REPORT OF THE MEETING OF THE OIE AD HOC GROUP
ON NON-TSETSE TRANSMITTED ANIMAL TRYPANOSOMOSES

Paris, 27 May 2001

The meeting of the Ad Hoc Group of the Office International des Epizooties (OIE) on non-tsetse transmitted animal trypanosomoses (NTTAT) was held at the OIE headquarters on 27 May 2001. The Agenda and List of Participants are given in Appendix I and Appendix II respectively.

Dr J.E. Pearson, head of the OIE’s Scientific and Technical Department, welcomed the participants on behalf of Dr B. Vallat, Director General of the OIE, who had been appointed by the opening session of the 69th General Session of the OIE International Committee, and passed on Dr Vallat’s welcome message. He then handed over the floor to Dr H.M. Solomon, Chief Livestock Project Officer of OAU/IBAR. The Secretary General of the Group, Dr L. Touratier, was appointed rapporteur.

After the Secretary General of the Group had presented the interim report, several presentations were made concerning the various items on the agenda.

1. Interim report of the Secretary General

1.1. Scientific meetings on trypanosomoses and the means for combating them

Third Internet Conference on Salivarian Trypanosomoses and other Trypanosomatidoses
(Tryplink-l@cenargen.embrapa.br, organised by FIOCRUZ, Brazil 17-18 October 2000, Contact: A. Davila)

Below are a few of the numerous documents sent on the various agenda items (biology and ultrastructure, biochemistry and development of medicinal products, molecular biology, immunology and pathology, epidemiology, vectors, phylogenesis and evolution):

- Pharmacokinetic study of suramin in Indonesian cattle from the Ongole and Madu breeds;
- Phylogenetic analysis of parasitic protozoans based on the elongation of genic factors;
- Partial purification of specific antibodies of sheep sera against strains of *Trypanosoma evansi*;
- Immunisation of cattle with cysteines proteases of *T. congolense*: targeting the disease rather than the parasite;
- Effectiveness of melarsomine (Cymelarsan) in mice infected with strains of *T. evansi* isolated in Argentina;
- What test should be used for *T. evansi* surveillance in Australia and New Guinea?

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1 OAU/IBAR: Organisation for African Unity/InterAfrican Bureau for Animal Resources
International Seminar on Parasites, Resistance and Access to Medicinal Products (Institute of Tropical Medicine of Antwerp, Belgium, 4-6 December 2000)

A large part of this seminar was given over to studying problems associated with African sleeping sickness. However, human and animal trypanosomoses had many aspects in common: resistance to trypanocides, lack of new specific substances, availability of existing medicinal products, etc. A number of papers illustrated these points:

- Surveillance of resistance (to trypanocides) in African sleeping sickness;
- Scarcity of medicinal products for treating “forgotten” diseases: inevitable stockout or curable public health problem?
- Pre-clinical and clinical development of new trypanocides: industry standpoint;
- Availability and supplies of medicinal products for African sleeping sickness;
- Proof of the activity of melarsoprol by means of an in vitro sensitivity test using the patient’s serum;
- Resistance to megazol in T. brucei.

Meeting of the French Parasitology Society [Société française de Parasitologie] (University of Versailles, France, 21-23 February 2001)

A paper stemming from large-scale international cooperation between CIRDES², Burkina Faso; the Freie Universität Berlin, Germany; the University of Guelph, Canada; the University of Glasgow, United Kingdom; the ILRI³ and OAU/IBAR, Kenya, was presented on: “Trypanosomoses of cattle and resistance to trypanocides in cattle from the province of Kenedougou, Burkina Faso”.

Third Biennial Parasitology Meeting of the CNRS/DGA/DCSSA/MENESR⁴: “Genes in the field”

More than 100 papers and/or posters were presented at this basic science meeting. Some referred to the study of trypanosomes in general:

- Developing a network and biocomputing tools for parasitologists;
- Components of a mitochondrial segregation mechanism for trypanosomes;
- Characterisation of a family of genes coding for fumarate reductase in T. brucei;
- Affinity of a trypanosome thioltransferase for a family of trypanocidal molecules and of this trypanosome’s chemotactic activity in vitro;

Fourth COST-B9⁵ Congress on Anti-Protozoal Chemotherapy (Lisbon, Portugal, 6-9 May 2001)

Numerous papers showed molecular biology’s contribution to studying the genomics of trypanosomoses. The close cooperation between the Tropical Medicine Research Institute and the Veterinary Science Faculty of the University of Khartoum (Sudan) which had taken T. evansi as a model for studying resistance to suramin, a trypanocide used against African sleeping sickness and against surra in camels, gave rise to a paper on “the characterisation of suramin-resistant T. evansi strains”.

