Summary: New information has been supplied on the prevalence of infections due to Trypanosoma evansi in Asia (India, Indonesia, the Philippines, Thailand, the People's Republic of China, Oman), in buffalo, camels and cattle, and also in pigs. Information has also been received concerning Africa (Chad, Kenya). In Chad, the disease of camels ("djoufar") is difficult to control due to the lack of effective drugs; in Kenya, it is the subject of a special research and disease control programme (KETRI).

Potential reservoirs of T. evansi (Macaca monkeys in the Philippines) and insect vectors such as certain species of Triatoma are being studied.

In view of the results communicated to the Working Group in previous years, certain diagnostic tests have been improved after field trials. They are aimed either at greater efficiency in the direct examination of blood, after centrifugation, to detect trypanosomes, or at the search for antigens or the corresponding antibodies, using enzyme immunoassay methods with or without monoclonal antibodies or slide agglutination. Field kits are made freely available to researchers on demand, by two institutes.

The development of a new, organically derived, trivalent arsenical trypanocide (Cymelarsan) is being tested on camels in Africa (Niger, Ethiopia, Kenya) and on buffalo and cattle in Indonesia. Curative doses are between 0.3 mg and 0.5 mg per kg live weight, with a chemotherapy index of 10 to 15.

KEYWORDS: Africa - Antrycide - Asia - Berenil - Buffalo - Cattle - Curative treatment - Cymelarsan - Enzyme-linked immunosorbent assay (ELISA) - Epidemiology - Monoclonal antibodies - Pigs - Slide agglutination - Trypanosoma evansi.

The ninth Meeting was held in the presence of twenty-three participants from nine countries, with Dr A.R. Njogu (Kenya) and Dr Farah Hussein (Somalia) acting as Chairman and Vice Chairman respectively. A short report of the Meeting was distributed on 20 May 1988 to all participants at the 56th General Session of the OIE.
INTERIM REPORT OF THE SECRETARY GENERAL

Since the eighth Meeting of the Group in May 1987 at the OIE Headquarters (Rev. sci. tech. Off. int. Epiz., 1987, 7 (2), 403-409), Trypanosoma evansi infections have been an object of considerable interest in a number of fields.

In basic research, mention can be made of the following: kinetoplast DNA studies on T. evansi by Borst et al. (1), an in-depth study of the flagellate apparatus of the parasite by Hiruki (5) and its polymorphism observed by Lun (7) in infected mice irradiated with Cobalt 60.

In relation to this work and the results of some astute research into the antigenic and isoenzymatic relationships of trypanosomes of the subgenus Trypanozoon, a possible revision of the nomenclature, suggested by Uilenberg (12) and supported by Gibson (3), should be considered.

In applied research, Hamers (4) reported the conclusions drawn from his work in South-East Asia on the diagnostic methods used for detection and control of T. evansi in buffalo and pigs. Luckins (6), in an article prepared in liaison with the International Working Group, has demonstrated the role of T. evansi in Asia. Furthermore, he describes the important role of Tabanidae as vectors of the parasite. This has been confirmed by the thesis of Manz (8), who also draws attention to the possible role of Reduvidae.

On the occasion of a world-wide survey conducted by the OIE at the demand of the EEC (10), on twenty-nine animal diseases of particular economic importance in eighty-two developing countries, trypanosomoses (including T. evansi infections) were reported by numerous countries as constituting a brake on development. Interesting information has been gathered, in particular, from:

- **India**, where surveys carried out on buffalo and dairy cows by the Anand Laboratory (Gujarat) and by the National Dairy Development Board laboratories clearly show the presence of T. evansi;

- **Indonesia**, where a coherent programme of T. evansi identification and control is being developed at the Bogor laboratory with the cooperation of Australia (James Cook University) and Great Britain (with the presence of a member of the Centre for Tropical Veterinary Medicine (CTVM), Edinburgh);

- **Oman**, where the Muscat veterinary laboratory performs experimental infection of laboratory animals with T. evansi and studies a variety of diagnostic techniques;

- the **Philippines**, where the College of Veterinary Science and Medicine, Luzon University, has carried out a survey on the incidence of T. evansi in pigs at the Santiago abattoir;

- **Thailand**, where the North-Eastern Veterinary Laboratory at Ta Phra/Khon Kaen is continuing studies on T. evansi infections in buffalo with regard to repercussions on the severity of haemorrhagic septicaemia epizootics.

