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

Paris, 23 May 2004

The meeting of the Ad Hoc Group of the World Organisation for Animal Health (OIE) on Non-Tsetse Transmitted Animal Trypanosomoses (NTTAT) was held at OIE headquarters on 23 May 2004. The Agenda and List of Participants are given in Appendices I and II respectively.

Since Dr B. Vallat, Director General of the OIE, was engaged in the opening session of the 72nd General Session of the OIE International Committee, Dr A. Schudel, Head of the OIE Scientific and Technical Department, greeted the participants on his behalf and passed on a welcome message from Dr Vallat before handing the floor to Dr H.M. Solomon, Chief Livestock Project Officer at AU/IBAR. Dr L. Touratier, the Group’s General Secretary, was appointed rapporteur.

After the General Secretary 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. A wide-ranging exchange of views then took place. Dr Solomon opened the session and handed the floor to Dr Touratier.

1. Interim report of the General Secretary (May 2003-May 2004)

1.1. Specialised International Meetings

- 19th International Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (New Orleans, USA, 10-14 August 2003)

Only a few of the reports and papers dealt with trypanosomoses:

- Combating tsetse flies and trypanosomes using molecular genetics.
- Symposium on donkeys and donkey diseases.
- Canine trypanosomosis in India (T. evansi).
- Studies on the antigenicity of the invariant surface protein of T. evansi.

1 AU/IBAR: African Union/Interafri can Bureau for Animal Resources
On 30 September 2003, the Ad Hoc Group held a round table discussion at this meeting, the minutes of which were sent to all participants.

During the plenary sessions, in addition to the many papers presented on tsetse-transmitted animal trypanosomoses, a number also dealt with NTTATs:

CLAES F. et al. – Link established between T. equiperdum, T. evansi and T. b. brucei by molecular fingerprinting

This gave rise to the following recommendation:

“The meeting agreed that the results should be confirmed by a larger number of samples taken from new isolated cases of dourine in different regions.

It would also be necessary to try to infect horses experimentally using new isolates.”

This recommendation therefore follows on from the research carried out by the Ad Hoc Group on NTTATs since 1999, the results of which are given below (see 1.2.). However, it does not propose any source of funding, the lack of which is currently holding back the programme’s development. This will be taken into account and the trials in progress will be published at the 28th meeting of the ISCTRC in Addis Ababa (Ethiopia) in September 2005.

Three of the many papers presented are worthy of special mention:

DESQUESNES M. & DIA M.L. – Mechanical vectors of pathogenic animal trypanosomes.

NJIRU Z.K. et al. – Detection of pathogenic trypanosomes using ITS1 based primers in a single PCR test in Kenya

This method is used to eliminate the non-pathogenic trypanosomes T. theileri, T. lewisi, which give no PCR product with the primers used.

MAJIWA PHELIX A.O. et al – Improvements in the detection of T. evansi infections

New developments were signalled primarily in the area of trypanocide research. Dr Reto Brun reported, in particular, on the interesting results obtained with pentamidine metabolites. Even though the aim of his research, conducted jointly with Prof. Tidwell’s consortium (University of North Carolina, United States of America) and supported by the Bill and Melinda Gates Foundation, is to develop active orally-administered trypanocides for treating sleeping sickness, certain compounds in the series studied are also active on T. evansi.

For the time being, one of these compounds, called DB 289, meets the criteria required for treating the human disease and is in phase II of testing.

At the same time, Dr Brun examined a series of compounds from the diamidine family, several of which are active on T. evansi.

Workshop on Cysteine Proteinases of Pathogens and their Role in Host/Parasite Relationships
(University of Bordeaux 2 (France), 2-4 March 2004)

At this basic science workshop funded by the European Union (DG XII), 48 researchers from 14 countries presented 29 papers and posters, 12 of which were devoted to human and animal trypanosomoses. The following two studies were of particular concern to NTTATs:

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2 PCR: Polymerase chain reaction
Could evansin be a pathogenic factor for *T. evansi*?

This study is by M.T. Gonzatti’s team (Simon Bolivar University, Caracas, Venezuela), which has been working on cysteine proteinases of *T. evansi* for around ten years.

