagues in Brussels:

- General research on the parasitology and biology of *T. evansi* was undertaken by E. Bajyana-Songa and R. Hamers, particularly the development of DNA probes and evaluation of the “Ro Tat 1.2.” CATT for epidemiological use. Also gene regulation associated with the differentiation of procyclic trypanosomes, immunological aspects of cloning of the gene for trypanothione reductase of *T. evansi*, and immunological studies on host-parasite relationships (roles of gamma interferon [INF] and TNF) and on the immunology of the parasite.

The role of TNF in mortality associated with treatment for trypanosomosis (from cytokine liberation) was investigated by R. Lucas, E. Bajyana-Songa, S. Magez, A. Darji, P. de Baetselier and R. Hamers.

Dr M.L. Dia then presented the results of his work done with G. Bourdoiseau and M. Gauthey at the Lyons Veterinary School on the role of TNF in experimental *T. evansi* infection in rabbits, which showed that *T. evansi* induces a “TNF effect”, which is not transferable passively, and which can be measured by the extent of hypertriglyceridaemia.

In the same field, Dr J.S. Ahmed commented on an article which he published with F. Horchner (1), demonstrating the influence of soluble antigens of *T. evansi* on interleukin 2 production, and the expression of interleukin-2 receptors by circulating lymphocytes in ponies.

L. Touratier asked if there were potential therapeutic applications of these lymphokines and Prof. M. Thiago de Mello replied that he was aware of experiments for this purpose conducted in Brazil, using a biological product containing a TNF derived from *T. cruzi*, but the experiments had ceased. However, new lines of research arising from the study of trypanosome cytokines should be encouraged.
Information concerning the field use of diagnostic kits

Prof. Van Meirvenne referred to a document about the CATT for *T. evansi* which was now available as a ready-to-use kit, thanks to collaboration between the Institute of Molecular Biology at the Free University of Brussels and the Antwerp Institute of Tropical Medicine. Each kit contains a box of reagents for 250 tests and a box of the equipment needed (syringes, bottles, special plastic cards, etc.). Trial kits may be obtained by specialists upon request from:

N. Van Meirvenne & E. Magnus
Institute of Tropical Medicine
Nationalestraat 155, B-2000 ANTWERP, Belgium
Telephone: (32.3) 247 6366/68 – Fax: (32.3) 247 6373.

Dr Masiga presented a communication by J.N. Waitumbi and V.N. Nantulya, received from Kenya, on the detection of antigen in the diagnosis of *T. evansi* by ELISA and the reliability of the test in infected camels. These authors concluded that the method is more reliable than microscopic examination of circulating blood for parasites when used from the eighth day of multiplication of trypanosomes in the blood.

A communication from Vietnam was presented on behalf of Ho Thi Thuan, Phang Hoang Dung, Le Thu Ha and Nguyen Ha Lien, entitled “Results obtained with ELISA in the diagnosis of *T. evansi* infection in cattle and buffaloes in South Vietnam”, which confirmed the reliability of the technique in comparison with conventional methods (blood smear and mouse inoculation).

A confirmation of this study was provided by Dr Luong To Thu, in his paper “Comparison of different methods for detecting antibodies to *T. evansi* among buffaloes in Vietnam (northern region)”. Four procedures were compared (ELISA, immunofluorescence, Ro Tat 1.2. CATT and direct agglutination on slides) and preference was given to CATT because of its simplicity and low cost.

The reliability of CATT did not necessarily apply to all zones of Vietnam, because Ro Tat 1.2. had not yet been tried with all the variable antigen types (VAT) of *T. evansi* present in Vietnam.

There were two other presentations concerning diagnosis:

- “Isoenzyme characterisation of eight strains of *T. evansi* isolated from various species of animals in China, and one strain of *T. b. brucei*” by Shen Jie, Ren Jian Zhen and Yu Fei Wang from the Shanghai Parasitology Laboratory. (The eight strains of *T. evansi* came from cattle, buffaloes, mules and horses in seven provinces.)

- “Use of a non-radioactive probe for identifying *T. evansi* isolates on microscope slides” by Drs Bajyana-Songa and Hamers. A probe obtained by cloning a fragment of kDNA minicircles was suitable for highly specific identification of *T. evansi* (kinetoplastid strains) to the exclusion of all other trypanosome species, including *T. equiperdum*.

New epidemiological findings

Dr Luong To Thu described “Some characteristics of *T. evansi* infection of buffaloes in Vietnam”. A comparison of the last three decades revealed:
– decline in mortality rates from between 50% and 60% in 1960-1970 to 10% in 1981-1990
– decline in the extent of parasitaemia
– systematic diagnosis from 1981 onwards: 25-30% of animals positive.

The constant improvement in the current situation in Vietnam may be compared with the account presented by Dr E.A. Wells to the Second Meeting of the Working Group in 1984 (12) and the report by Prof. D. Zwart to the Fourth Meeting of the Group in 1985 (13).

Trypanocides

Three communications were presented:

“Cymelarsan®: a new treatment for \textit{T. evansi} infections in camels. Results of two clinical trials in Sudan” by Dr F. Van Gool.

(Note that the main features of this organic arsenical have been presented at earlier meetings of the Working Group. Following trials on several hundred dromedaries in Niger, Ethiopia, Kenya and Somalia, a clinical trial was conducted in two Sudanese herds of camels. Cymelarsan® had a reliable action at 0.25 mg/kg body weight by intramuscular injection, which lasted for three months in the absence of reinfection.)

“Assay of the new trypanocide \textit{T}_{46} in biological specimens by reverse HPLC [high performance liquid chromatography], and its pharmacokinetics in mice” by Li Dong Feng, Shen Jie and Wang Ming Jie, of the Institute of Veterinary Parasitology in Shanghai.