Special mention must be made of the variety of research carried out by the CTVM (Edinburgh) in the fields of epidemiology, immunology and diagnostics — notably the development of an enzyme immunoassay (ELISA) technique to detect antigen or circulating antibodies in the camel, to study the resistance of T. evansi to drugs, and to study new approaches to the development of molecules acting on this parasite.
In the sphere of general works dealing with parasitological disciplines, including trypanosomes, the publication of the following should be noted: *Traité de Protozoologie médicale comparée* by J. Euzéby (2), and a work dealing with all aspects of parasitology, *Parasitology in Focus. Facts and Trends* by H. Mehlhorn (9). Furthermore, an international expert committee of the World Association for the Advancement of Veterinary Parasitology (WAAVP) has suggested a *Standardised Nomenclature of Animal Parasitic Diseases* (SNOAPAD), systematically adopting the suffix “ose” in French and “osis” in English, to designate the different parasitic diseases, rather than “ose” and “iase” in French and “osis” and “iasis” in English (13).

**NEW INFORMATION REPORTED AT THE MEETING**

**Epidemiological information**

In Kenya, Dr A.R. Njogu considers that *T. evansi* infection constitutes the major obstacle to the development of camel production in the dry regions of the country. Several projects have been set up to study the diseases, nutrition and animal husbandry conditions of camels, in arid zones in particular. These projects have received support from producers, for the camel is well suited to local conditions and may offer a means of milk production where dairy cows cannot be maintained. The existing national camel population is about 750,000 and the government wishes to increase this figure.

Camel trypanosomosis due to *T. evansi* is principally studied at KETRI (Kenya Trypanosomosis Research Institute).

With regard to Chad, Dr F. Brückle (GTZ) read the summary of a report from Dr D. Schillinger, following a mission carried out in March 1988 in the Province of Ouaddai, in collaboration with the IEMVT. “Djoufar”, the local name for camel trypanosomosis, is considered to be the most important disease of camels in the region. The main symptoms are anorexia, weight loss, agalactia in females and abortion. Diagnosis is based on clinical signs and on the odour of the urine which, according to herdsmen, is characteristic, due to the presence of ketonic compounds. Nomads believe that biting insects play the most important role in disease transmission but that the infectious agent may also be excreted in urine and faeces, and thus picked up again by biting insects. The prevalence of the disease is particularly high after the rainy season, notably in camels in the Southern Ouaddai (high multiplication of biting insects). Camel trypanosomosis is not transmitted by tsetse, which are absent from this province of Chad. There is no treatment for “djoufar” using traditional medicines. Sometimes the advice of the veterinarian is sought, but, where this is impossible, trypanocide drugs are obtained from the Sudan – for example, Antrycide and Berenil. However, these drugs are rarely used at the correct dosages since the instructions are in English. This results in under-dosage, incorrect administration (intravenous instead of subcutaneous or intramuscular), insufficient concentrations of the active principles and the use of inappropriate instruments for the injections.

In the Philippines, Prof. H.K. Dennig is carrying out research into the possible existence of a reservoir of *T. evansi* in the various species of monkey of the genus *Macaca*. To date, he has found a very low level of infection. With regard to transmission by insects other than *Tabanidae* and *Stomoxys* spp., he and his research team have found evidence for the possible role of some species of *Triatoma* (8).
In the People's Republic of China, Dr Zhang Zheng Qing of the Parasitology Section of the Shanghai Provincial Veterinary Laboratory indicated that in recent years *T. evansi* infections in domestic animals have been identified in greater numbers in several provinces of Southern China, particularly in Anhui, Yunnan and Zhejiang. In 1985, a total of four million large animals (cattle and buffalo) were tested in Anhui Province. Of these, 110,000 were subjected to the indirect haemagglutination test (IHT) and to the complement fixation test (CFT): 62,758 were found to be infected with *T. evansi* and 8,087 died, resulting in a 12.88% mortality rate. In Yunnan Province, 400,000 cattle subjected to the same tests gave 8% positive reactions (32,000 animals). In the Province of Zhejiang, a 15.3% positive rate was obtained (52,000 animals infected out of 340,000 cattle tested).

**Suggested diagnostic techniques for epidemiological surveys**

*a)* Professor N. van Meirvenne suggested a method using a detergent to detect trypanosomes in blood.