Characterisation was done using molecular carotyping to compare the isolates of 16 strains of T. evansi by pulse field gel electrophoresis. The isolates came from Sudan’s eastern, central and western regions. It was shown that the type XII malic enzyme is an indicator of resistance to suramin in T. evansi.

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² CIRDES: International Research Centre for the Development of Animal Husbandry in Humid and Sub-Humid Regions
³ ILRI: International Livestock Research Institute
⁴ CNRS/DGA/DCSSA/MENESR: Centre national de la recherche scientifique/Direction générale de l’armement/Direction centrale du service de santé des armées/Ministère de l’éducation nationale: enseignement, science et recherche
⁵ COST: European Cooperation in the field of Scientific and Technical Research, funded by the European Union (DGXII) and sponsored by the International Organisation for Chemical Science in Development (IOCD)
There were other interesting contributions:

- Therapeutics for African sleeping sickness (six trypanocides are used at present: melarsoprol; suramin (which has ceased to be produced); dimetridazole (commonly used in animals); nifurtimox; difluoro-methylornithine or DFMO). In recent discussions with the WHO, two pharmaceutical companies had agreed to donate large quantities of melarsoprol (Arsobal from Aventis) and suramin (Naganol from Bayer) to treat the 300,000 sick people in Africa who face certain death unless they get treatment.

- Functional genomics and validation of the target by RNAi. RNAi is a recently described phenomenon in which the presence of double-stranded RNA leads to the specific degradation of homologous messenger RNA.

- Pterin metabolism and resistance to antifolate medicinal products in trypanosomatidae;

- Absorption, distribution and metabolism of DB289, an active orally-administered pro-drug for treating African trypanosomoses.

- Pentamidine-type molecules as medicinal products to combat trypanosomiasis and leishmaniasis. A new look at an old therapeutic agent.

The above two papers reported on a study of the metabolites (and their derivatives) of pentamidine, which was formerly used as a chemoprophylactic agent for treating trypanosomoses. They may open up a new research avenue and are currently being studied by a group of American researchers at the University of North Carolina, with large-scale international collaboration between America, Africa and Europe. The powerful international William and Melinda Gates Foundation is funding the research and the first results from monkeys infected with *T. rhodesiense* appear promising.

1.2. Bibliographical data

Numerous articles were devoted to the study of *T. evansi* in various countries:

- General expression of the variable type antigen RoTat1.2 in *T. evansi* isolates of various origins;

- Prevalence of trypanosomosis by *T. evansi* in camels in West Niger;

- Prevalence of *trypanosomosis* by *T. evansi* in camels in the district of Likipia in Kenya;

- *T. evansi* infection in dromedaries in Jordan;

- Detection of *T. evansi* in the brains of hog deer in Thailand that had been naturally infected with biotin/streptavidin using immunochemistry;

- Trypanosomosis of dromedaries in the Canary Islands: determination and seroprevalence using CATT/*T. evansi* and parasitological tests;

- Health of camel herds and productivity in eastern Ethiopia;

- Biological and biochemical characterisation of *T. evansi* isolates in the Pantanal of Mato-Grosso, Brazil;

- Comparative study of methods for diagnosing trypanosomosis in experimentally infected buffalo in Vietnam;

- Ten years of research into the tropical parasitology of dromedaries, cattle and small ruminants in Mauritania;

- Comparison of serological tests in natural infections with *T. evansi* in rice-paddy buffalo in North Vietnam;

- Comparative assessment of parasitological tests and of PCR for diagnosing *T. evansi* in experimentally infected buffalo;

- Interaction between *T. evansi* and *H. contortus* infections in goats in India.

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6 WHO: World Health Organization
7 RNAi: ribonucleic acid interference
8 mRNA: messenger ribonucleic acid
9 CATT: Card agglutination trypanosome test
10 PCR: Polymerase chain reaction
2. Information communicated to the Office International des Epizooties by the Member Countries

The documents which the Member Countries had provided to the 69th General Session on their animal health situation included the following references to NTTATs:

**Dourine (T. equiperdum)**

*South Africa* (18 outbreaks, 46 cases, 4 slaughtered).

