The meeting of the Ad hoc Group of the Office International des Epizooties (OIE) on Non-Tsetse Transmitted Animal Trypanosomoses (NTTAT) was held at OIE headquarters on 28 May 2002. The Agenda and List of Participants are given at Appendix I and Appendix II, respectively.

Since Dr B. Vallat, Director General of the OIE, was engaged in the opening session of the 70th General Session of the OIE International Committee, Dr J.E. Pearson, Head of the OIE Scientific and Technical Department, welcomed the participants on his behalf and passed on a welcome message from Dr Vallat before handing the floor to Dr J.T. Musiime, Acting Director of AU/IBAR. The General Secretary 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 regarding the various items on the agenda. In addition, a number of papers were presented, either by the participants themselves, or by the General Secretary on behalf of the authors of written papers who had been unable to attend the meeting. This was followed by wide-ranging discussions. Dr Musiime opened the session and handed over to Dr Touratier.

1. Interim report of the Secretary General (May 2001–May 2002)

1.1. Scientific meetings on trypanosomoses and means for combating them

- **Sixth Meeting of the Society for Tropical Veterinary Medicine** (Pilanesberg National Park, South Africa, 22–26 July 2001)

  Two papers were presented on:
  - Progress with research into the problems currently posed by dourine and differentiation between the two types of trypanosomes: *Trypanosoma equiperdum* and *Trypanosoma evansi*.
  - Expression of the variable antigen RoTat 1.2 of *T. evansi* and *T. equiperdum*.

- **Eighteenth Conference of the World Association for the Advancement of Veterinary Parasitology** (Stresa, Italy, 26–30 August 2001)

  Of the papers and posters presented, the following deserve special mention:
  - Clinical and pathological response in buffaloes infected in India with *T. evansi* and subsequent immunosuppression.
Chronic surra in dromedaries in the desert region of northwest India.

T. evansi infection in royal Bengal tigers in Dhaka Zoo, Bangladesh.

Impact of surra on several breeds of dromedary: a pilot study in the district of Laikipia, Kenya.

Influence of nutrition on the physiopathology of animal trypanosomosis.

Response of buffaloes infected with T. evansi to treatment with a combination of diminazene aceturate and procaine.

**Twenty-Sixth Meeting of the International Scientific Committee for Trypanosomiasis Research and Control (ISCTRC)** (Ouagadougou, Burkina Faso, 1–5 October 2001)

As in previous years, many reports, papers and posters were presented at this conference. The most relevant ones, directly concerning NTTATs or general trypanosomosis mechanisms, include:

**Mechanical vectors of trypanosomes:**

- Trypanosomosis in Sudan: past, present, future and prospects.
- Introduction to the study of insects that might be mechanical vectors of trypanosomoses in West Africa.
- Studies of the ecology of Tabanidae in the State of Khartoum.
- Comments concerning the problem posed by trypanosomosis outside the tsetse fly belt.
- Epidemiological aspects of mechanically transmitted trypanosomoses to cattle (T. vivax and T. evansi).

**Diagnosis:**

- A serodiagnostic test (card agglutination trypanosomosis test – CATT) for animal trypanosomosis in Uganda.
- An indirect ELISA\(^2\) cross-reaction suitable for trypanosomoses.

**Trypanocides:**

- Remarks and future prospects concerning a brief history of trypanocides for animal use.
- Study of the metabolism and distribution of phenanthridine trypanocides in T. b. brucei.
- Effectiveness of oral administration of a pentamidine metabolite in green monkeys infected with T. b. rhodesiense.
- Effectiveness of a combination of diminazene aceturate/procaine in dromedaries infected with T. evansi.
- Pharmacokinetics and residues in bovine tissues of C14-labelled diminazene.
- Relationship between the concentration of the medicinal product, its pharmacokinetic properties and chemical prophylaxis with homidium bromide.
- Determination of the diminazene content of various products using high-performance liquid chromatography (HPLC).

The need for new trypanocides was again raised during the discussions that followed the various presentations. However, although basic research into various potential trypanocide derivatives continues, there does not seem to be any real likelihood of new compounds being marketed. The pharmaceutical industry argues the problem of profitability due to the high costs of research, development and registration of such products, which requires research into acute and chronic toxicity, pharmacokinetics, bioavailability and effectiveness for each target animal species in order to comply with the provisions of the Codex Alimentarius.

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\(^2\) ELISA: enzyme-linked immunosorbent assay
Round table on NTTATs

Thanks to the good offices of the organisers of the 26th Meeting of the ISCTRC, a round table was held during a break in a session. Eighteen participants from the following countries attended: Burkina Faso, France, Germany, Kenya, Mali, Nigeria, Sudan, Tanzania, Uganda, and a representative from the ILRI.

