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

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Summary: There is increasing interest in many parts of the world in trypanosomoses other than those transmitted by tsetse flies, as shown by numerous research projects and field studies.

The refinement of techniques for studying the behaviour of trypanosomes (techniques of molecular biology) in axenic culture or in the parasitised host has led to progress in diagnosis and immunology, and a rational approach to chemotherapy and chemoprophylaxis of these infections.

Field trials of enzyme-linked immunosorbent assays in Africa, Asia and South America have shown that these tests may now be regarded as reliable in demonstrating antibodies or antigens for Trypanosoma evansi infection in buffalo, cattle and camels, and for mono-infection with T. equiperdum in equines. However, it is not yet possible to differentiate reliably between infections with T. evansi and T. equiperdum in equines. The card agglutination trypanosomosis test (CATT) has been adapted to T. evansi infection and can also be recommended.

Immunosuppression induced by T. evansi infection inhibits the immune response to vaccination against Pasteurella haemolytica.

In areas freed from tsetse flies (Cameroon, Central African Republic, Zambia) it has been observed that Trypanosoma vivax can be transmitted mechanically by other biting insects, which are at present being identified.

Research on trypanocides has led to the toxic factor for Trypanosoma brucei or T. equiperdum present in human or simian serum being localised to the high density lipoprotein of serum lipoproteins. Various derivatives are being tested under laboratory conditions, and the efficacy of some (e.g. ronidazole) is being checked at present, while others are ready to pass to the development stage (e.g. IMOL 881). Melarsomine, already available commercially (as Cymelarsan®) for the treatment of T. evansi infection in camels, is being studied for possible use in other species of animals.


* 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). In the present paper, the suffix "-iasis" has been retained for the titles of official bodies, institutions and/or publications which use the latter form.

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INTRODUCTION

The Thirteenth meeting of the Office International des Epizooties (OIE) Ad hoc Group on Non Tsetse-Transmitted Animal Trypanosomoses (NTTAT) was attended by fifteen participants from nine countries, and was chaired by Dr W.N. Masiga and Dr G. Uilenberg. A brief summary of the discussions, available to all participants attending the 60th General Session of the OIE, drew attention to the following points:

- *Trypanosoma vivax* infection persists in certain countries (notably northern Cameroon, the Central African Republic and Zambia) from which tsetse flies have been eradicated by efficient vector control procedures (insecticides and fly traps). This trypanosome is therefore being transmitted by other insect vectors.

- Information was provided on the existence of *T. vivax* infection in cattle in South America (Colombia) and of *T. evansi* infection among camels, cattle, buffalo and mules in the People’s Republic of China.

- Good results have been achieved in the field in many countries of South America, Africa and Asia with diagnostic kits supplied free of charge by the Institute of Tropical Medicine in Antwerp (Belgium), utilising the card agglutination trypanosomosis test (CATT) for *T. evansi* infection.

- An enzyme-linked immunosorbent assay (ELISA) has been developed for *T. equiperdum* infection at the Onderstepoort (South Africa) and Weybridge (UK) laboratories.

- It is difficult to differentiate inapparent infections with *T. evansi* and *T. equiperdum* in equines in areas where both trypanosomes are present.

- Molecular biology techniques have opened up many avenues for research to find chemicals active against trypanosomes.

- Further research has been conducted on the activity, pharmacology and pharmacokinetics of melarsomine (Cymelarsan®).

INTERIM REPORT OF THE SECRETARY GENERAL

The report of the Twelfth meeting of the Group, held on 15 May 1991, was published in the OIE *Scientific and Technical Review* (43), after incorporation of corrections submitted by participants.

Information derived from scientific literature and specialist meetings

In addition to publications in the principal scientific journals, presentations were made of the results of the following four major meetings attended by members of the Group between May 1991 and May 1992:


- Trypanosomosis seminar (Antwerp, Belgium, 11-12 December 1991) organised jointly by the Belgian Institute of Tropical Medicine and the British Society for Parasitology
Keystone Symposium on Molecular and Cellular Biology (Los Angeles, United States of America, 13-16 January 1992)

First International Camel Symposium (United Arab Emirates, 2-6 February 1992), organised by the Camel Study Centre of Dubai.

Subjects of interest to this Group which were discussed at these meetings included recent, detailed studies of molecular biology applicable to field diagnostic tests and to the study of trypanocides.