**Characterisation of vivapains, *T. vivax* cysteine proteinases**


The works presented included:

- **MATTIOLI R.** -- *Control of animal trypanosomoses*
- **MELVILLE S.J.** -- *The brucei genome project: access to data analysis*

**International Symposium on Parasitology of the Russian Academy of Science** (Biology Section, Russian Institute of Parasitology, Moscow, Russia, 14-16 April 2004)

This symposium was held to celebrate the 125th anniversary of the birth of K.I. Skryabin. It was devoted to the main achievements of parasitology and prospects for its development. The following report was presented at the symposium:

- **TOURATIER L.** -- *Current status of research and new prospects in knowledge about NTTATs*

This symposium enabled the General Secretary of the Ad Hoc Group to make or renew contact with Russian protozoologists and other parasitologists from all over Russia.

**International meeting on camel husbandry in Central Asia** (Ashkabad, Turkmenistan, 19-22 April 2004)

This meeting, financed by NATO\(^3\), brought together thirty or so specialists from a number of Central Asian, European, North African and Middle Eastern countries. CIRAD/EMVT\(^4\) is due to publish the proceedings and send them to the Group Secretariat.

### 1.2. Dourine

The following article was published (February 2004) in collaboration with the OIE reference laboratory for dourine (VIEV, Moscow, Russia), the University of Berlin (Institute of Tropical Medicine), the Onderstepoort Veterinary Institute (South Africa), the Antwerp Institute of Tropical Medicine (Belgium) and OIE Central Bureau.


This article takes stock of current knowledge on dourine and its identification, in view of the problems arising when diagnosis of the disease is uncertain using complement fixation - the only recognised method for identifying dourine for international trade in live equidae. At the same time the article summarises the main elements of the plan which the OIE Ad Hoc Group on NTTATs has been proposing since 1999 to attempt to resolve the problem:

- Isolation of new *T. equiperdum* strains from clinical cases.
- Identification of specific markers for *T. equiperdum* to allow it to be differentiated from other species of the *Trypanozoon* subgenus.

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\(^3\) NATO: North Atlantic Treaty Organization

\(^4\) CIRAD/EMVT: Centre of International Cooperation in Agronomic Research for Development/Department of Livestock and Veterinary Medicine
– Experimental infection of horses with freshly isolated strains of *T. equiperdum* to compare their pathogenicity with that of the strains currently used in national diagnostic laboratories and with strains of *T. evansi*.

– Phylogenetic studies.

– Proposal and validation of a reliable new diagnostic test that is internationally recognised.

It is all the more important to establish a new specific diagnostic test for dourine since, during the period under review (May 2003/2004), positive and/or suspect reactions to CFT in equidae were noted in very different geographical zones, and the animals were put immediately into quarantine. As in 1998, these reactions became negative after two months and the equidae under observation were released from quarantine.

F. Claes’ PhD thesis, defended in June 2003 at the University of Louvain (Belgium) after conducting laboratory work at the Antwerp Institute of Tropical Medicine from 1999 to 2003, has already demonstrated that most of the *T. equiperdum* strains stocked in national diagnostic laboratories are either strains of *T. evansi*, or linked with *T. evansi*, and that only the OVI (Onderstepoort) and BoTat1 (Bordeaux) strains are “pure” *T. equiperdum* strains. However, trials on horses have yet to be carried out, which is difficult for the time being due to lack of funding. The trials could take place at the same time as evaluating the pathogenicity of new strains.

**Search for new strains of *T. equiperdum***

The following countries, which have acknowledged the existence of dourine in their territory, are again being called upon: Russia, Kazakhstan, Mongolia and Ethiopia. Up to now, only Kazakhstan and Mongolia appear to have successfully isolated *T. equiperdum*, but it is difficult to resolve the problem of transferring these isolates on account of highly restrictive international regulations.

In Ethiopia, an epidemiological survey of trypanosomoses of equidae (*T. evansi* and *T. equiperdum*) has been set up with the assistance of CIRAD/EMVT, the Free University of Berlin and the Antwerp Institute of Tropical Medicine, under the supervision of Prof. Abebe Getachew of the Debre-Zeit Veterinary Faculty (Ethiopia), with one of the Professor’s assistants having received training at the Antwerp Institute of Tropical Medicine.

A first sample survey on 400 equines is due to be carried out in late 2004. The British Donkey Sanctuary foundation, represented by Dr A. Trawford, together with Prof. Feseah Gebreab, will participate in the survey.

In Kazakhstan, Dr G. Ilgekbaeva and Dr Claes were able to use CATT to examine several camel and horse breeding centres at the kind invitation of Dr Zh. Kumekbaeva, President of the national veterinary association of horse and camel breeders, with material support from France’s Equine Veterinary Association. The aim was to conduct serological surveys to evaluate the reliability and ease of use of CATT in the field. All the data collected will be analysed and published in French in early 2005 in the equine veterinary journal *Revue de Pratique Vétérinaire Équine*.

