Eleventh International Meeting on Trypanosoma evansi: Report of the Working Group *
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Summary: Three objectives have been achieved by the Working Group since its creation in 1983:

— more detailed information (and consequently better awareness) of zones infected by T. evansi;
— refinement of diagnostic techniques and the development of test kits suitable for field use;
— development of a new synthetic trypanocide.

Exchange of strains between specialist laboratories should be encouraged in order to compare isolates from Africa, Asia and South America by using current techniques of biotechnology, and to open the way to better knowledge of the pathogenicity of T. evansi and to the discovery of effective prophylactic measures.

Research reported to the meeting was concerned with the taxonomy and genetics of T. evansi, the cloning and sequencing of nuclear and kinetoplast DNA (kDNA), chromosomal polymorphism in relation to antigenic variation, the detection of lymphocytic interleukin 2 and its receptors in infected ponies, the use of monoclonal group antibodies to detect T. evansi, and the importance of natural receptivity of the host.

A text concerning the diagnosis of surra (T. evansi infection) was drafted and forwarded to the Office International des Epizooties (OIE) for incorporation in the OIE Manual of recommended diagnostic techniques and requirements for biological products. A concise dossier was presented on the pharmacology and pharmacodynamics of the new trypanocide, melarsomine (proprietary name Cymelarsan®). The recommended active dosage was 0.25 mg/kg body weight, given as a single intramuscular or subcutaneous injection. Laboratory tests were also reported with ronidazole, demonstrating its trypanocidal activity in rats.

KEYWORDS: Africa - Asia - Cattle - Diagnosis - ELISA - Horse - Interleukin - kDNA - Melarsomine - Monoclonal antibody - Ronidazole - South America - Synthetic trypanocide - Taxonomy - Trypanosoma evansi.

* Since the 59th General Session of the OIE, renamed: the OIE Ad hoc Group on Animal Trypanosomoses Not Transmitted by Tsetse Flies.

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INTRODUCTION

The Eleventh Meeting was attended by twelve participants from five countries and chaired by Dr J.W. Thomson of the Organisation of African Unity (OAU) and the Inter-African Bureau for Animal Resources (IBAR, Kenya) and advisor to Dr W.N. Masiga. A brief summary of the discussions was made available during the 58th General Session of the OIE. The following points were raised in the course of the Meeting:

- the position of the Working Group on the binomial designation of species of the subgenus *Trypanozoon* (request to the International Commission for Zoological Nomenclature);
- new basic research (cloning and sequencing *T. evansi* strains from different geographical regions), and a renewed request for the exchange of strains between specialist laboratories (standardisation of diagnostic antigens and biochemical studies to open the way to new trypanocides);
- contribution to the *OIE Manual of recommended diagnostic techniques and requirements for biological products*;
- new epidemiological findings;
- new trypanocides;
- recent research conducted in various laboratories.

This outline of discussions did not attempt to summarise the activities of the Group since its creation in 1983, nor did it refer to the interim report of the Secretary, presented as two separate working documents and included in the proceedings presented below.

SUMMARY OF ACTIVITIES OF THE WORKING GROUP

In accordance with the objectives established at the First Meeting in May 1983 during the 51st General Session of the OIE (23), the Group has met:

- seven times during OIE General Sessions in Paris between 1983 and 1989;
- three times during meetings between the OAU, IBAR and the International Scientific Committee for Trypanosomiasis Research and Control (ISCTRC) devoted to African trypanosomoses, held in Harare in 1985, Lomé in 1987 and Mombasa in 1989;
- once during the 5th International Conference of Institutes of Tropical Veterinary Medicine in Kuala Lumpur in 1986.

In addition, members of the Group have made use of other occasions to meet, notably at the World Veterinary Congress in Montreal in 1987, and the 13th Conference of the World Association for the Advancement of Veterinary Parasitology in Berlin in 1989.

After each meeting, a draft report was compiled by the Secretary and sent to participants for corrections and/or amendments, before publication in the *Scientific*
and Technical Review of the OIE between 1984 and 1990 (in the three official languages of the organisation).