The issues discussed included:

- A reminder of the tasks which the OIE Committee had entrusted to the Ad hoc Group and of the actions completed or in progress since its creation in May 1983.
- The persistence of *T. vivax* infections in the absence of tsetse flies (certain areas in Chad and Sudan).
- Experiments in progress to establish a new dourine diagnostic procedure capable of differentiating between *T. equiperdum* and *T. evansi*.
- The use of CATT/T. evansi for the field diagnosis of surra in dromedaries and/or equidae.

Tenth International Veterinary Congress (Moscow, 11–13 April 2002)

The General Secretary of the Ad hoc Group presented the following report during the ‘Equine Section’ of the Congress:

- Epidemiology of equine trypanosomoses (including diagnostic problems), with particular reference to dourine and surra.

International workshop on surra diagnostic techniques (Bogor, Indonesia, 29 April–3 May 2002)

This workshop was held at the national veterinary laboratory (BALITVET) in Bogor, with the support of the Australian Centre for International Agricultural Research (ACIAR).

It had been decided at a meeting held in March 2001 in Los Banos, Philippines, at the headquarters of the Philippine Council for Agriculture, Forestry and Natural Resources, Research and Development (PCARRD), attended by ILRI Philippines, ACIAR and the Central Murdoch University from Perth, Australia, represented by Dr Simon Reid. Dr Reid, who had been commissioned by the Australian Quarantine Inspection Service (AQIS) to experimentally infect Australian marsupials with *T. evansi*, was appointed as the project manager.

At Indonesia’s invitation, the BALITVET national laboratory was chosen because of its experience with surra and its modern facilities enabling practical laboratory demonstrations to be carried out.

The General Secretary of the Ad hoc Group was invited to participate. The workshop was divided into two stages:

- Presentation of reports: Philippines, Indonesia.
- Practical laboratory demonstrations.

The main reports concerned:

- Importance of surra as an NTTAT and the role of the OIE Ad hoc Group in understanding this infection, illustrated by the Symposium on Surra, held in Obihiro, Japan, in August 1998.
- Trypanosomoses caused by *T. evansi* (surra) in Indonesia (cattle, buffaloes, equidae).
- Differential diagnosis of surra in the region (Jembrana disease, malignant catarrhal fever, bovine viral diarrhoea, mucosal disease, arboviruses).
- Pathology and pathogenesis of surra.
- Studies conducted on surra in the Philippines among farm animals at the University of Southern Mindanao.
- Prevalence of *T. evansi* infection in farm animals in the province of Agusan del Sur, Philippines.

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3 ILRI: International Livestock Research Institute
4 CATT: Card agglutination trypanosome test
– Prevalence and distribution of trypanosomosis in cattle and buffaloes in the north of Sumatra and in Aceh.
– Trypanosomosis cases in a few districts of Java.

The practical laboratory demonstrations consisted of:

– An introductory course describing the main diagnostic tests used and the value to be accorded to them; epidemiological surveys (modelling and analysis, with a practical example of a survey conducted on 1,861 animals in the Philippines).
– Laboratory handling procedures with the techniques used for preserving, amplifying and preparing *T. evansi* antigens.
– Laboratory demonstrations of the main tests used: CATT, ELISA, MAECT5, HCT6 and PCR7.
– Abattoir sampling.

A discussion on currently available trypanocides once again revealed the current lack of therapies and the slim likelihood of new products appearing on the market.

1.2. Bibliographic references

Many articles were devoted to the study of *T. evansi*, especially in Asia and South America:

– Overview of publications in Thailand since 1997 on *T. evansi* and surra in various domestic and wild animals: cattle, buffaloes, equidae, hog-deer (*Rusa porcus*) and elephants.
– Anatomic pathology and histopathology of a dog experimentally infected with *T. evansi* (Indonesia).
– Many studies in Vietnam (Vietnamese/Belgian cooperation) on the diagnosis of surra in buffaloes and cattle and on *T. evansi*-induced immunosuppression in buffaloes and its influence on vaccination against hemorrhagic septicemia.
– Evaluation of an immunoenzymatic test for diagnosing surra in cattle (Australia).
– Publications on animal reservoirs of *T. evansi* in South America (coati, capybara = *Hydrochoerus hydrochaeris*) and a plan for *T. evansi* control and containment in Australasia.
– Much fundamental research work on *T. evansi*: total absence of kinetoplastic DNA in Brazilian strains isolated from domestic and wild animals, studies of genetics and molecular biology (France, Brazil, Venezuela).

2. Information reported to the Office international des Epizooties by Member Countries

The documents provided by Member Countries to the 70th General Session regarding their animal health status included the following references to NTTATs:

**Dourine (T. equiperdum)**

*South Africa* (14 outbreaks, 34 cases).
*Botswana* (11 outbreaks, 11 cases, 3 slaughtered).
*Kirghizistan* (142 cases, 142 slaughtered).
*Lithuania* (677 horses tested with negative results).
*Namibia* (12 outbreaks, 28 cases).
*Pakistan* (presence of the disease).
*Russia* (presence of the disease, 142 horses slaughtered).