*Botswana* (11 outbreaks, 13 cases).

*Kyrgyzstan* (4 outbreaks, 94 cases, 94 horses slaughtered).

*Lithuania* (1,131 tested, 2 positive to the CFT\(^{11}\), no clinical signs observed).

*Namibia* (4 outbreaks, 10 cases).

*Pakistan* (present).

*Peru* (295 sera were subjected to the CFT in the departments of Cuzco, Cajamarca, Piura, Ayacucho, Lima, Ica and Apurimac with negative results. The samples for the tests were taken from horses of different origins, as well as from donkeys and mules, demonstrating that the country is free from the disease.

*Russia* (50 outbreaks, 910 cases, 8 deaths, 902 slaughtered).

**Surra (T. evansi)**

*Argentina* (presence limited to specific zones).

*Brazil* (presence limited to specific zones, 1 outbreak, 1 case in horses).

*Egypt* (2 cases in buffalo).

*Eritrea* (present in horses).

*India* (3 outbreaks, 15 cases in camels).

*Indonesia* (present in horses).

*Iran* (9 outbreaks, 26 cases in camels).

*Jordan* (2 outbreaks, 6 cases in horses).

*Pakistan* (present in cattle and horses).

*Tunisia* (3 outbreaks, 59 cases in camels).

Note: By adding information drawn from scientific publications (see point 1.2.) to the data provided by the central administrations of the above ten countries, we note that infections by *T. evansi* were reported in the following 17 countries in 2000: Argentina, Brazil, Canary Islands (Spain), Egypt, Eritrea, Ethiopia, India, Indonesia, Iran, Jordan, Kenya, Mauritania, Niger, Pakistan, Thailand, Tunisia and Vietnam.

**Infection by T. vivax (in the absence of tsetse flies)**

*Sudan*: recent epidemiological surveys in the tsetse fly free zone, covering Khartoum and Central Sudan, have revealed an infection rate of 2% by *T. vivax* in dairy cows. Tabanidae and Stomoxes had been captured during the survey. The disease was reported in two districts.

3. Dourine and differentiation between *T. equiperdum* and *T. evansi*

3.1. Comparative tests in the OIE dourine reference laboratory (VIEV, Moscow, Russia) on the reagents used in seven countries to diagnose the disease by means of the complement fixation method

Dr Solomon handed over the floor to Dr Touratier who presented the now-complete table of results.

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\(^{11}\) CFT: Complement fixation test
Table 1

Results of comparative tests on the complement fixation tests used in seven countries to diagnose dourine

<table>
<thead>
<tr>
<th>Origin of the serum</th>
<th>Antigen of <em>T. equiperdum</em></th>
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<tbody>
<tr>
<td></td>
<td>China titre 1:16</td>
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<tr>
<td>USA positive titre 1:20</td>
<td>1:10 2+</td>
</tr>
<tr>
<td>USA positive titre 1:20-1:40</td>
<td>1:40 3+</td>
</tr>
<tr>
<td>USA positive titre 1:40</td>
<td>1:320 3+</td>
</tr>
<tr>
<td>Russia positive titre 1:10</td>
<td>1:10 2+</td>
</tr>
<tr>
<td>Russia positive titre 1:40</td>
<td>1:40 2+</td>
</tr>
<tr>
<td>France positive titre 1:10</td>
<td>1:40 2+</td>
</tr>
<tr>
<td>Germany positive titre 1:80</td>
<td>1:40 2+</td>
</tr>
<tr>
<td>Germany positive titre 1:320</td>
<td>1:80 3+</td>
</tr>
<tr>
<td>Italy positive titre 1:96</td>
<td>1:40 3+</td>
</tr>
<tr>
<td>South Africa positive titre 1:32 4+</td>
<td>1:40 2+</td>
</tr>
<tr>
<td>USA negative</td>
<td>negative</td>
</tr>
<tr>
<td>Germany negative</td>
<td>negative</td>
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<tr>
<td>Russia negative</td>
<td>negative</td>
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<tr>
<td>Italy negative</td>
<td>negative</td>
</tr>
<tr>
<td>South Africa negative</td>
<td>negative</td>
</tr>
</tbody>
</table>

(Source: V.T. Zablotsky, Ch. Georgiu, *The All-Russian Research Institute of Experimental Veterinary Medicine, Laboratory of Protozoology, 109472, Moscow, Kuzminki, Viev*)

The table, drawn up mainly in May 2000, did not include South Africa. The reagents sent by South Africa’s Onderstepoort Veterinary Institute had, in fact, been rendered unusable by defective transport and storage and the second dispatch reached the VIEV only in autumn 2000.