The following results have been achieved:

- collection of much epidemiological data for refining and expanding the distribution map for *T. evansi* in Africa and Asia;
- free distribution of diagnostic kits for detecting *T. evansi* in the field, through the participation of the following specialist laboratories:
  a) Kenyan Trypanosomiasis Research Institute (KETRI) and the International Laboratory for Research on Animal Diseases (ILRAD) (Kenya)
  b) Bogor (Indonesia)
  c) Free University of Berlin (Federal Republic of Germany)
  d) Institute of Animal Husbandry and Veterinary Medicine in Tropical Countries (IEMVT) (France)
  e) Institute of Tropical Medicine in Antwerp and the Institute for Molecular Biology of the Free University of Brussels (Belgium),

and employing various techniques: direct microscopy improved by using a detergent, slide agglutination test, enzyme-linked immunosorbent assay (ELISA) for antibodies or antigens, and the card agglutination test with a cold probe (CATT);

- greater interest by many countries in a more detailed study of *T. evansi*, either in relation to camel breeding (Morocco, Egypt, Ethiopia, Kenya, the Sudan, India, USSR) or in relation to other animals, e.g. buffaloes, cattle, goats, asses, horses and pigs (India, the People’s Republic of China, Indonesia, Malaysia, Thailand, Vietnam, Venezuela);

- research, synthesis and development of the first new trypanocide for thirty years, melarsomine (Cymerlarsan®) – active against *T. evansi* and other members of the genus *Trypanozoon* and recommended for use in camels, buffaloes and cattle;

- favourable laboratory tests of ronidazole (already used in veterinary therapy) against *T. evansi*, together with two other candidate products known as “IMOL 881” (Belgium) and “T 46” (People’s Republic of China);

- exchange of information with the International Commission on Zoological Nomenclature concerning possible revision of the nomenclature of the genus *Trypanozoon*.

**INTERIM REPORT OF THE SECRETARY GENERAL**

The report of the Tenth Meeting of the Group on 24 May 1989 was published in the *Scientific and Technical Review* of the OIE, 1990, Vol. 9 (4), 1209-1219, after incorporation of corrections submitted by the participants.

**Information published in the literature**

The period between May 1989 and May 1990 was marked by the increasing interest of Asian countries in *T. evansi*. 
In particular, much research was conducted:

- in **India**, on the subcellular structure of *T. evansi* (16), antigen formation (17), and on serological modifications (20) and lymphocytic changes (21) in infected buffalo calves;

- in **Indonesia**, on the place of *T. evansi* among other blood parasites of cattle (15) and survival of *T. evansi* in samples of buffalo blood (22);

- in **Kuwait**, on the characterisation of *T. evansi* by enzymic electrophoresis (2);

- in **Malaysia**, on the prevalence of *T. evansi* among cattle (19);

- in **Taiwan R.O.C.**, on experimental infection of dogs (11, 12) and a comparison of ELISA and the haemagglutination-inhibiting antibody (HIA) test for diagnosing *T. evansi* infection in goats (10);

- in the **Philippines**, on the epidemiological role of the crab-eating macaque (*Macaca fascicularis*) (8);

- in **Thailand**, on the use of CATT for detecting *T. evansi* infection, in collaboration with the Institute for Molecular Biology of the Free University of Brussels (4).

The following research has been conducted in Africa:

- in **Somalia**, on the seasonal incidence of blood parasites, including *T. evansi* in cattle (7), and trypanosomes (and their vectors) in camels (9).

- in **Ethiopia**, the testing of Cymerlarsan® on camels infected with *T. evansi* (24).

- in the **Sudan**, in collaboration with researchers at the Centre for Tropical Veterinary Medicine (CTVM) in Edinburgh, United Kingdom, on immuno-enzyme techniques (ELISA) for diagnosing *T. evansi* infection in dromedaries (18).

At the same time, research has continued in European countries on the detection of *T. evansi* infection. There have been reports on the use of CATT tests for serological diagnosis of *T. evansi* (3, 5), on their suitability for use in tropical countries (14) and on antigenic variability of *T. evansi* in rabbits (6).

**Other information**

With the help of the French Institute for Research in Cooperative Development (ORSTOM) in Montpellier (France), links have been established with **Bolivia** for a Franco-Bolivian research programme on substances of vegetable origin active against trypanosomes and species of *Leishmania*. The Free University of Brussels has been consulted by the **Colombian** Veterinary Services on the possibility of controlling *T. evansi* infection in llamas.

Through the CTVM at Edinburgh, contact has been made between the Secretary of the Group and the Division of Biological Sciences at Simón Bolívar University, Caracas, on two major projects involving *T. vivax* and *T. evansi* infections in cattle in **Venezuela**. ELISA is already in use in Venezuela to diagnose *T. evansi* infection in horses. Research is also being conducted by this Division on cloning certain strains, their preservation and biochemical properties and on modifications to diagnostic tests.
In Egypt, tests for *T. evansi* infection in dromedaries and buffaloes are being developed by the Department of Parasitology of the Giza Veterinary Faculty, based on diagnostic kits (microscope identification with the use of a detergent, and/or agglutination-lysis on slides) kindly supplied by the Tropical Institute of Antwerp.