**Diagnosis**

The following research has been conducted on diagnostic methods:

- evaluation of CATT in the diagnosis of *T. evansi* and *T. rhodesiense* infections occurring in countries of Africa, Asia and South America (1)
- evaluation of ELISA with monoclonal antibody for detecting *T. evansi* infection in dromedaries (28, 37, 49)
- detection of trypanosomes in mice infected and subsequently treated with a trypanocide, by using polymerase chain reaction (PCR), a technique for enzymatic amplification of deoxyribonucleic acid (DNA) (11)
- comparison of various procedures (23).

**Infected countries**

*T. evansi* infection has been studied in working and breeding buffalo (35), in cattle, buffalo and horses (33), in adult cattle and calves in Indonesia (34); also in dromedaries in Sudan and other countries (18).

**Biology of trypanosomes**

Studies have been conducted on the following aspects of the biology of trypanosomes:

- the promoter of glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) identified in *T. brucei* by means of molecular biology (3)
- the metabolism of *Kinetoplastida*, particularly glycolysis (32)
- kinetoplast DNA of *T. evansi* (30)
- the growth of, and pyruvate kinase production by *T. evansi* in axenic culture (38)
- conservation of the receptor on trypanosomes for low density lipoproteins, using biochemical and molecular techniques (4).

**Pathogenesis**

It was demonstrated that *T. brucei* does not pass the blood-brain barrier, but remains confined to circumventricular organs (15). *In situ* multiplication of *T. evansi* was observed at the site of cutaneous infection in rabbits and cattle (19). Pathogenesis of *T. evansi* was studied in guinea-pigs and horses in Argentina (24) and in goats in Kenya (27).

Studies continued on the tumour necrosis factor (TNF), which was shown to have a toxic role in animals treated with a trypanocide (2, 17) but seemed to protect laboratory mice in the initial phase of experimental parasitaemia (16).
Cell-mediated immunosuppression was induced by trypanosomes (5), particularly \textit{T. evansi}, which altered the immune response to \textit{Pasteurella haemolytica} vaccine (29).

\textbf{Trypanosoma equiperdum and dourine}

There was renewed interest in this trypanosome as a laboratory model (36, 39, 40), either to develop a diagnostic procedure for dourine in inapparent carriers (50, 51, 52) or to differentiate \textit{T. evansi} and/or \textit{T. equiperdum} infections in equines (20).

\textbf{Trypanocides}

Difficulties encountered in developing a vaccine against trypanosomoses, due to antigenic variation among the various trypanosome species, seems to have stimulated research into curative or prophylactic trypanocides.

There is a particular need for trypanocides because active drugs such as nifurtimox and efllornithine, effective in treating human trypanosomoses, are being abandoned by their manufacturers due to poor profitability (47).

Basic research has concentrated on resistance factors which form in the serum of parasitised subjects, inhibiting development of the parasite (8), and this has led to separation of the trypanocidal factor present in the high density lipoprotein (HDL) of human serum, capable of neutralising \textit{T. brucei} (10, 31) or \textit{T. equiperdum} (48).

Experimentation and efficacy testing of existing trypanocides has continued (26, 42, 54), including attempts to dissociate the toxicity of propylene glycol from that of melarsoprol (21), to define the molecular basis of drug resistance (25), and using an ELISA to measure serum concentrations of isometamidium (8).

Potentially useful trypanocidal activity has been found in some new compounds, such as the incorporation of homidium bromide in various polymers for slow release within the body (6), a new organic arsenical (IMOL 881) (22), polyunsaturated n-3 fatty acids and lysophosphatidylcholine derivatives (45). In addition, combinations of two or more trypanocides have proved to be effective (12), particularly 5-nitroimidazole with melarsomine and suramin (13). The activity of melarsomine against \textit{T. evansi} infection in dromedaries has been confirmed (53) and its mode of use defined (46).

\textbf{Classification and nomenclature}

A new classification has been proposed for species of the subgenus \textit{Trypanozoon} (44) and the use of computers has been suggested to assist in evaluating species, subspecies and groups of strains (41).