Dr Devolz considered these results to be satisfactory. However, over a six-month period, sometimes positive, sometimes negative temporary reactions to the complement fixation test (CFT) were observed in racehorses imported in 1998. Perhaps the same thing could happen with any other diagnostic method currently available.

Filip Claes pointed out that CFT was a group diagnosis: it could only establish a diagnosis for the *brucei* group, which included three trypanosome species pathogenic for horses: *T. brucei brucei*, *T. evansi* and *T. equiperdum*, with no possibility of distinguishing between the three species.

Dr Musiime agreed with his comment and hoped that supplementary research would lead to the development of a diagnostic method that was not only more accurate than CFT but also more reliable.
3.2. Follow-up: isolation of new strains of *T. equiperdum* and differentiation between *T. evansi* and *T. equiperdum*

Dr Touratier said that the tests carried out at the VIEV had been suggested by the Ad Hoc Group at its annual meeting in May 1999 and that Dr Avilov, the then head of the main veterinary department of Moscow’s Ministry of Agriculture, had asked for the aforementioned tests to be carried out as a starting point for subsequent studies. In October 1999, Professor Büscher drafted this research project, which was submitted to the Director General of the OIE. Whilst awaiting funding, the Institute of Tropical Medicine of Antwerp had guided a student, Filip Claes, towards a PhD thesis whose main guidelines coincided with that of the research project. The first results from this plan had already been presented in May 2000 and consisted of a current state of knowledge report on research into dourine (small number of *T. equiperdum* strains available in the world, test to identify variable antigen types (VAT) of these strains, choice of molecular biology techniques for attempting to precisely identify the two trypanosome species).

Dr Solomon then handed over the floor to Dr Filip Claes for a presentation of the results of the research programme into dourine and the differentiation between *T. equiperdum* and *T. evansi*.

The programme had involved three phases:

(i) *Expanding the collection of available strains in the Antwerp laboratory’s cryobank*

In addition to the 38 *T. evansi* strains, it had been possible to obtain 11 strains of *T. equiperdum* (one Antwerp strain, one “BoTat 1.1” Bordeaux strain, one “OVI” Onderstepoort strain, three USA strains, one Canada strain, one Switzerland strain, and three Germany strains).

(ii) *Analysis of serological cross-reactions between *T. equiperdum* and *T. evansi***

For *T. evansi*, it was established that the RoTat 1.2 strain is an early and ubiquitous variable antigen (VAT) whose antibodies can be detected in the serum of infected animals (rabbits, camels, buffalo, cattle). This made it possible to monitor the development of the antibodies in rabbits infected with this strain using immune trypanalysis.

Moreover, cross-reactions were studied between the RoTat 1.2 *T. evansi* strain and the infection in rabbits inoculated with the various strains of *T. equiperdum*. The early responses to the RoTat 1.2 antibodies were analysed using three tests: CATT/*T. evansi*, ELISA/12/*T. evansi*, immune trypanalysis.

All of the *T. equiperdum* strains responded positively to the three tests, except the OVI strain (negative in all three tests) and the BoTat 1 strain (negative only to the immune trypanalysis test).

The other strains are therefore closely related to *T. evansi* and yet they are used in national laboratories to diagnose dourine using the CFT.

An article had been prepared for publication in the *Annals of the New York Academy of Sciences*.

(iii) *Molecular biology: variable surface glycoprotein (VSG) of the RoTat 1.2 strain of *T. evansi***

The DNA/13 sequence of the VSG of *T. evansi* RoTat 1.2 was used as the basis for identifying a pair of primers from the non-homologous region of the VSG sequences. Subsequently, polymerase chain reaction yielded a product that was detected in all the *T. evansi* strains and in seven out of nine *T. equiperdum* strains. In this case, too, only the OVI strain and the BoTat 1 strain of *T. equiperdum* were negative using PCR. Similarly, the PCR carried out using the isolated DNA of *T. b. brucei*, *T. b. gambiense*, *T. b. rhodesiense*, *T. congolense*, *T. vivax* and *T. theileri* did not yield this amplification product.