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5 MAECT: Miniature Anion-Exchange Centrifugation Technique
6 HCT: hematocrit
7 PCR: Polymerase Chain Reaction
Surra and mal de Caderas (*T. evansi*)

*Argentina* (presence of the disease).

*Brazil* (3 outbreaks, 3 cases).

*Egypt* (27 cases in cattle, 21 cases in buffaloes, 11 cases in camels).

*United Arab Emirates* (presence of the disease in camels).

*Eritrea* (presence of the disease).

*India* (1 case in camels).

*Indonesia* (presence of the disease in equidae).

*Jordan* (142 cases in equidae).

*Myanmar* (2 outbreaks, 2 cases in equidae).

*Pakistan* (presence of the disease in cattle and equidae).

*Philippines* (188 cases, 32 deaths in equidae).

*Thailand* (11 cases in equidae).

*Tunisia* (3 outbreaks in camels).

*Uganda* (presence of the disease in equidae).

*Venezuela* (presence of the disease in equidae).

*Vietnam* (1,943 cases in cattle, 128 deaths, 8 slaughtered; 2,835 cases in buffaloes, 162 deaths, 12 slaughtered; presence of the disease in equidae).

This information was supplemented by conversations with a number of permanent Delegates during the General Session:

*In Australia*, the central service constantly monitors the possible extension of the geographic area infected with *T. evansi* in the Indonesian archipelago, and in particular the situation in Irian Jaya in western Papua New Guinea, where *T. evansi* antibodies were detected in young wallabies. We know that experiments carried out on confined wild Australian marsupials – reported to the Ad hoc Group in 1999 – have shown that these animals are extremely susceptible to *T. evansi* (around 90% mortality).

*In Cambodia*, serological surveys conducted by the CTVM⁸ on buffaloes using the enzyme-linked immunosorbent assay test (ELISA) showed a high frequency of surra antibodies (15% to 20% in infected animals). The disease is also common in horses.

*In Indonesia*, surra in all species of domestic animals is considered to be the most serious disease to control after foot and mouth disease, haemorrhagic septicaemia and Jembrana disease in Bali. Unfortunately, now that production of suramin, the oldest of the trypanocides, has stopped, it is not possible to treat surra effectively in horses. We still need to re-evaluate the potency of a few other compounds that act on *T. evansi*, which are still available (melarsomine, diminazene aceturate, iso-metaminiun, quinapyramine), in order to determine suitable dosage schedules whilst preventing secondary effects of toxicity and/or chemoresistance.

*In Mongolia*, the central veterinary inspection services is focusing special attention on the serological survey being jointly conducted by a German/Mongolian team to determine the infection rate of *T. equiperdum* and *T. evansi* among the equine population (around 3 million heads). The provisional results revealed 7% positive results. This is why the research programme, launched by the Ad hoc Group and being conducted by the Institute of Tropical Medicine in Antwerp, is attracting keen interest. The Director General of the Ulaanbaator laboratory would like to participate in the meeting of the Ad hoc Group in May 2003.

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⁸ CTVM: Centre for Tropical Veterinary Medicine
In the Philippines, there is a national surra control programme supported by a diagnostic laboratory with a team of several veterinarians, two of whom participated in the Bogor workshop (see above). In September–October 2001, a purported ‘major epizootic’ of surra in horses had been reported on the Internet by a private veterinarian, which alerted specialised laboratories worldwide and elicited a flurry of questions and answers by email. In fact, following a survey by the specialised Philippine laboratory, only five horses were found to be infected with *T. evansi* and no mortality was reported. In contrast, surra in buffaloes causes serious problems to small farmers due to the significant weakening and even death of their plough animals. The development of chemoresistance to the trypanocides in current use is starting to cause severe problems. The national surra control programme is carrying out its activities in coordination with the ACIAR.

In Ethiopia, the Ministry of Agriculture’s central veterinary service acknowledges that dourine is posing serious problems among equidae (horses and donkeys) in a number of provinces. Donkeys are particularly numerous in Ethiopia (4 to 5 million), since they are extensively used for transporting all types of goods, as well as people. This information is needed, in particular to ascertain whether the infections detected were due to *T. equiperdum* or to *T. evansi*, the latter being widespread in the country, in order to adopt effective control measures.

In Sudan, the various members of the Animal Resource Ministry attending the meeting entirely agreed with the points made at the meeting: persistence of *T. vivax* outside the tsetse fly belt; high prevalence of surra in dromedaries; cases of dourine in equidae, especially donkeys.