\textbf{COURSE OF THE MEETING}

\textbf{Field use of diagnostic kits}

Following the proposal of Prof. N. Van Meirvenne, made at the Twelfth Meeting of the Group, several users have received the free diagnostic kits prepared specially by the Institute of Tropical Medicine in Antwerp (Belgium). In particular, Prof. P. Kageruka stated that there had been requests from Saudi Arabia, Thailand, Tunisia, Vietnam and the United Arab Emirates, all interested in CATT. He concluded that CATT could now be recommended for practical use in identifying \textit{T. evansi} infections.
Transmission of *Trypanosoma vivax* in Africa by insects other than tsetse flies

Transmission of *T. vivax* was reported by Dr D. Cuisance in the Cameroon and by Dr H.G.B. Chizyuka in a small area of Zambia; in each case, the area was free from tsetse flies.

Dr Cuisance presented research conducted by F. D’Amico and colleagues on vectors of animal trypanosomes in the humid savanna of the Central African Republic. In this area, the habitual vector (*Glossina fuscipes fuscipes*) had been practically eradicated by trapping. *T. vivax* infection of cattle persists at considerable prevalence, as approximately 79% of cattle were found to be carrying the parasite on examination by standard parasitological techniques. This is in contrast to the low density of *G. f. fuscipes*. Consequently, carriage by other vectors was suspected and investigations conducted to date have shown that *Stomoxys* spp. are abundant on grazing land, while *Tabanidae* spp. and *Hippoboscidae* spp. are rare. However, Dr Cuisance recalled that *Glossina* spp. can mechanically transmit certain types of trypanosome.

Prof. R. Hamers reported that in areas where several species of trypanosome pathogenic for livestock coexist (e.g. *T. vivax*, *T. brucei*, *T. congolense*), the animals become infected first with *T. vivax* and then with *T. congolense*. The phenomenon reported by Dr Cuisance is therefore important for the general prophylaxis of trypanosomoses of livestock.

Investigation of the epidemiology of non tsetse-transmitted animal trypanosomoses in South America

Dr J. Otte commented on the situation in South America and introduced a paper written in collaboration with J.Y. Abuabar and E.A. Wells, entitled “Epidemiology of *T. vivax* in northern Colombia and its impact on livestock productivity”.

A study of 104 herds in the Cordoba region confirmed the prevalence of infection, with considerable variation from herd to herd, occurring in general at two peak periods each year: during the transition from dry to wet seasons, and at the end of the wet season. A controlled transmission experiment strongly incriminated *Tabanidae* spp. as vectors of *T. vivax*. Infected animals showing no clinical signs suffered a 20-25% reduction in growth in comparison with healthy animals. Sequelae among clinically-infected cows included a pronounced drop in milk yield, loss of weight and abortion, and an increase was noted in the death rate.

In the case of *T. evansi* infection in horses, Dr Otte stated that a major epizootic had occurred in the eastern plains of Colombia (*llanos orientales*) in 1990-1991, causing some deaths. The situation was aggravated by a lack of quinapyramine for treating affected horses. *T. evansi* infection did not occur along the northern coast of Colombia, which has a large horse population.

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

Dr Uilenberg reported that, as indicated by Dr S.M. Touré shortly before the meeting, it was clear that the diagnosis of inapparent *T. equiperdum* infection in equines remains a problem in sub-Saharan Africa. The Onderstepoort and Weybridge laboratories have developed an ELISA, but the problem of differentiating species within the *Trypanozoon* subgenus remains unresolved.

Prof. Hamers stated that this question is under investigation at the Institute of Molecular Biology in Brussels, particularly the genomes of *T. evansi* and *T. equiperdum*, as detailed in a recent publication (9).
Prof. Hamers, Prof. Kageruka and Dr M.L. Dia discussed the difficulty of correctly isolating strains of *T. evansi* and *T. equiperdum*. Prof. Kageruka remarked that very few strains of *T. equiperdum* are available for comparison of virulence, because most research workers use strains which have been maintained for years in the laboratory. It is essential that new, freshly isolated strains be obtained.

Dr Dia preferred to passage newly-isolated strains in rabbits in order to obtain a stable strain, because of the eclipse phase, as detailed in his paper entitled “Comparison of the pathogenicity of *T. evansi* strains from Mauritania, Kenya, Niger, Chad and the People’s Republic of China”.

Mice injected with these strains survived for between 2.1 and 6.5 days in the case of isolates from Kenya, Niger or China, and 12.5-21.88 days in the case of isolates from Mauritania and Chad.

Dr L. Touratier presented another contribution, submitted from the Shanghai Institute of Animal Parasitology by Drs Wang Yunfei, Shen Jie and Zheng Renjian, entitled “Characterisation of isoenzymes of *T. evansi* strains isolated from various species of animals in the People’s Republic of China.”