**MINUTES OF THE MEETING**

**Nomenclature of the subgenus *Trypanozoon* in relation to other members of *Trypanosomatidae***

Considering proposals made by the Tenth Meeting of the Group, the International Commission on Zoological Nomenclature has made a detailed reply, three paragraphs of which are worth citing:

"[...] if the two names *Trypanosoma evansi* and *T. brucei* are true synonyms, the International Code requires that these parasites be known by the older of the two names, i.e. *T. evansi.*"

"[...] it follows that use of the trinomial designation *T. brucei evansi* is not in accordance with the International Code, and that the correct designation is *T. evansi evansi.*"

"[...] if it is recommended that the specific name *evansi* is applied to a *Trypanozoon* transmitted mechanically by insects, the name *equiperdum* to a *Trypanozoon* transmitted by the genital route in equines, and the name *brucei brucei* to a *Trypanozoon* having cyclic development, transmitted by the tsetse fly and not infectious for human beings [...], the consequence is a nomenclature based, at least partly, on the mode of transmission of the parasite rather than its morphological or physical characteristics [...]. A solution to this dilemma would be to accept that the considerable difference between the biological cycles of *T. brucei* and *T. evansi* (presence or absence of cyclic development in a vector) is sufficient to assure specific differentiation [...]."

After discussion, it was decided that the system of nomenclature presented at the previous meeting was the most appropriate.

**Basic research**

Professor Th. Baltz reported the results of cloning and sequencing obtained in his laboratory (Bordeaux University II) with Y.C. Ou on four strains of *T. evansi* from buffaloes and horses in the Philippines, the People's Republic of China (Jilin and Shanghai provinces) and Ethiopia.

Comparison of kinetoplastic DNA (kDNA) sequences demonstrated the heterogeneity of the strains (within the homogeneous group which characterises *T. evansi*), and probes specific for *T. evansi* were identified.

Professor R. Hamers reported a similar observation with strains from buffaloes and pigs in Thailand. Dr E. Bajyana-Songa used seven VSG (variant surface glycoprotein) probes to confirm by nuclear DNA analysis the finding derived from kDNA that all strains of *T. evansi* are members of a group which is very homogeneous in terms of sequential divergence. Moreover, their work showed that *T. evansi* is more
closely related to West African *T. brucei* brucei and to *T. brucei* gambiense than to other African trypanosomes.

Professor N. Van Meirvenne confirmed the findings mentioned above and stressed the additional results: heterogeneity between individual strains within the same group of trypanosomes.

**Other activities at specialist laboratories**

*Exchange of strains*

Very few exchanges of strains between specialist laboratories have been reported since the formation of the Group. In the opinion of Dr L. Touratier, supported by Professor D. Mehlitz, this was a major gap to be filled. Comparison of numerous *T. evansi* strains of different geographical origin could lead to important developments:

- standardisation of diagnostic antigens;
- studies of variability in drug resistance in different geographical areas, with reference to a single trypanocide or several trypanocides, utilising the latest techniques in biotechnology, which could elucidate failures in certain chemotherapeutic and/or chemoprophylactic schemes, and might lead to new active compounds.

*Evaluation of diagnostic techniques and other research*

Dr G. Saint-Martin of the Institute of Animal Husbandry and Veterinary Medicine in Tropical Countries (IEMVT) reported briefly on attempts to detect *T. evansi* in cattle and dogs in French Guiana by means of CATT supplied by the Institute for Molecular Biology of the Free University of Brussels. Previously, detection had relied on the indirect immunofluorescence (IIF) test, which gave negative results. Some of these were false negative results, for CATT made it possible to detect positive animals more accurately.

Professor Mehlitz summarised research conducted on *T. evansi* at the Institute of Parasitology and Tropical Veterinary Medicine of the Free University of Berlin over the last five years:

- the influence of *T. evansi* infection on lymphocyte function in ponies (study of the Fc receptor in peripheral blood, inhibition of mitogenic T cells, detection of specific antibodies to *T. evansi* variants in serum from infected animals);
- the demonstration of antigenic variation *in vitro* and *in vivo*;
- polyclonal activation of pony lymphocytes by an antigen prepared from *T. evansi*. Stimulation of the lymphocytes enhanced the production of interleukin 2 (IL-2) and the expression of receptors for IL-2 (1). Further work, at present awaiting publication, dealt with the production of IL-2 and the expression of IL-2 receptors by lymphocytes in circulating blood of ponies, following stimulation by a soluble fraction of *T. evansi*.