### 3. Animal health status in NTTAT-infected countries and epidemiological surveys

Dr Musiime asked Dr A. Delafosse to present his paper:

**DELAFOSSE A & DOUTOU M A.A.(2001) – Epidemiology of trypanosomosis in dromedaries (surra) in Eastern Chad.** (1 volume: 258 pages, 233 tables and figures, 6 appendices)

The study was conducted in eastern Chad, a traditional livestock-farming region that is home to one million dromedaries, where livestock farmers frequently use trypanocides (especially quinapyramine and diminazene di-aceturate) to rid their animals of the ‘djuflar’ (meaning ‘bad urine smell’) or surra. However, cases of chemoresistance have been reported.

The epidemiological survey was carried out between November 1998 and January 2000, in order to include both the rainy and dry seasons. It included:

- A cross-sectional study to assess the prevalence of surra.
- A cohort study (seasonal incidence assessment).
- An entomological survey using Challier-Laveissière and NZI traps.

Regular blood samples were taken from 2,935 dromedaries, marked with a ring in the ear. Parasitological diagnoses were conducted at the same time as assessing the PCV, by means of blood smears on slides stained with May–Grünwald–Giemsa, followed by double observation under an optical microscope. The serological diagnosis was done using CATT/*T. evansi*.

The results showed:

- A parasitological prevalence of 5.3% (± 0.8%) and a serological prevalence of 30% (± 5%).
- An estimated annual incidence of 15%.
- An apparent prevalence per herd (with a single infected animal leading the entire herd to be considered infected) of 93% using CATT and an estimated actual prevalence of 60%.
- 2,443 insects were captured for the entomological survey: 1,272 stomoxes, 945 tabanidae and 226 hippoboscidae. All these species were identified and linked with the season and their capture site.
- All the infected dromedaries were treated with melarsomine.

In conclusion, the study confirmed that surra is common in dromedaries in this part of Chad and showed that it was highly dependent upon livestock farming and transhumance practices.
Dr Musiime then asked Dr Delafosse to outline his second report concerning *T. vivax* infection in cattle in the absence of tsetse flies:

- DELAFOSSE A. & THEBAUD E. (2001) – *Epidemiology of trypanosomosis in cattle in the Lake Chad region* (1 volume: 75 pages, 123 tables, 6 appendices)

The study was carried out in the Lake Chad basin, in a zone situated between the 13th and 14th parallel of latitude north of cattle herds of the ‘Kouri’ breed, which is well adapted to its environment and supplies meat and milk. At the outset, 1,000 animals from 17 herds were studied. The study extended from January 1998 to January 2000 at the start of the dry season.

The blood samples were examined at CIRDES\(^9\) in Bobo-Dioulasso, both by microscopic examination of blood smears on a slide stained with May–Grünswald–Giemsa and by indirect ELISA.

The prevalence of *T. vivax* infection differed depending on the method used:
- 1.8% (± 0.8%) by parasitological examination
- 42% (± 1.2%) by serological examination

Each animal received an injection of trypanocide (3.5 mg/kg of diminazene di-aceturate).

All the results obtained were subjected to an analysis to elucidate the sources and other factors involved.

It has therefore been possible to determine the annual incidence (around 6%) and seasonal incidences, which vary in line with the places where herds are based.

This study confirms the presence of *T. vivax* trypanosomosis in the Lake Chad basin, even though there are no tsetse fly populations in this area, with two main periods of infection risk:
- At the end of the dry season (March to June) on the islands in the lake.
- At the start of the dry season (October to January) on the banks of the lake.

Dr Musiime opened the discussion. Dr Van Gool said that cases of chemoresistance had been reported in Cameroon. Dr Musiime added that the situation was similar in Uganda. Dr Touratier had always been surprised at the use of diminazene di-aceturate on camels because this trypanocide had never been recommended for that species, in particular due to the risk of toxicity, as had been pointed out in a toxicology journal\(^10\). Dr Delafosse pointed out that Chad’s livestock farmers used a low dose of diminazene on their dromedaries, which produced an apparent but temporary recovery from a mild infection, whilst at the same time leading to the gradual emergence of chemoresistant *T. evansi* strains.

Dr Morton asked whether the vectors of *T. evansi* in dromedaries and of *T. vivax* in cattle had been studied in Chad. Dr Delafosse said that Dr Cuisance had carried out field surveys, which had been reported to meetings of the Ad hoc Group about ten years previously and published by CIRAD/EMVT\(^11\). Dr Touratier added that the data provided in d’AMICO’s doctoral thesis concerning the studies carried out in the Central African Republic were also available. Dr Morton asked whether the chemoresistant strains of trypanosomes could also be transmitted and play an iatrogenic role during chemoprophylaxis campaigns. In Dr Musiime’s view, this was a major problem and could become a part of the epidemiology of trypanosomoses.