These authors used electrofocusing of the isoenzymes in agarose gel or a thin layer of acrylamide. A total of six stocks of *T. evansi* were grouped: two (from buffalo in Guangdong and Zhejiang) were regarded as reference stocks and the remaining four (a camel strain from Xiangjiang, a donkey strain from Guangxi and buffalo strains from Xuyi and Juangsu) were examined individually for ten enzymes. The results showed that these stocks formed two zymodemes.

Because of this conflicting result, Prof. Kageruka asked if mixed infections with *T. evansi* and *T. equiperdum* occur in China as well as in South America. He proposed that the Group should make a recommendation that samples of the implicated strains be obtained. With the agreement of Prof. Hamers, the Group adopted the following text:

“It is necessary to develop ways of distinguishing *T. equiperdum* from *T. evansi*. Numerous strains of *T. evansi* are available and have been characterised at the molecular level, but this is not the case with *T. equiperdum*, where all research has been conducted on a very small number of strains. For this reason, an appropriate number of *T. equiperdum* strains should be isolated from field samples from areas where *T. evansi* does not occur in endemic form, so that they can be characterised at molecular level. In this way, it would be possible to distinguish *T. evansi* from *T. equiperdum*.”

A letter had been received from Dr V. Zablotsky (All-Russian Institute for Veterinary Experimentation [VIEV], Moscow) requesting a strain of *T. equiperdum* adapted to laboratory animals.

Chemotherapy

Dr F. Van Gool referred to documents presented at the 21st Meeting on African trypanosomoses and the First International Camel Symposium (46, 53) and confirmed that the effective dose of melarsomine (Cymelarsan®) is 0.25 mg/kg body weight for dromedaries and camels infected experimentally or spontaneously with *T. evansi*. Pharmacokinetic studies showed that the drug was eliminated rapidly.

Dr Cuisance enquired whether *T. b. gambiense* and *T. b. rhodesiense*, which constitute a danger to human beings, could be eliminated from the animal reservoir (mainly pigs and small ruminants in East Africa) by injecting an effective trypanocide.
With regard to reservoirs of sleeping sickness, Kageruka and colleagues (14) have shown that baboons are both receptive and tolerant to *T. b. gambiense*. In addition, serum contains a factor which is toxic against *T. b. brucei* but not against *T. b. gambiense*. This factor has been identified as HDL, as in human serum.

In reply to the question posed by Dr Cuisance, Dr Touratier stated that the results obtained at the Kenya Trypanosomiasis Research Institute (KETRI), Kikuyu (Kenya) in monkeys infected experimentally with *T. b. gambiense* and *T. b. rhodesiense* in 1987-1989 show that melarsomine injected at the cerebrospinal stage has a curative action which lasts for eighteen months. Although the infection subsequently recurs, melarsomine could probably be used effectively in animals carrying “human” trypanosomes.

Prof. Hamers drew attention to the mode of action of arsenicals on trypanosomes, and believed that some failures could be due to cytokine liberation during the disease. Arsenicals alter the secretion of trypanothione by the parasite and drug resistance is probably due to an enzymatic reaction. As for propylene glycol, this caustic fluid has been chosen as an excipient for melarsoprol (Arsobal®) to facilitate passage of the drug through the blood-brain barrier. However, the peak blood concentration of melarsoprol is reached rapidly after intravenous injection, and the drug does not pass through the blood-brain barrier; Dr Van Gool indicated that the same applies to melarsomine.

With regard to the arsenical IMOL 881 prepared at the Laboratory of Molecular Biology of the Free University of Brussels, Prof. Hamers announced that laboratory testing (for tolerance and efficacy) has been completed and the development stage is commencing.

Prof. Kageruka is continuing his experiments with ronidazole administered in drinking water, with good results against *T. evansi* and *T. congolense*; it had not been tested against *T. vivax*.

**Problems of classification and nomenclature**

Dr G. Uilenberg presented a note on the “Nomenclature of trypanosomes of the subgenus *Trypanozoon*” in which he discussed the proposal of Travassos Santos Dias (44) to create a new group for *T. equiperdum*, but he opposed this and suggested adherence to the decision of the Group, submitted to the International Commission of Zoological Nomenclature in 1989. Moreover, *T. equiperdum* which has been adapted to existence in tissue and venereal transmission tends to behave like *T. evansi* upon successive syringe passage in laboratory animals.

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**REFERENCES**