- Principle: After several dozen detergents had been screened for removal of erythrocytes and leukocytes, one was chosen for the detection of motile trypanosomes in lysed blood. In the concentrated form, the detergent may be used to clarify and facilitate classical wet blood film preparation and to speed up examination by optical microscope. Readings may then be carried out at a magnification of 100 x. In the diluted form, the detergent can be used to process large quantities of blood. Trypanosomes are detected in the centrifugation deposit.

Prof. van Meirvenne will supply a demonstration kit on demand, free of charge, enclosing a description of the technique and the procedure to be followed. A publication is in preparation.

In reply to a question from Dr R. Boid, Prof. van Meirvenne stated that he had used the technique extensively in the field in Uganda.

*b)* Dr V.N. Nantulya presented an enzyme immunoassay method using monoclonal antibodies to detect trypanosomal antigens of *T. evansi*.

This method appears to be highly specific and has been tested, with the collaboration of Prof. R. Hamers and Dr E. Bajyana-Songa, on sera from different animal species: buffalo and pigs (Thailand, Indonesia), camels (the Sudan), cattle (Colombia).

Dr R. Boid asked if a correlation existed with experimental parasitaemia and if the monoclonal antibody was strictly specific to *T. brucei evansi* and thus excluded *T. brucei brucei*. He also felt it would be interesting to know how long the antigen persists after efficacious treatment with a trypanocide.

Dr L. Touratier wished to know if such a method was economical and if the test itself could be carried out on the spot.

Dr Nantulya stated that the antibody used for the test recognised a membrane antigen common to the *brucei* group, but that it was *T. evansi*-specific. Seropositivity is not necessarily linked to parasitaemia since it was possible to detect (in the pig) animals which did not present any trypanosomes on direct examination and because the antigen disappeared from peripheral circulation in one to two weeks after efficacious treatment with a trypanocide. As for the cost of using the technique and its application in the field, these have not yet been determined.
c) Dr Boid presented some aspects of the work carried out at the CTVM (Edinburgh) in the form of brief summaries of articles in press:

- Improving the diagnosis and control of trypanosomosis and other vector-borne diseases of African livestock using immunoassay methods, by A. Luckins (a research programme under the auspices of the International Atomic Energy Agency with the financial support of the Netherlands);
- Serological diagnosis of *T. evansi* in camels in the Sudan, by P.F. Rae, M. Thrusfield, A. Higgins, C. Aitken, T.W. Jones and A.G. Luckins;
- Isolation of a *T. evansi*-specific antigen for use in an ELISA test to detect circulating antibodies in *T. evansi*-infected animals, by R. Boid and T.W. Jones.

d) With regard to the detection of antibodies, the subject of the research commented on by Dr Boid in his report, Prof. N. van Meirvenne drew attention to a “slide agglutination test for the detection of anti-trypanosomic antibodies” which he has developed and which are presented in the form of kits ready for use. Such kits are freely available, on request, for field evaluation.

Using the Card Agglutination Trypanosomosis Test (CATT) which Prof. Hamers had supplied him the year before, Dr P. Christy carried out an epidemiological survey on the presence of *T. evansi* in camels in Mauritania. The results were presented in tabular form by Dr M. Clair. Some discrepancies were observed between the results of blood smear examination and those given by the CATT (smears positive whereas CATT remained negative). However, in the ensuing discussion it became clear that other species of trypanosomes (*T. brucei brucei*, *T. vivax*) may interfere with results since, in certain zones of Mauritania, camels may be bitten by *Glossina*. Trials must therefore be continued in light of this new information.

Prof. R. Hamers and Dr E. Bajyana-Songa then presented a new CATT which uses an early *T. evansi* antigen (VAT RoTat 1.2). In collaboration with E. Wittouk, they have written an article which is currently in press: “*Trypanosoma evansi*: serological and kinetoplast sequence evidence for a homogeneous species and diagnostic perspectives”.

In their research they used k-DNA probes to confirm the specificity of *T. evansi* and allow a differential diagnosis with other trypanosomes carrying kinetoplasts of the species *T. brucei brucei* and *T. brucei equiperdum*. The problem remains, for the present, of differentiating between akinetoplastic strains of the latter two species. A variety of strains from Africa, South-East Asia and South America were compared.