The molecular biology results concurred with the serology results and suggested that there were at least two groups of *T. equiperdum* strains in the laboratory’s collection.

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12 ELISA: enzyme-linked immunosorbent assay
13 DNA: desoxyribonucleic acid
An article had been prepared for publication in *Experimental Parasitology.*

In view of these results, it would now be a good idea to define two diagnostic tests:

- An easy-to-use, 100% sensitive screening test (e.g. the direct or indirect agglutination test)
- Followed by a specific test for 100% confirmation (e.g. PCR).

Tests on horses should then be carried out using the available strains and perhaps also recently isolated strains in order to verify whether or not the *T. equiperdum* strains stored for decades in the cryobanks of the official diagnostic laboratories had suffered antigenic variations.

In answer to the audience’s questions about funding for this research programme, F. Claes said that up until then the Flemish government had funded the work carried out by the Institute of Tropical Medicine of Antwerp, but that funding for the tests on horses was not yet confirmed. These tests would be carried out at the Onderstepoort laboratory in South Africa as part of a budget that had yet to be established.

Dr Jeggo suggested that an AbELISA would make it possible to differentiate between the two similar trypanosome species. Dr Touratier agreed, but added that this AbELISA had to be prepared from a true strain of *T. equiperdum,* recently isolated from a case of clinical dourine and that the crux of the problem was to isolate it.

On a more general issue, Dr Desquesnes had sent the secretariat a letter with the preliminary results of a project being conducted with Dr Dávila on “the detection and identification of trypanosomes using a single PCR”. Unfortunately the PCR had been unable to distinguish the species within the *Trypanozoon* sub-genus.

In another article, published jointly with his colleagues from CIRDES, Dr Desquesnes analysed the cross-reactions detected using AbELISA in ruminants with natural multiple infestation from *T. vivax, T. congolense* and *T. b. brucei* and compared them with the cross-reactions from sheep experimentally infected with *T. evansi, T. vivax* and *T. congolense.* However, the results had been specific only by genus and not by the different species.

Dr Solomon asked Dr C.A. Monzón to present his paper:

**MONZÓN C.M., MANCEBO O.A. & RUSSO A.M. – Antibodies follow-up in *T. evansi* infected horses after treatment with quinapyramine sulphate analysed by indirect ELISA test**

Fourteen horses naturally infected with *T. evansi* (detected using the Woo method and inoculating the blood into mice controlled twice weekly for 60 days by taking twice-weekly samples from the tail vein) were left out to pasture with six horses that had been found to be free using ELISA and parasitological examination. After that, all of the horses were treated with quinapyramine sulphate (3.5 mg/kg of live weight sub-cutaneously), then tested using the ELISA method and parasitological tests three, four, ten, twelve and twenty-two months after treatment. The antibodies, detected using indirect ELISA gradually diminished before disappearing at varying times. Two horses relapsed after four and 12 months, but these could have been reinfections which are common in areas like Formosa, where equine trypanosomosis is endemic. Graphs showed the evolution of the antibodies.

Filip Claes was interested in Dr Monzón’s paper because tests carried out by the Institute of Tropical Medicine of Antwerp are currently being carried out in Vietnam on horses infected with *T. evansi.*

Dr Solomon then asked Dr Touratier to present the paper of the Chinese researchers from the Shanghai Institute of Animal Parasitology:

**LIAO DANGJIN & SHEN JIE. – Isolate of cDNA fragments from *T. evansi* involved in quinapyramine (Antrycide) resistance**

Using molecular biology techniques, the creation and study of numerous cDNA fragments from *T. evansi* allowed a regulator gene to be isolated which is inhibited by the activity of antrycide, which would explain the resistance of *T. evansi* to this trypanocide. However, a number of genes might be responsible for this phenomenon.
Dr Jeggo said that the above-mentioned papers aptly illustrated the growing problem of trypanocide resistance and current research to find out what underlying mechanism caused them to appear. In view of the very small number of effective trypanocides, it was very worrying to see this phenomenon counteracting their effectiveness, since no vaccine existed at present that could immunise animals against trypanosomoses.