Dr Durand believed that the ‘pour on’ technique using appropriate insecticides could indirectly prevent this problem in the areas where it was observed. However, Dr Delafosse said that livestock farmers disliked the practice.

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\(^9\) CIRDES: Centre International de Recherches pour le Développement de l'Elevage en Zone Humide et Sub-Humide


\(^11\) CIRAD/EMVT: Centre de Coopération Internationale en Recherche Agronomique pour le Développement/Département d'Elevage et de Médecine Vétérinaire Tropicale
Next it was Dr Ali Almahid’s turn to present a summary of his paper:

- **ALMAHID A. – Non-Tsetse Transmitted Animal Trypanosomoses (NTTATs): status of Sudan**

NTTATs caused by *T. evansi* and *T. vivax* are of average prevalence in the Sudan and are a potential threat to the development of livestock farming in a country where 90% of the herds are exposed to the risk of trypanosomosis. Nagana has been discovered in tsetse fly-free zones in southern Kordofan and urgent studies are needed to determine why the disease persists under these conditions, so research is being carried out into other biting insects. Around 74 species of *Tabanidae*, two species of *Stomoxys* and three species of *Haematobia* have been identified.

In a camel herd of around three million heads, surra (*T. evansi*) is considered to be the most important disease. *T. evansi* antibodies have been detected in goats and the antigen has even been identified in goats and sheep. However, the role of the different animal species in the epidemiology of surra remains to be clarified.

Control of the disease relies on chemotherapy, with all its inherent problems (need for a precise diagnosis, chemoresistance to quinapyramine).

The studies to be undertaken include: effectiveness of certain traps for capturing NTTAT vectors, epidemiological surveys to assess the impact of these diseases and the role of reservoir species, chemoresistance, the use of PCR.

Dr Musiime asked Dr Touratier to briefly present the following paper, in the absence of its authors:


Based on the experimental infection of two cattle with *T. vivax*, it was possible to transmit the infection to other cattle in the same enclosure, even though they were thoroughly protected from tsetse fly intrusion by a net with a very fine mesh, by means of other biting insects (*Tabanidae* of the *Atylotus agrestis* species).

Dr Davila, from the Oswaldo Cruz Institute, Rio de Janeiro, had sent the summary of the PHD science thesis he had recently defended, the text of which was given to the participants:

- **DAVILA – Animal trypanosomoses in South America: Epizootiology, evolution and information.**

*T. vivax* and *T. evansi* have an economic impact on South America, particularly in the Peruvian Amazonia and Brazilian Pantanal regions. The study was on the phylogenetic relationship between various South American and African strains of *T. vivax*. Furthermore, modern information technology was used in order to hold scientific debates on trypanosomes over the Internet.

In **South America**, the epizootiological situation is characterised by low-level parasitemias which can usually only be detected by PCR. However, epizootic outbreaks can occur. A PCR was developed for molecular differential diagnosis, showing for example that the South American strains of *T. vivax* bear more phylogenetic similarity to West African strains than East African strains (use of ITS rDNA). Reference is made to the launch of the electronic journal 'Kinetoplastid Biology and Disease'.

In **Asia**, a press release\(^\text{12}\) had been issued on 16 March 2001 to describe the situation on surra control in the Philippines, entitled: **Being Sore and Sorry for Surra.**

\(^{12}\) Press release no. 033 of the Philippine Council for Agriculture, Forestry and Natural Resources (PCARRD)
This plan was handed to each participant. It stated that the surra control plan had been approved by ACIAR and that Dr Simon Reid had been appointed Head of Project to develop appropriate diagnostic techniques and effective control methods. This surra plan would be carried out in a zone covering four countries: the Philippines, together with Vietnam, Indonesia and Thailand.

The Ad hoc Group received an important review of the surra situation in the Philippines through the good offices of Dr Douglas Gray from ILRI Philippines, which Dr Musiime asked Dr Touratier to present:


Since its introduction into the Philippines, \textit{T. evansi} has gradually come to infect all ruminant species and dogs, as well as equidae, in which the first surra outbreak was identified in 1901. Since then the number of cases has increased and in recent years there has been a steady rise in the number of infection reports.

After studying publications and laboratory reports, we can conclude that \textit{T. evansi} is currently widespread in the Philippines but that its impact is not fully appreciated. Consequently, research is needed on the diagnosis, epidemiology and economic impact of surra so that effective control programmes can be developed. According to the information gathered, the problems posed by surra in the Philippines, and probably in other South-East Asian countries too, appear to have been underestimated and call for better appraisal. As surra is neither an infectious disease, nor a problem affecting a single animal species, any control programme will need to cater for an epidemiological complex that encompasses numerous hosts, vectors, predisposing diseases and possible stress factors. The impact on livestock, poverty and the environment will need to be precisely assessed before such programmes are developed.

