Tenth International Meeting on *Trypanosoma evansi*: Report of the Working Group  
Paris, 24 May 1989  
L. TOURATIER *

**Summary:** Some epidemiological surveys have provided information on the incidence of *Trypanosoma evansi* infection among dromedaries in Mali, and among buffaloes in Java and Indonesia. The disease among camels has been reported again from Kazakhstan in the USSR, with the coexistence of *T. evansi* and *Cephalopina titillator* in animals which developed acute infection. The disease has been studied among horses in Venezuela and among buffaloes in Vietnam and Indonesia, and suspected among horses in Brazil.

Diagnostic kits for rapid and reliable detection of *T. evansi* are being made available free of charge, upon request, by the institutes which have developed these new techniques, namely: detecting the parasite by agglutination-lysis; detecting antibody (by a modification of CATT); detecting antigen (by using monoclonal antibodies).

Once these various diagnostic procedures developed by competent institutes have been evaluated and used widely, the next step will be to standardise the techniques and the antigens.

Differential diagnosis of *T. evansi* and *T. equiperdum* is still difficult in the case of akinetoplastic strains.

For improved evaluation of *T. evansi* isolates, a proposal has been made to form collections of complementary DNA (cDNA) with a view to exchanging these copies and the original strains.

The advice of the International Commission on Zoological Nomenclature has been requested for definitive adoption of a binomial designation for the species *T. evansi*.

With more extensive data on the pharmacology and pharmacokinetics of Cymelarsan and laboratory testing of a new trypanocide called "IMOL 881", research on trypanocides continues.

**KEYWORDS:** Africa - Agglutination tests - Antigens - Buffaloes - Camels - cDNA - Cymelarsan - Dromedary - Epidemiology - IMOL 881 - Monoclonal antibodies - Standardisation - Trypanocides - Trypanosoma evansi.

* 228, boulevard du Président Wilson, 33000 Bordeaux, France.
The Tenth Meeting was attended by nineteen participants from eight countries, with Dr W.N. Masiga, Director of the Inter-African Bureau for Animal Resources, Organisation of African Unity (IBAR/OAU), Nairobi, Kenya, as Chairman. The agenda and a brief summary of the meeting held on 26 May 1989 were distributed to all participants of the 57th General Session. The summary focused on the following principal points of discussion:

- a request to the International Committee for Zoological Nomenclature for binomial designation of species of the genus *Trypanozoon*

- an examination of various diagnostic procedures which the Group had already considered in previous meetings, and which are currently being evaluated in the field in Africa and Asia

- a new request for laboratory exchanges of *T. evansi* strains, under the aegis of the OIE

- the details of new work on Cymelarsan and announcement of the first tests on laboratory animals of a new trypanocide (“IMOL 881”).

**INTERIM REPORT OF THE SECRETARY GENERAL**

The report of the Ninth Meeting of the Group, held at the OIE on 18 May 1988 (*Rev. sci. tech. Off. int. Epiz.*, 1989, 8 (4), 1047-1052) was supplied to all participants of that meeting, and was presented at a small symposium on *T. evansi* held in Mombasa (Kenya) on 11 April 1989 as part of the Twentieth International Scientific Committee for Trypanosomiasis Research and Control (ISCTRC) Meeting organised by the IBAR/OAU.

**New epidemiological information**

According to correspondence with the Bernhard Nocht Institute in Hamburg (Federal Republic of Germany), *T. evansi* infection of horses in Brazil was traced to capybaras (*Hydrochaerus hydrochaerus*), which act as a reservoir host for the trypanosomes. A detailed investigation, based on the results presented to the Group by Kageruka in 1985 (4), is under way.

A recent publication by Toro Benitez *et al.* (10) of the Veterinary Research Institute at Maracay (Venezuela) drew attention to experimental infection of rats and horses by a strain of *T. evansi* isolated from a naturally infected horse, resistant to the therapeutic effect of isometamidium.

In Kazakhstan, Ergaliev *et al.* (2) found larvae of *Cephalopina titillator* in the nasal cavity of camels with acute surra. This was supported by the J.L. Jacquemin abattoir survey of dromedaries in southern Algeria; reported to the Group in 1987, the survey found *T. evansi* in association with the same oestrid larvae.

Walter-Toews *et al.* (11) reported to the Fifth International Symposium of Veterinary Epidemiology and Economics (Copenhagen, Denmark, 25-29 July 1988) that *T. evansi* was a major cause of illness and death among buffaloes imported from Australia for fattening or work in Indonesia, particularly Java.
An article by Luckins (5) described the historical and current status of surra in Asia, and mentioned the work of the International Group on *T. evansi*.