However, Filip Claes pointed to the development of a measure of surra immunity among buffalo in Vietnam. This prompted Dr Solomon to mention the following summary, sent by colleagues from the Shanghai Institute of Animal Parasitology:

ZHOU JINLING, WANG QUAN, ZHOU YONGZHI & SHEN JIE – *A preliminary experiment on immune protection according to regularity antigenic variation in Trypanosoma evansi*

Three batches of mice were used to test the effect of a complex antigen containing the VAT of the ShTat 1 and ShTat 1.2 strains of *T. evansi*. After injecting one or other, or both, of these strains, the mice were challenged with infection from 100 ShTat 1.2 trypanosomes. All of the mice that had been immunised with both strains were still alive 30 days following the infective test. So it appeared that the test might benefit from a certain regularity antigenic variation of *T. evansi* to produce a preventive effect.

Dr Touratier compared these results with the work carried out by the Simón Bolívar University in Caracas, Venezuela where a *T. evansi* protein, known as ‘evansina’, had been identified as the ‘congopain’ of *T. congolense* currently used in prevention tests in Africa, not against the causal trypanosome, but against the disease it causes (programme financed by the European Union in accordance with an INCO-DGXII14 and accepted by GFAR15).

This seemed to be an appropriate time to report on a study on the experimental infection of thoroughbred horses by a cameline strain of *T. evansi*, the publication of which was announced on 28 May 2001 by Dr Tom Norton, Veterinary Adviser, Department of Animal Wealth, Dubai (United Arab Emirates). The text had been sent to the Ad Hoc Group’s secretariat at the end of the OIE’s 69th General Session. The study was submitted to Group members for comments.

WERNERY U., ZACHARIAH R., MUMFORD J.A. & LUCKINS A.G. – *Preliminary evaluation of diagnostic tests using horses experimentally infected with *T. evansi***

Seven surra-free thoroughbred horses were intravenously inoculated with an infectious dose of $3 \times 10^6$ *T. evansi* of cameline origin. One horse was used as a control. Then various diagnostic tests were used:

- The microhematocrit centrifugation test (MHCT) was the most sensitive, isolating parasites between one and three days following infection;
- The AgELISA test detected the parasites between three and ten days following infection;
- The latex agglutination test (LAT) gave the first positive result three days following infection;
- After treating the horses with quinapyramine sulphate and/or melarsomine, the PHCT and the mouse inoculation test became negative;

According to the LAT, the antigen levels declined and returned to normal in five out of six horses during the observation period (92 to 279 days).

According to the AgELISA, the antigen levels also declined but failed to return to the pre-infection levels in any of the six inoculated horses.

Three techniques were used to detect antibodies: AbELISA, CATT and IFAT16 detected antibodies in seven infected horses but not in the control.

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14 INCO-DGXII: International Cooperation of the European Union
15 GFAR: Global Forum on Agricultural Research
16 IFAT: indirect fluorescent antibody technique
However, the AbELISA was unable to clearly distinguish antibodies between the infected horses and horses that had come directly from the United Kingdom. The antibodies increased following infection in six horses but not the seventh, except after it relapsed.

The IFAT detected the antibodies 15 days following infection.

The CATT gave positive results for all seven horses between seven and nine days following infection. However, differences were observed in the degree of agglutination, ranging from low to very high.

Professor Büscher from the Institute of Tropical Medicine of Antwerp sent the secretariat the following remarks on this article:

i) The idea of a study to compare the performance of several diagnostic tests for isolating *Trypanosoma evansi* infection in horses is excellent, but the presentation of “Equipment and methods” in this article is incomplete.

ii) In fact, in the case of CATT, the authors appear to be confusing CATT/*T. gambiense*, developed by E. Magnus for African sleeping sickness, with CATT/*T. evansi*, developed by B. Songa for *T. evansi* infections in animals. While the principle of the two tests is the same, the antigens used are completely different. The authors did not say which of the two tests they had used. This confusion persists in the discussion.

iii) Furthermore, according to “Equipment and methods” the CATT had been carried out using a Tittertek stirring rod. It is uncertain whether the recommendations in the CATT instructions had been followed and the Tittertek was possibly not suitable for carrying out the test. This could explain why pre-infectious sera were positive and why the degrees of agglutination could not be interpreted.

iv) The authors rightly stated that the CATT results could not be interpreted. This is why we propose to test the sera once again, if they are still available, using a CATT/*T. evansi* and trained laboratory personnel before deciding on the procedure for comparing the different tests.

v) Meanwhile, our laboratory has developed other tests (latex agglutination, ELISA and immune trypanolysis) which could be applied to the sera collected in this study on experimentally infected horses.