**Basic research**

Besides the work carried out by the team of Prof. Hamers and his collaborators mentioned above, and that of the CTVM notably on enzyme modifications of *T. evansi* in order to explain the occurrence of drug-fast strains, other participants gave details of their current research.

Dr A.R. Njogu mentioned the activity of KETRI in the following fields: differing transmissibility of trypanosomes according to vectors; isoenzyme and kinetoplast DNA studies of trypanosomes; study of differing sensitivity of certain strains of trypanosomes resistant to other trypanocides.

Prof. F. Hörchner presented an article currently in press: “Antigenic variation of a *Trypanosoma evansi* clone in vivo and in vitro”, by W. Beck, E. Rudolf,
J. Ahmed and F. Hörchner, in which the authors examine the occurrence and sequence of antigenic variation of *T. evansi* clones in rabbits and mice as well as in rabbit fibroblasts. Using an immunolysis test it was concluded that the VAT-repertoire of *T. evansi* during an infection is rather small in comparison with its genetic potential.

**Trials using new trypanocides**

At present, only the arsenical derivative already reported on during the two previous meetings of the Working Group has been subjected to tolerance and efficacy tests in the field, though it has not yet been commercialised.

Dr J.P. Raynaud gave a documented presentation of this new molecule, synthesised by the Rhône-Mérieux pharmaceutical laboratory. It is a new trivalent arsenical derivative, given the code name **Mel Cy**, chemically related to Melarsoprol (registered name: Arsobal) and used in Africa for the curative treatment of human trypanosomosis due to *T. gambiense* and *T. rhodesiense* (sleeping sickness). The product is presented as a dry powder highly soluble in water or saline solution. Once prepared, the solution must be used rapidly, by subcutaneous or intramuscular injection.

**Mel Cy** has proved to be purely curative, having no prophylactic effect, in respect to *T. brucei brucei* and *T. brucei evansi*, as has been demonstrated in comparative tests with Melarsoprol in mice.

The **toxic dose** is between 5 mg and 7.5 mg per kg live weight according to results observed in dogs, goats, sheep, cattle, buffalo, horses, pigs and camels.

The **curative dose** would appear to be between 0.3 mg/kg and 0.5 mg/kg according to tests carried out on different animal species in Indonesia, the Niger, Ethiopia and Kenya.

In reply to questions, Dr Raynaud stated that he was ready to help those wishing to carry out tests by sending them the product and the experimental procedure which must be strictly followed.

Prof. T. Baltz reminded participants that he could evaluate the trypanocide activity of the sera of treated animals, using the method which he had presented to the Working Group in 1986.

Dr M. Clair then presented a summary of trials carried out in Niger, in 1985, using **Mel Cy** (registered name: Cymelarsan) thanks to cooperation between the IEMVT and the INRAN (Institut National de Recherches Agronomiques du Niger). These trials are reported in an article, currently in press, to be published in *Revue d’Elevage et de Médecine vétérinaire des Pays tropicaux* (11).

**Other matters**

Dr W.N. Masiga reminded participants that, at the 20th Meeting of the "Conseil Scientifique International pour la Recherche et la Lutte contre les Trypanosomoses" (CSIRLT) to be held in Mombasa, Kenya, from 10 to 14 April 1989, a special section on *T. evansi* was programmed, and that representatives from countries outside Africa where *T. evansi* infections are rife, were cordially invited to attend.
CONCLUSIONS

In a number of fields, the information received and the research presented during the ninth Meeting highlighted encouraging points in the following areas:

- in epidemiology, with improved knowledge of the distribution of *T. evansi* infections, thanks to information concerning India, Indonesia, Oman, the Philippines, Thailand, Kenya, Chad and the People's Republic of China,

- in diagnostics, with the proposal of several tests developed to detect *T. evansi* and the free supply of kits, on demand, by the following: Prof. N. van Meirvenne for the microscope examination of lysed blood cells and the slide agglutination test, Prof. R. Hamers for the CATT and Dr V.N. Nantulya for the enzyme immunoassay test using monoclonal antibodies,

- in therapeutics, with the proposal by Dr J.P. Raynaud for participants to experiment with a trypanocide which has already produced promising results with large animals in a variety of countries. All these developments are in accordance with the aims set by the first Meeting of the Working Group in May 1983.

* REFERENCES *