**Diagnostic techniques**

Subsequent to action taken by the OIE to include reliable, well-adapted and simple diagnostic tests for *T. evansi* in its *Manual of recommended diagnostic techniques and requirements for biological products for Lists A and B diseases*, the following documents were sent to the Group Secretariat:

- Van Meirvenne N. – A haemolytic detergent concentration method for detection of trypanosomes. Institute of Tropical Medicine, Antwerp.

- Kageruka P. – Diagnosis of *Trypanosoma (Trypanozoon) brucei evansi* infection (Surra). (Comprehensive paper describing all methods currently in use.) Institute of Tropical Medicine, Veterinary Department, Antwerp.


- Shen Jie, Fang Weimin & Sun Jilan. – Diagnosis of *T. evansi* using the ELISA technique in buffalo. Shanghai Institute of Animal Parasitology, Chinese Academy of Agricultural Sciences.


**Consideration of nomenclatural problems within the subgenus Trypanozoon**

Recent research on the kinetoplastic DNA (kDNA) of various *T. evansi* strains done in Europe by W. Gibson (3), in Africa by Masiga and Gibson (6), and in Asia by Zainal-Abidia et al. (12) has demonstrated the need to define the position of *T. evansi* within the *Trypanozoon* subgenus.

**Research on new trypanocides**

Two important meetings were held in Kenya in 1988:

- a WHO meeting held at Nairobi on 13-16 March 1988
– a joint meeting arranged by the WHO, International Atomic Energy Agency (IAEA) and FAO at the KETRI Headquarters in Kikuyu from 11 to 20 December 1988.

Research trends in the biochemistry and molecular biology of trypanosomes were examined at the first meeting, with emphasis on the mode of action of existing trypanocides: interference with antigenic variation, anchoring of variable surface glycoproteins (VSG) on the membrane, and either inhibition of certain biosynthetic processes or activation of certain enzymes.

At the second meeting the principal topics were:

– the current situation regarding the chemotherapy of African trypanosomiases and new drugs under development
– the pharmacokinetics of trypanocides, and residues in meat
– the slow release of trypanocides by using new pharmaceutical formulations
– in vitro detection of trypanocidal activity for the study of new compounds. (This topic had been previously examined by the Group and a solution was proposed in 1987.)

Workshop on veterinary drug registration in Africa

This was held during the Eighth Conference of the OIE Regional Commission for Africa in Arusha (Tanzania) from 19 to 20 January 1989.

Twentieth Meeting of the ISCTRC, organised by the IBAR/OAU in Mombasa (Kenya), 10-14 April 1989

As mentioned above, a symposium of the Group was held during this meeting, and its conclusions were included in the final report distributed by the IBAR/OAU.

Many hundreds of communications were presented in most of the fields relating to African trypanosomiases of man and animals. In particular, five documents (one communication and four poster presentations) dealt with Cymelarsan chemotherapy of T. evansi infection:

NEW FINDINGS REPORTED TO THE MEETING

The meeting was opened by Dr Masiga at 9.30 a.m., and the points of the agenda were examined successively.

Nomenclature of the subgenus *Trypanozoon*

After discussion between members of the Group, particularly between Professors and/or Drs Uilenberg, Njogu, Baltz, Boid, Nantulya, Hamers and Van Meirvenne, it was concluded that:

* a) the name *T. evansi* has priority over *T. brucei*

* b) consequently, the trinomial designation *T. brucei evansi* is incorrect

* c) the following provisional nomenclature is recommended to simplify usage and avoid confusion involving *evansi* in a trinomial system:
  
  - *Trypanosoma (Trypanozoon) evansi* for the *Trypanozoon* species transmitted by insects
  
  - *Trypanosoma (Trypanozoon) equiperdum* for the *Trypanozoon* species transmitted by the sexual route in equines
  
  - *Trypanosoma (Trypanozoon) brucei brucei* for the *Trypanozoon* species transmitted after passage through tsetse flies and non-infectious for man
  
  - *Trypanosoma (Trypanozoon) brucei gambiense* and *Trypanosoma (Trypanozoon) brucei rhodesiense* for the *Trypanozoon* species for human sleeping sickness caused by *gambiense* and *rhodesiense*

* d) The help of the International Commission for Zoological Nomenclature should be requested to resolve the nomenclatural problem of subspecies *Trypanozoon*.