4. **Infection by *Trypanosoma vivax* in the absence of tsetse flies: Africa and South America**

Dr Solomon said that Sudan had sent a report on this point to the secretariat of the 26th meeting of the CSIRLT17, which was due to take place in Ouagadougou (Burkina Faso) from 1 to 5 October 2001.

Dr Jeggo and Dr Musiime successively took the floor to underline their mutual belief that the appearance of *T. vivax* infections in cattle was impossible in the absence of tsetse flies. The cases that had been reported in Sudanese cattle outside of the tsetse belt had probably been caused by some stray tsetse flies that had escaped the detection surveys or had been blown in by the wind. Detailed entomological surveys needed to be carried out. Dr Jeggo recalled the considerable success that had been achieved in Zanzibar through a joint IAEA18/FAO19 programme using the sterile male method, allowing a 700 km² zone to be rid of tsetse flies. It showed that, by using this method in association with the programmed use of insecticides, it was reasonable to expect gradual eradication, extending bit by bit to other African countries.

However, Dr Touratier recalled the facts that had been reported to the various meetings of the Ad Hoc Group in previous years on the presence and persistence of *T. vivax* infections in cattle in areas that had been rid of tsetse flies (see Dr Chizyuka’s statement on Zambia in 1993; Dr Y. Chollet’s report on North Cameroon in 1995; Dr D. Cuisance’s paper on the Central African Republic in 1996). He also cited the Internet discussions in 1999, which had been prompted by the appearance of numerous cases of *T. vivax* infection in cattle in Brazil (Pantanal), as well as several articles on the subject in the *Revue de Médecine Vétérinaire des Pays Tropicaux* from 1996 to 1999. Outbreaks had been periodically reported in Venezuela, Colombia, Brazil and,

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17 CSIRLT: International Scientific Council for Trypanosomiasis Research
18 IAEA: International Atomic Energy Agency
19 FAO: Food and Agriculture Organisation of the United Nations
more recently, in Bolivia. Even though the conditions between the African and South American continents varied greatly, this problem deserved to be studied in more detail because, after eliminating tsetse flies, very careful surveillance of the areas in question would certainly be necessary for a time and that the South American example would be particularly useful.

Dr Monzón confirmed that T. vivax infections had been observed in Argentina and they had extended as far as the northern region.

Meanwhile, the secretariat had received the following short paper:

**DELAFOSSE A. – Epidemicology of T. vivax infection in cattle in a zone of Lake Chad (December 1998-January 2000).**

An epidemiological study had been conducted (prevalence and incidence) using two methods: parasitological examination and indirect ELISA on a cohort of one thousand cattle in an area of Chad that was free from tsetse flies. The two methods produced very different results:

- For prevalence: 1.5% with the parasitological examination and 42% using indirect ELISA;
- For average incidence: evaluated at 15%.

This disparity in prevalence and incidence could be explained by the massive use of trypanocides by livestock producers in the area under study (especially Diminazene diaceturate).

The analysis of seasonal incidence helped to explain the existence of Trypanosoma infection during the transhumance season on the lake’s islands – dry season – and during the resting season on dry land – rainy season and start of the dry season.

The existence of populations of *Glossina* spp. in this area was very improbable since *G. tachinoides* were observed way back in 1966 along the Chari River as far as the southern bank of Lake Chad (Gruvel, 1966). Recent entomological surveys (D. Cuisance, 1996) had shown that the range of *G. tachinoides* had shrunk considerably. Nowadays this species was trapped only close to the 10\(^{\text{th}}\) parallel of latitude north (more than 500 km south of the area under study). This reduction had been explained by the destruction of the gallery forests that previously extended alongside the river. Finally, the lake was situated in the Sahelian zone (not to say the Sahel/Saharan zone) around the 250m/m isohyet. This climatic factor appeared to be potentially damaging to isolated populations of *Glossina* spp.

With regard to Y. Chollet’s epidemiological work in Northern Cameroon in 1994/95, in the vicinity of the Garoua national veterinary laboratory, there had been no follow-up after his departure. The site for his study in this zone of Garoua was very close to the 10\(^{\text{th}}\) parallel of latitude north. The zone bordered the Mayo-Kebbi area of Chad, which was infested with *Glossina* spp., so certain local infestations could occur.

5. **Information on the development of programmes against African trypanosomoses (PAAT and PATTEC)**

Dr Solomon explained in more detail the activities of the PAAT\(^{20}\) and the objectives of the PATTEC\(^{21}\).