(The authors provide no information on the chemical family to which \textit{T}_{46} belongs. The active dose was about 1 mg/kg body weight, and its chemotherapeutic index was between 2 and 3. Reverse HPLC was capable of detecting 2.4 µg/ml in tissues and 0.1 µg/ml in blood plasma. Pharmacokinetic studies in mice showed that \textit{T}_{46} passed the blood brain barrier with difficulty unless a second injection was given seven days after the first. A withdrawal period of 35 days was proposed for meat for human consumption.)

“The \textit{in vitro} and \textit{in vivo} sensitivity of various strains of \textit{T. evansi} and \textit{T. equiperdum} to current trypanocides: suramin, diminazene, quinapyramine, isometamidium and \textit{MelCy} (Cymelarsan®)” by Z.Q. Zhang, C. Giroud and Th. Baltz (work performed at Bordeaux University II).

(Determination of the thresholds of sensitivity \textit{in vitro} in the axenic media described by Th. Baltz and colleagues, and \textit{in vivo} in mice, using eleven strains of \textit{T. evansi} and one strain of \textit{T. equiperdum} (South Africa). Resistance developed to some trypanocides, and this could be overcome by using different trypanocides. Cross-resistance was studied. \textit{In vitro} and \textit{in vivo} techniques for testing new trypanocides were compared. It was concluded that the \textit{in vitro} method was suitable for screening new trypanocides and rapid detection of drug-resistant strains.)
Other topics

Professor Thiago de Mello drew attention to a brief communication entitled "Trypanosoma minasense in a squirrel monkey (Saimiri ustus) in Rondonia State, Brazil".

This trypanosome was identified in Giemsa stained blood smears from squirrel monkeys captured as a result of flooding around Porto Jelho in north west Brazil. This is the first identification of the trypanosome in this host. Investigations to establish the presence of trypanosomes in wild animals are being carried out by a Wildlife Study Centre in Rio de Janeiro.

Dr G. Saint-Martin of the Institute of Animal Husbandry and Veterinary Medicine in Tropical Countries (IEMVT), France, presented an illustrated document about the Coordinating Unit for Camel Production.

EXPANSION OF THE ACTIVITIES OF THE WORKING GROUP

After submission of a review of the work of the International Working Group on Trypanosoma evansi Infections to the Director General of the OIE and the OIE Administrative Commission, as decided at the Eleventh Meeting of the Group in May 1990, it emerged that:

– on the one hand, the Group could become better integrated in the major orientations of OIE

– on the other hand, its activities could be extended to all animal trypanosomoses not transmitted by tsetse flies.

Consequently the terms of reference were defined jointly with the Director General, accepted by the Administrative Commission and finally approved by the OIE Permanent Committee at its plenary meeting on 16 May 1991. The Group will be known as:

"The OIE Ad hoc Group on Non Tsetse-Transmitted Animal Trypanosomoses" (NTTAT), attached to the FMD and Other Epizootics Commission of the OIE.

The full text of the terms of reference of the NTTAT Group is provided in the Appendix.

FORTHCOMING MEETINGS

First International Seminar on Non Tsetse-Transmitted Animal Trypanosomoses (Annecy, France, 14-16 October 1992)

This seminar is being supported by the Marcel-Mérieux Foundation and the Laveran Foundation, with sponsorship by the United Nations Food and Agriculture Organisation (FAO) and the OIE, and the participation of the World Health Organisation (WHO).
The principal topics on the agenda are:

- economic impact of these infections
- epidemiology (diagnosis, epidemiological surveys, characterisation of strains, vectors, reservoirs)
- disease situation in individual countries
  role of international organisations
  chemotherapy
- fundamental research on the parasites
- control methods
  other topics.

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Appendix

APPOINTMENT OF AN OIE AD HOC GROUP ON NON TSETSE-TRANSMITTED ANIMAL TRYPANOSOMOSES

Background

On account of the success of the work carried out by the International Working Group on *Trypanosoma evansi* Infections between 1983 and 1990, the OIE Administrative Commission decided, at a meeting in February 1991, to extend the scope of this Working Group to all non tsetse-transmitted animal trypanosomoses around the world. Designated as an ad hoc expert group, the Group will be attached to the OIE Foot and Mouth Disease and Other Epizootics Commission.

The aim of the Group will be to study the problems related to non-cyclically transmitted trypanosomoses in animals.

The Group will meet once a year in May.

Terms of reference

The terms of reference for the Ad Hoc Group on Non Tsetse-Transmitted Animal Trypanosomoses (NTTAT) are to study, discuss and inform OIE Member Countries of the following points:

a) the pathological and economic impact of NTTAT in Africa, Asia and America

b) the possible interference of NTTAT with other diseases and immune responses to vaccinations for other diseases (e.g. foot and mouth disease and haemorrhagic septicaemia)

c) the reliability of diagnostic tests, the costs involved and the ease with which trypanosomes may be differentiated from each other (e.g. *T. evansi/T. brucei, T. evansi/T. equiperdum, T. evansi/T. vivax*, etc.)
d) the similarities between strains, isolates and stabilates of different origin, so that possible differences in their genetic, immunological and biochemical characteristics may be determined

e) the problems of chemoresistance to trypanocidal drugs

f) the current research into new drugs and drug evaluation

g) new means of control of NTTAT.

The relationship between the Ad hoc Group and other organisations

The work of the Group will be conducted in close cooperation with other bodies which are working in related fields, for example:


b) The FAO Trypanosomosis Unit

c) The WHO Tropical Diseases Division Steering Committee on Chemotherapy, Immunology and Pathology of African Trypanosomosis

d) The International Atomic Energy Agency (IAEA).

**REFERENCES**