Diagnostic procedures for epidemiological surveys

**Identification and/or comparison of strains of different origin**

Professor Baltz described research, conducted in his laboratory at Bordeaux University II with the assistance of a Chinese veterinarian from Chang Chun Veterinary Institute in Jilin Province and another from the Shanghai Institute for Parasitology, on strains of *T. evansi* from these two regions. Provisional results, obtained by comparing four Chinese strains with a museum strain of *T. equiperdum*, showed that two of the Chinese strains were similar (they had the same kinetoplast DNA as that described for Group A strains by Borst *et al.* (1) in 1987). Professor Van Meirvenne and Dr Bajyana-Songa took part in the discussion. Dr Bajyana-Songa suggested extending the comparison of *T. evansi* and *T. equiperdum* by sequencing techniques.

According to Professor Hamers, *T. evansi* proved to be a homogeneous species when examined for serodemes and kinetoplast DNA. The kinetoplast could be labelled with probes specific for *evansi* and thereby help identify *T. evansi* by the "dot blot" and *in situ* hybridisation techniques applied to blood smears of non-radioactive strains. For dyskinetoplastic isolates, nuclear DNA could be used
to identify *T. evansi*, again owing to the homogeneity of the species, as in the case of *T. b. gambiense*. Some of the strains from African dromedaries seemed to belong to *T. b. brucei* (heterogeneous, distinguished by their kinetoplasts).

Dr Boid described work done at the Research Institute of Veterinary Science (RIVS) in Bogor, Indonesia (Director: Dr Purnomo Ronohardjo) in collaboration with the Tropical Veterinary Medicine Unit in Edinburgh, Scotland (Director: Professor Brocklesby) and the Overseas Development Administration (ODA) on the epidemiology of *T. evansi* in the Republic of Indonesia (R.C. Payne, T.W. Jones, R. Boid and A. Wilson). This work has so far concentrated on:

- the collection of parasitological and serological data in areas of Indonesia where cattle are kept
- natural *T. evansi* infection in calves and buffaloes
- the detection of *T. evansi* among buffaloes imported from Australia
- the effect of working on *T. evansi* infection in buffaloes.

Taken altogether, the findings support previous studies made in Indonesia, Vietnam and Thailand.

Drs Uilenberg and Nantulya drew attention to the need for experiments on the role of maternal antibodies in protecting calves and buffaloes. Dr Boid explained that *T. evansi* infections mainly affect young buffaloes imported from Australia. He and his colleagues are attempting to establish a correlation between the genes responsible for variation in surface glycoproteins of *T. evansi* (VSG genes) and mortality in buffaloes. Responding to a question from Professor Mulegeta, Dr Boid said that the mortality could not be due to any other cause.

A similar situation exists in Vietnam, according to Professor Hamers, who recently visited the Laboratory of the Animal Husbandry Department in Bac Mai, near Hanoi, and was told that many surveys had been done on buffaloes infected with *T. evansi*.

For improved evaluation of *T. evansi* isolates, Dr Boid suggested the preparation of a collection of types of cDNA of *T. evansi*, as there is a lack of information on such collections. It was suggested that all persons or laboratories preparing or possessing a collection of cDNA should transmit the information to the OIE through the Group Secretariat.

Information concerning the existence of kinetoplastic DNA (kDNA) probes, kDNA maps and kDNA sequences should also be made available to Group members for purposes of comparison and/or utilisation. Such information would facilitate the comparison of museum strains present in laboratories throughout the world. Of course, the DNA must have been extracted from *T. evansi* and not from *T. b. brucei*.

**Field use of test kits supplied by different laboratories**

A report by Dr Y. Ozawa, who was unable to attend the Meeting, was summarised by Dr Touratier. This dealt with OIE proposals for a Pan-African Centre for diagnostic procedures. Such a centre would introduce and develop reliable, simple, safe and economical procedures to be used in the field. The Centre would promote standardisation of reagents and diagnostic techniques, evaluate the work of field teams, provide liaison between national laboratories and enhance the OIE information system on animal diseases.
These proposals corresponded to the request that the Group participate in drafting the chapter on *T. evansi* for the O.I.E. *Manual of recommended diagnostic techniques and requirements for biological products for Lists A and B diseases*.