A paper on Cambodia was handed to the participants thanks to the good offices of Dr Luckins:


The survey was carried out by a member of the CTVM in Edinburgh. Sera had been collected from 341 buffaloes, randomly selected from seven villages in the Kompong Chan province. Further sera were collected from 179 buffaloes in the Prey Veng province.

The ELISA method was used: the estimated prevalence of surra was 27% in the first group and 19% in the second group. Furthermore, the examination of refrigerated blood from 67 buffaloes failed to reveal a single trypanosome. However, 10 of the 67 buffaloes had a PCV of less than 26%.

Next came a presentation of a summary sent by researchers from the Shanghai Institute of Animal Parasitology (Chinese Academy of Agricultural Sciences):

- JINLIN ZHOU, JIE SHEN & YONGZHI ZHOU – Cloning and sequencing of an ADNc clone: ShTat1.3 gene of a variable surface glycoprotein (VSG).

The ShTat1.3 gene was derived from a strain of \textit{T. evansi} from the province of Anhui, isolated from buffaloes in 1986. After cloning and sequencing, the oligonucleotide sequence obtained (presented in the summary) was confirmed by the BLAST (Basic Local Alignment Search Tool) programme and filed in the GeneBank database under number AF 418 693.

4. Follow-up of the research programme in progress (year two) on dorine and differentiation between \textit{T. equiperdum} and \textit{T. evansi}

Dr Musiime asked Dr Giovanna Samproni to present the memorandum from Italy’s ‘G. Caporale’ Institute: Serological tests conducted at the Zooprophylactic Institute in Teramo between 1 January and 30 April 2002.
The table presented showed that:

- For the year 2000, of the 1,274 samples tested, 1,267 were negative and seven were positive.
- For the year 2001, of the 2,618 samples tested, 2,617 were negative and only one was positive.
- For the first four months of 2002, of the 505 samples tested, 502 were negative and three were positive.

Dr Morton asked whether tests other than CFT\textsuperscript{13} were used at Teramo. Dr Samproni replied that the Institute was perfectly familiar with the other diagnostic tests (IFA, ELISA, PCR), but that CFT was still the only officially recognised diagnostic test in international trade and commerce in equidae.

Dr Musiime then handed over to Dr Filip Claes, head of the joint Antwerp Institute of Tropical Medicine (ITM)/OIE Project, who presented the other three papers:

- **Joint Antwerp ITM/OIE project on the differential diagnosis of T. equiperdum/T. evansi and development of new methods for diagnosing dourine**

The annotated text is based on the conclusions and recommendations of the OIE Ad hoc Group at their 1998 and 1999 annual meetings. The Antwerp Institute of Tropical Medicine adopted them and put them into application in 1999 and the ‘laboratory’ part was financed by the Flemish Government’s Institute of Research and Technology.

The objectives of the project were to:

- Isolate new strains of *T. equiperdum* (not possible since 1982) in order to renew the antigens – which were both very old and very rare – in the various national laboratories responsible for diagnosing dourine.
- Identify predominant variable antigen types (VATs) for a differential diagnosis of *T. equiperdum* and *T. evansi*.
- Characterise *T. equiperdum* and *T. evansi* at the molecular level in order to define the two species by their DNA sequences.
- Develop accurate diagnostic tests for each species, taking into account the response of antigens/antibodies or of specific DNA sequences and to validate these new tests.

**CLAES F. – Progress with the research programme on T. equiperdum and T. evansi. Catholic Faculty of Louvain and Antwerp Institute of Tropical Medicine, Belgium**

Since progress with serological research had already been reported in 2001, only molecular biology research was summarised.

Based on the eleven *T. equiperdum* strains available for the tests (one OVI strain from South Africa, one BoTat1 strain from Bordeaux, three strains from the United States of America, one strain from Canada, one strain from Switzerland, three strains from Germany, one strain from Antwerp), it was shown by serological study that only the OVI and BoTat1 strains differed from the *T. evansi* RoTat1.2 strain that had been taken as the reference.

The molecular biology tests by PCR, RAPD\textsuperscript{14} and AFLP\textsuperscript{15} again showed that OVI and BoTat1 differed from all the other strains of *T. equiperdum*.

However, all the other strains labelled *T. equiperdum* are closely linked with the *T. evansi* group. Therefore the question is whether:

- Most of the strains used in diagnostic laboratories as *T. equiperdum* are in fact strains of *T. evansi*.
- Or whether *T. equiperdum* is the same as *T. evansi*.

\textsuperscript{13} CFT: complement fixation test
\textsuperscript{14} RAPD: random amplified polymorphic DNA
\textsuperscript{15} AFLP: amplified fragment length polymorphism
Tests on target animals (equidae) would resolve this question by using two types of strain currently labelled *T. equiperdum*, meticulously observing the resulting pathology: clinical and anatomopathological signs.