The PAAT had been created five years previously under the aegis of three United Nations organisations: WHO, FAO and IAEA, in liaison with OAU/IBAR, in order to create an international forum in fields associated with combating tsetse flies and trypanosomoses: provision of experts, organisation of specialist meetings, sustainable development of animal production and agriculture, etc.

PATTEC was the result of a declaration from OAU heads of state largely based on the IAEA’s work in eradicating tsetse flies (campaign successfully conducted on Zanzibar Island using the sterile male technique), according to the joint IAEA/FAO programme and the IAEA’s technical cooperation department.

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\(^{20}\) PAAT: Programme Against African Trypanosomoses

\(^{21}\) PATTEC: Pan African Tsetse and Trypanosomosis Eradication Campaign
The CSIRLT’s 26th conference (Ouagadougou, Burkina Faso, 1-5 October 2001) had been chosen to officially launch PATTEC. The application programmes were currently being finalised and would gradually be implemented, zone by zone. The first tranche would cover Ethiopia and Uganda, with the aim of totally eradicating tsetse flies.

Dr Musiime described the major phases that would have to be achieved: establishment of the implementation programme, possible reorientation of the programme, European Union funding following a more detailed evaluation and reintroduction of cattle into sanitised zones. All this would take place with the involvement and assistance of all of the governments of beneficiary countries.

6. Combating non-tsetse transmitted animal trypanosomoses

6.1. Trypanocides

In spite of basic research efforts, no new substance was currently being developed to control animal trypanosomoses because there was no applied research due to a lack of public or private funding.

The secretariat had received two short papers which reported favourable results obtained using a combination of diminazene diaceturate, antipyrine and procaine (TRYPAN):

ZHANG XICHEN, YAO LONG QUAN & BOURDICHON A.J. – *The pharmacokinetics and tissue residues of Diminazene, Diminazene liposomes and TRYPAN in rabbits.*


6.2. Global Forum on Agricultural Research (GFAR)

The Ad Hoc Group secretariat had received no specific information on the Forum since its creation in Dresden in May 2000. The organisation was gradually being set up, in liaison with the World Bank in Washington (United States of America), where it had a representative, with the ILRI in Nairobi (Kenya), with FAO in Rome (Italy) and with CIRAD/EMVT in Montpellier (France). The fight against animal trypanosomoses had been the subject of a detailed paper from GFAR at the previous meeting of the Ad Hoc Group in May 2000 and an INCO had been created with European Union funding (DG XII) in order to expand the study into improved methods for controlling animal trypanosomoses and for inducing trypanotolerance in susceptible cattle.

6.3. European cooperation in the field of scientific and technical research (EU/DG XII)

The activity of this European Union programme has already been described above under section 1.1. on its Lisbon Congress and on the scientific information collected there. COST could play a useful role in the search for active substances to treat animal trypanosomes animals, by encouraging basic research.

7. International meetings concerning non-tsetse transmitted trypanosomoses in the near future

- **Fifth COST Meeting**, London, United Kingdom, from 21 to 25 June 2002, London School of Tropical Medicine and Hygiene. Information: Dr Simon Croft.

- **Tenth International Congress of Parasitology, ICOPA X**, Vancouver, Canada, from 4 to 10 August 2002.

- **World Veterinary Congress**, Tunis, Tunisia, September 2002.

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22 CIRAD/EMVT: Centre de Cooperation Internationale en Recherche Agronomique pour le Développement, Department of Animal Husbandry and Tropical Veterinary Medicine
REPORT OF THE MEETING OF THE OIE AD HOC GROUP ON NON-TSETSE TRANSMITTED ANIMAL TRYPANOSOMOSES

Paris, 27 May 2001

Agenda

1. Interim report of the Secretary General
2. Information communicated to the Office International des Epizooties by the Member Countries
3. Dourine and differentiation between *T. equiperdum* and *T. evansi*
4. Infection by *Trypanosoma vivax* in the absence of tsetse flies: Africa and South America
5. Information on the development of programmes against African trypanosomoses (PAAT and PATTEC)
6. Combating non-tsetse transmitted trypanosomoses
7. International meetings concerning non-tsetse transmitted trypanosomoses in the near future
REPORT OF THE MEETING OF THE OIE AD HOC GROUP ON NON-TSETSE TRANSMITTED ANIMAL TRYPANOSOMOSES

Paris, 27 May 2001

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Appendix II