Recalling the report (written in collaboration with workers in sixteen countries) by Dr H.R.P. Miller to the 57th General Session held on 23 May 1989 on internal parasitism and immune response, Dr Njogu stated that this report should be made available to all members of the Group. He further stated that the International *T. evansi* Group should recommend those diagnostic techniques which had reached a degree of development sufficient for field trials and had been made available to centres possessing *ad hoc* facilities for evaluating them.

Existing institutes for this purpose are KETRI in Kikuyu, Kenya, the RIVS in Bogor, Indonesia, the Laboratoire Central Vétérinaire (LCV) in Bamako, Mali, and also such European and international institutes as the ILRAD in Nairobi, the Institut d’Elevage et de Médecine Vétérinaire des Pays Tropicaux (IEMVT) in Maisons-Alfort, the Vrije Universiteit in Brussels, the Tropical Institute in Antwerp and the CTVM in Edinburgh (which maintains constant liaison with all of the aforementioned institutes). Funds necessary for such verification can only be obtained by bilateral or multilateral agreements.

For example, KETRI has facilities for experiments on camels in insect-proof buildings. Forty km from the Institute is a herd of about 150 dromedaries, some infected with *T. evansi*. At Galana Ranch, 400 km distant, KETRI could conduct trials on 300 dromedaries infected with *T. evansi* and living within a tsetse fly zone. Owing to good relations with the owners of thousands of dromedaries in Nguruvit, Marsabit District, KETRI has a field laboratory suitable for conducting epidemiological surveys and/or trials of trypanocides on these animals. Dr Njogu requested that the International *T. evansi* Group take note of these unique facilities and encourage Group members to make use of them.

Professor Van Meirvenne was surprised that no one had requested the diagnostic kits, one for detecting trypanosomes in circulating blood (using a special detergent) and the other a slide agglutination test, which he announced were available in May 1988. Both kits are rapid, reliable and suitable for a preliminary epidemiological survey in the field, and are available free of charge upon request.

Dr Bajyana-Songa is working with other researchers to develop diagnostic tests; he is collaborating in the field, for example, with Dr G. Duvallet of the Centre de Recherches sur les Trypanosomoses en Afrique (CRTA), Bobo-Dioulasso, Burkina Faso and in the laboratory with Professor Baltz (Parasite Immunology and Biology, University of Bordeaux II).

Dr O. Diall of the LCV in Bamako, Mali, exchanges information with various centres, particularly ILRAD, the Instituut voor Moleculaire Biologie in Brussels, and KETRI.

Dr Nantulya (7, 8) summarised the three main techniques proposed:

- lysis test with detergent to detect the parasites
- card agglutination of trypanosomes (CATT) using a new variant, VAT (Rota 1-3) for detecting antibodies
- ELISA with monoclonal antibodies for detecting antigens.
These three tests have so far been evaluated on a small scale, and the antigen-detection test is at present being tried in Kenya by KETRI, in Mali by the LCV and in Sudan by the Faculty of Veterinary Sciences at Khartoum (Dr El Amin).

The Group recommended that these three tests be evaluated in parallel by several centres.

Offering their cooperation for this purpose were: Dr Njogu on behalf of KETRI, Kenya; Dr Diall on behalf of the LCV, Mali; Dr Boid for the joint CTVM/RIVS/ODA project at the Bogor Laboratory in Indonesia; Dr Uilenberg on behalf of the IEMVT in Mauritania, Chad and Djibouti.

Dr Njogu proposed eventual standardisation of methods and antigens. He offered to collaborate with Professor Van Meirvenne and, with Dr Nantulya of ILRAD, to evaluate his lysis technique.

Dr Boid, supported by Dr Nantulya, drew attention to the importance of exchanging information, techniques, materials and strains of *T. evansi* in order to reproduce the tests under identical conditions.

Professor Hamers believed that such exchanges should be extended to southeast Asia and the Far East; with EC financing, he is attempting to verify, and eventually to develop, CATT among buffaloes of the ricefields of Thailand.

Drs Bajyana-Songa and Boid stressed that it was necessary to have well-documented sera to determine the serodemes present in a given area. For example, antisera have been prepared and exchanged between the Instituut voor Moleculaire Biologie at the Vrije Universiteit in Brussels and the CTVM in Edinburgh.

Dr Nantulya, whose study of the *brucei* group was published in 1988, emphasized the differences between other groups of trypanosomes in Kenya, Mali and Indonesia. In cooperation with the IAEA (Vienna) and the Australians responsible for technical cooperation in Indonesia, he has already started to evaluate ELISA with monoclonal antibodies in buffaloes and horses.