Dr A.G. Luckins and Dr R. Boid of CTVM (Edinburgh) were unable to be present, but Dr Touratier read the three summaries he had received from them, as follows:

- "An ELISA to detect *T. evansi* by using group-specific monoclonal antibodies" (A.G. Luckins *et al.*).
— "Importance of natural receptivity of the host to *T. evansi* infection" (T.W. Jones & C.D. McKinnell).

— "Relationships between chromosome polymorphism in *T. evansi* and the range of antigenic variants" (R. Boid & T.W. Jones).

Regarding proposals for the *OIE Manual of recommended diagnostic techniques and requirements for biological products*: the text prepared by Dr P. Kageruka was adapted by Dr Y. Ozawa after a few minor changes for inclusion as Chapter 20 "Surra (*T. evansi*)" in Volume III of the *Manual*.

New epidemiological information

**Brazil:** according to Professor Mehlitz, it is now recognised and proven that capybaras (*Hydrochoerus capybara*) can act as a reservoir for *T. evansi*.

**Colombia:** as mentioned above, Drs Hamers and Bajyan-Songa have identified *T. evansi* in blood samples from llamas.

**Southern Algeria** and **Southern Morocco:** *T. evansi* infection in dromedaries ("debab") is frequently underestimated, according to a report presented by Professor A. Dakkak at the Maghreb Veterinary Conference in Algeria in May 1990. The infection, often insidious, has never been reported to the Veterinary Services, but camel owners are well aware of its existence and treat dromedaries at the least suspect symptom, using low doses of diminazene aceturate (Berenil). However, this is not a specific drug, and its use may result in a carrier state.

**Trypanocidal drugs**

*Development of studies on melarsomine (Cymelarsan®)*

Dr J.P. Raynaud reported trials with melarsomine, initially on laboratory animals infected with camel strains of *T. evansi* and subsequently on experimentally and naturally infected dromedaries in Niger, Ethiopia, Kenya and the Sudan. He confirmed the results given at previous meetings of the Group concerning the toxicity, pharmacokinetics and biological availability of melarsomine in different species of animals, and its consistent efficacy against *T. evansi* in a single dose at 0.25-0.5 mg/kg body weight, administered by intramuscular or subcutaneous injection. Dr Raynaud recommended the dose rate of 0.25 mg/kg as giving the most consistent results.

Melarsomine will be marketed, as recommended by a Resolution of the 20th ISCTRC Meeting in Mombasa in April 1989.

This move is particularly important as melarsomine will be the first effective trypanosomosis drug to be marketed since 1965, during which time many international organisations concerned with this disease and its consequences for developing countries have expressed the wish for an effective drug.

*Other trypanocides under investigation*

No information has been supplied by the Laboratory for Animal Parasitology at Shanghai on the chemical group to which compound "T 46" belongs. It was reported in 1989 that this compound was effective against *T. evansi* in buffaloes and cattle in the People’s Republic of China.
Compound "IMOL 881", which showed promise after initial tests on laboratory animals at the Free University of Brussels, is still being evaluated.

Professor Kageruka reported good results following administration of ronidazole to rats in drinking water. This veterinary drug, available commercially for some time to combat haemorrhagic enteritis in pigs, protected all the experimental animals from \textit{T. evansi} infection when given at 68 mg/kg body weight for nine consecutive days (13). Trials on the target species will be undertaken.

**OTHER TOPICS**

**Economic impact of \textit{T. evansi} infections**

Professor Mehlitz stressed the need to conduct surveys in order to discover the actual impact of \textit{T. evansi} infections in various species of domestic animals. The diagnostic kits introduced by the Group, easy to use and cheap, could greatly assist the performance of such surveys.

**Announcement of a meeting**

*First International Seminar on Animal Trypanosomoses Not Transmitted by Tsetse Flies, Annecy, 14-16 October 1992*

This seminar is being sponsored by the OIE, the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO), with the participation of the Laveran Foundation and the Marcel Mérieux Foundation, which will provide accommodation for participants and a conference room. It will be organised jointly by this Working Group, CTVM (Edinburgh) and the Laboratory for Molecular Biology of Bordeaux University II.

The provisional agenda is as follows:
- Economic impact
- Epidemiology of, and epidemiological surveys for \textit{T. evansi}, \textit{T. vivax} and \textit{T. equiperdum}
- Diagnosis
- Basic research on these parasites
- Chemotherapy and chemoprophylaxis
- Methods of control.

**REFERENCES**