This part of the programme is planned to take place at the Onderstepoort Institute, but is currently awaiting funding.

**Saruultuja Chuluun – Sero-epidemiological research on the prevalence of *T. equiperdum* (Doflein, 1901) in horses in Mongolia. Veterinary Institute, Ulaanbaatar, Mongolia**

In Mongolia, a country that has around three million horses, a serological survey was carried out in the central province (Tuv-amag) between May and July 2000. Blood samples were taken randomly from 1,190 horses using CFT and the ELISA method (antibody search). Out of 1,122 useable sera, 85 tested positive (7.6%) to CFT and 75 (6.7%) to ELISA. Since these results were distributed over different herds, PCR was then used in herds with at least three positives, using a pair of specific primers of the *Trypanozoon* subgenus. The PCR seems to have revealed a few more positives than did CFT and ELISA.

The clinical examination of all these horses led to the identification of only one stallion with an oedematous plaque and a scrotal and preputial oedema. The sample taken from this stallion tested negative using CFT but positive using PCR and ELISA.

Three mares with vaginal discharge tested seropositive using CFT and ELISA but negative using PCR.

Four horses with cutaneous nodules undoubtedly caused by stomoxes came out positive to all the tests. Blood smears taken from horses that had tested positive with CFT and ELISA, stained with Giemsa, revealed no trypanosomes under microscopic examination.

Thus the prevalence of a trypanosomal infection was revealed by the CFT, ELISA and PCR tests, but none of these tests allowed differentiation between *T. equiperdum* and *T. evansi*. However, the clinical data and negative results from parasitological examinations would indicate dourine.

Longitudinal studies of heavily infected herds are needed in order to identify the causal trypanosome.

**5. Validation of diagnostic tests for *T. evansi***

As Chapter 1.1.3. of the OIE Manual of Standards for Diagnostic Tests and Vaccines (Principles of validation of diagnostic assays for infectious diseases) recommends, every diagnostic technique must be validated. For *T. evansi* infections, in particular, it was noted that *T. evansi* surveillance in Papua New Guinea, Australia and the Philippines: ‘…is limited by the low sensitivity and inadequate validation of existing diagnostic tests and by the lack of information on the determinants of the infection’.

Dr Musiime agreed with this remark and handed over to Dr D. Verloo so that he could present his paper:

**Verloo D., Magnus E. & Büschel Ph. – Assessment of the sensitivity and specificity of two diagnostic tests for *T. evansi* in the absence of a ‘Gold Standard’. ITM, Antwerp, Belgium.**

An assessment of the sensitivity and specificity of a new diagnostic test calls for a ‘gold standard’.

In the case of trypanosomosis, detection of the parasite is usually seen as a ‘gold standard’ but the lack of sensitivity of this detection method leads to biased results.

According to Hui and Walter, if two tests are applied to comparable individuals from two populations with different pathological prevalence rates, assuming the independence of errors of the two tests, it is possible to estimate the diagnostic sensitivity and specificity of these two tests and the real prevalence rates in the two populations.

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This was demonstrated by data collected in the field using two tests: the direct agglutination test CATT/ 
*T. evansi* and the microhaematocrit centrifugation technique practised on dromedaries in Niger.

The CATT operating instructions were presented as a complement to this presentation: the Ad hoc Group has 
recommended this test for the diagnosis of surra in camelidae since 1996 because it allows the disease to be 
identified on the herds in situ, with no need to send samples to the laboratory and using only light equipment 
(off-road vehicle or mobile laboratory).

The instructions state that this test was assessed for the diagnosis of surra in dromedaries in Mali, Mauritania, 
Niger and the Canary Islands, as well as in buffaloes from Vietnam.

A list of relevant bibliographic references supplements the operating instructions for the test kits.

6. **Information from FAO** on the Programme Against African Animal Trypanosomoses (PAAT) 
and the Pan African Tsetse Eradication Campaign (PATTEC)

In the absence of Dr Y. Cheneau, who had sent his apologies, Dr Musiime asked the General Secretary of the 
Ad hoc Group to present the report sent by FAO entitled:

- **FAO – Tsetse flies and trypanosomoses: a sustainable integrated strategy.**

African trypanosomoses are in evidence in both humans (sleeping sickness) and animals (nagana). They cover 
approximately 10 million km² in 37 countries south of the Sahara, threatening around 50 million inhabitants 
and affecting 48 million cattle.

FAO has identified an expansion in farming in the area in question, pointing out that 80% of the population 
there depends on farm products.

**Programme Against African Animal Trypanosomoses (PAAT)**

The PAAT was created five years ago under the aegis of three United Nations organizations: WHO
d and IAEA, in liaison with AU/IBAR. Its aim is to control all direct and indirect aspects of animal 
trypanosomosis.