Dr Touratier drew attention to the booklet produced by the OIE in the three official languages of that organisation (English, Spanish and French), “Immuno-enzyme ELISA techniques in diseases of animals and plants” (Technical Series No. 7, 2nd edition), aimed at laboratory personnel and for refresher courses.

Dr Mulegeta stated that the Ethiopian Veterinary Service would be interested in the techniques described by Dr Nantulya.

**Differentiation of *T. evansi* and *T. equiperdum***

Professor Hamers proposed that this differentiation be made by using kinetoplastic strains. There is also the possibility that *T. equiperdum* is a sufficiently homogeneous species for identification by a battery of appropriate nuclear DNA probes. Dr Njogu requested a better definition of the parameters for reliable differentiation of *T. equiperdum* from *T. evansi*.

**New epidemiological findings***

Dr Diall briefly described work done at the northern frontiers of Mali, where blood samples were taken from dromedaries to identify and collect several isolates of the
brucei group. Plans were made to type these isolates by means of DNA probes, pulse-field electrophoresis and iso-enzyme electrofocusing, in collaboration with ILRAD and the Instituut voor Moleculaire Biologie in Brussels. Two recent serological surveys of dromedaries in the Nara district (northwestern Mali) gave similar infection rates (10.5%) for 1987 and 1988, even though the 1987 survey was done during the dry season and the 1988 survey during the rainy season. A third survey, done at the start of 1989 in the Gao area (northeastern Mali), showed that only 7 of 165 dromedaries were carrying trypanosomes of the brucei group.

Professor A. Dakkak drew attention to the camel breeding situation in the Maghreb. A programme was under way in Morocco to develop camel breeding with dromedaries imported from Mauritania, which could result in more cases of camel trypanosomiasis in Morocco. The disease is well-known to camel owners in southern Morocco and, as in other North African countries, seems to be increasing. Professor Dakkak requested that his laboratory at the Hassan II Agronomic and Veterinary Institute (Rabat) be associated with other laboratories more experienced in this field. He suggested that:

a) Dr Diall maintain contact with him about testing imported animals

b) animals found to be infected should be treated upon arrival in Morocco with trypanocides of known efficacy

c) ILRAD be asked to train a Moroccan assistant

d) the ODA supply aid.

Dr Mulugeta recalled previous aid from the ODA to conduct sampling of Ethiopian dromedaries, coupled with treatment of infected animals. Ethiopia currently requires aid on this problem and will establish contacts with ILRAD.

New trypanocides

As already reported above, Cymelarsan was the subject of five communications or posters presented at the Twentieth ISCTRC Meeting held in Mombasa from 10 to 14 April 1989. KETRI is conducting pharmacological and pharmacokinetic studies of this compound.

Professor Hamers and Dr Bajyana-Songa provided initial results obtained with a new trivalent arsenical derivative referred to as “IMOL 881”, synthesised at their Institute. Tests on mice revealed a good chemotherapeutic index and significant activity against various strains of T. evansi (between 1 and 5 mg/kg body weight), with a lethal dose of 500 mg/kg and efficacy against melarsoprol-resistant strains. The activity of this new compound, which had been reported previously at the workshop organised by the WHO, IAEA and FAO in Kikuyu in December 1988, might be extended to T. congolense and T. vivax. Further research is necessary, however, and the results will be communicated to the Group at some future date.

Other topics

After consulting Dr Masiga and other participants, Dr Bajyana-Songa suggested that the Secretary write a summary report of the work of the Group since its foundation in May 1983, to be presented at the 1990 meeting. Dr Touratier accepted this task and proposed that the report be submitted, after approval and any changes
or corrections by members of the Group, to the Twenty-first ISCTRC Meeting to be held in Côte d’Ivoire in 1991. He stressed that, although some progress has been made by the initiatives of the Group (in matters of diagnosis, epidemiology and therapy), there has been little progress in the exchange of strains between laboratories. Much research is still required to understand the action mechanisms of *T. evansi*, and thereby develop an effective strategy for controlling this parasite and related organisms.

* * *

REFERENCES

*Note: Documents have been received from the Shanghai Institute for Animal Parasitology concerning research conducted in the People’s Republic of China by Zheng Renjian et al. (13) on proteins and antigens of *T. evansi*, and by Shen Jie et al. (9) on comparison of the activity of four trypanocides against *T. evansi*. These documents have been distributed to participants.*