**Pan African Tsetse Eradication Campaign (PATTEC)**

The PATTEC stemmed from a declaration of African Union Heads of State based mainly on the IAEA’s work 
to eradicate tssete flies (campaign successfully conducted on the island of Zanzibar using the sterile male 
technique), in accordance with the joint IAEA/FAO programme and the IAEA’s technical cooperation 
department. The Prime Minister of Burkina Faso launched the PATTEC following the 26th meeting of the 
ISCTRC in October 2001.

The two programmes have come into operation and the PAAT has already made numerous advances, the 
results of which are regularly reported by FAO.

7. **Control of non-tsetse transmitted animal trypanosomoses**

Next Dr E. Camus presented an overview of the Global Forum on Agricultural Research (GFAR):

- **Global Partnership Programme: genetic resources management and biotechnology, control of 
trypanosomoses**
  - Planned duration of the programme: five years
  - Objectives: to increase productivity through the integrated control of trypanosomoses
  - Alleviation of poverty

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17 FAO: Food and Agriculture Organization of the United Nations
18 WHO: World Health Organization
19 IAEA: International Atomic Energy Agency
- Improvement of food safety
- Justification: decision to control trypanosomoses, which impose major constraints on the development of livestock farming in Africa, Asia and Latin America, as well as on human health (sleeping sickness in Africa)
- Main phases of this programme since its creation in January 2000 (acceptance by WECARD at its meeting in Dresden in May 2000; establishment of a worldwide network and presentation of a proposal to the EFARD20; cooperation with the PAAT and OIE Ad hoc Group on NTTAT; world survey of trypanosomosis research programmes).

Dr Camus stressed the special importance of the world survey to identify the different research projects being planned or developed, quantifying them and stating the origin of funding agencies. All of these projects were designed to halt the spread of trypanosomoses in order to achieve sustainable development.

Dr Van Gool asked what results had been obtained by the scientific groups responsible for developing an ‘anti-disease approach for the immunological control of cattle trypanosomoses’, which was the subject of an INCO financed by the European Union (OIE Report, 69 SG/16). Was a vaccine in sight? Dr Camus pointed out that the aim of this project was to vaccinate against the disease and not against the causal parasite. In practice, the project was endeavouring to create a kind of trypano-tolerance among susceptible bovine breeds.

Dr Musiime reviewed the summaries received on the subject of trypanocides and chemoresistance and asked for brief presentations.

Mr Bourdichon summarised the following paper:

- **KAUR P., JUYAL P.D., SRIVASTAVA A.S. & BOURDICHON A.** – *Therapeutic and kinetic effectiveness of diminazene in combination with antipyrine and procaine (Trypan) in the experimental infection of calves with T. evansi.*

The described trypanocide combination does not differ significantly from the plasmatic levels of the active substance and its pharmacokinetic parameters. The effective dose for treatment is 4.04 mg/kg, followed by 3.5 mg/kg after 14 days.

Dr Touratier briefly presented the paper from the Chinese researchers:


This paper complements the one on resistance to quinapyramine, presented by the same authors in 2001. They had reported that many genes could cause quinapyramine resistance. This time, again using resistant strains of ADNC, they obtained the TbTA1 gene. The subsequent sequencing of this gene between *T. evansi* and *T. brucei* showed a difference of 10 nucleotides.

The following summary was presented after Dr Clausen had sent a brief information note on the trypanocide research undertaken by Berlin’s Institute of Pharmacy:


This chemical family of quinones, several derivatives of which were tested in monolayer fibroblast cultures, has shown some degree of trypanocide activity.

Due to lack of time, the discussion was limited to addresses from Dr Durand, who underlined the need to use quality-controlled trypanocides in the field, and from Doctors Van Gool and Shan, who stressed that pure trypanocides should be used.

20 EFARD: European Forum on Agricultural Research for Development
MEETING OF THE OIE AD HOC GROUP ON
NON-TSETSE TRANSMITTED ANIMAL TRYPANOSOMOSES

Paris, 26 May 2002

Agenda

1. Interim report by the Secretary General
2. Information reported to the Office International des Epizooties by Member Countries
3. Animal health status in NTTAT-infected countries and epidemiological surveys
4. Follow-up of the research programme in progress (year two) on dourine and differentiation between \textit{T. equiperdum} and \textit{T. evansi}
5. Validation of diagnostic tests for \textit{T. evansi}
6. Information from FAO on the Programme Against African Trypanosomiasis (PAAT) and the Pan African Tsetse Eradication Campaign (PATEC)
7. Control of non-tsetse transmitted animal trypanosomoses
MEETING OF THE OIE AD HOC GROUP ON NON-TSETSE TRANSMITTED ANIMAL TRYPANOSOMOSES

Paris, 26 May 2002

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