**1.3. A few opinions on problems with differentiating *T. evansi* from *T. equiperdum***

In addition to the work carried out by F. Claes, instigated by the Ad Hoc Group’s decision cited earlier, at least four recent articles highlight these problems:

- **GIBSON W.** – *Species concepts for trypanosomes: from morphological to molecular definitions? Kineto plastid Biology and Disease*, 2004, 10 pp. 51 ref.

  A number of remarks were made:

  – Neither *T. evansi* nor *T. equiperdum* are transmitted by tsetse flies.

  – *T. evansi* lacks a mitochondrial genome and its kinetoplastic DNA contains only homogeneous minicircles.

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5 CATT: card agglutination trypanosome test
– *T. equiperdum* sometimes lacks kinetoplasts, but when it does have them, it contains maxicircles and minicircles, like *T. brucei*.

– An examination of DNA polymorphism using isoenzymes, RFLP⁶, karyotype, minisatellites or phylogenetic analysis, shows no appreciable differences between *T. evansi*, *T. equiperdum* and *T. brucei*.

“In a sense, *T. evansi* and *T. equiperdum* can both be regarded as natural mutants of *T. brucei*. However they must be considered as two distinct species because they each meet the definition of a species: the impossibility of interbreeding. However, since genetic exchange in *T. brucei* occurs during its cyclical development in the tsetse fly, it precludes the involvement of *T. evansi* and *T. equiperdum*”.


In both of the above studies, the authors conclude that it is impossible to differentiate the two species of trypanosome by biochemical or immunological means.

### 1.4. Other data drawn from examining literature


– Hilali M. *et al.* - Evaluation of CATT/*T. evansi* for detecting the *T. evansi* infection in the buffalo (*Bubalus bubalis*) in Egypt.

– Uzcanga G. *et al.* - Purification of a 64 kDa antigen from *T. evansi* that exhibits cross-reactivity with *T. vivax*.

– Ventura R.M. *et al.* – Characterisation of *T. vivax* by amplification of an intergenic sequence.


– Lejon V. *et al.* – Application of the enzyme-linked immunosorbent assay method (ELISA) for detecting *T. congolense* and *T. vivax* in goats.

Furthermore, a group of researchers (cooperation between Belgium and Togo) showed that *T. vivax* infections were relatively frequent parasitoses of small ruminants in Togo.

### 1.5. Data drawn from correspondence

Following the ACIAR⁷ development course held at the Research Institute for Veterinary Science (Bogor, Indonesia) in April/May 2002 on the diagnosis and epidemiology of *T. evansi* in Southeast Asia, contacts were maintained with the participants. In early 2004, Dr G. Buenviaje, Dean of the School of Veterinary Medicine, University of Southern Mindanao, was put in charge of research into surra in the Philippines, in partnership with the Mindanao Unified Surra Control Approach (MUSCA) run by Dr R. Mercado. The project is being funded by ACIAR.

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⁶ RFLP: restriction fragment length polymorphism

⁷ ACIAR: Australian Centre for International Agricultural Research
The two colleagues have provided the following details:

- Surra is a major disease in the Philippines, particularly in Mindanao, where not only horses, but also cattle, water buffaloes (carabaos), goats, pigs and dogs are also affected.
- The repercussions of surra-induced immunosuppression have been evaluated by risk analyses. It turns out that cattle and buffaloes infected with *T. evansi* have a two to five times greater risk than healthy animals of contracting haemorrhagic septicaemia (*Pasteurella multocida*).
- *T. evansi* strains in the Philippines are seen to be more pathogenic than those of other Asian countries or Indonesia owing to the high mortality and morbidity rates caused by surra among cattle, buffaloes, goats and sheep. Hence the need to search for possible genetic markers that could distinguish the strains found in the Philippines from those of other countries.
- The surra control methods rely essentially on treatment using trypanocides. For the time being, only diminazene aceturate (DMZ) is commercially available but it does not kill 100% of trypanosomes.
- It was not possible to precisely determine the economic impact of surra. A precise directive is needed to study the surra problem. The only criterion that can be used at present is the market value of animals in the case of mortality. But how can one accurately evaluate the losses associated with the non-availability of draught animals, or loss of earnings when selling infected animals for meat or when livestock producers are unable to hire out their animals for farming work?
- Finally, surra is highly seasonal: the disease is most prevalent during the rainy season. During the 1994 epizootic that raged in the region, deaths occurred mainly among horses and carabaos used as draught animals, which were heavily infested with parasites according to microscopic examinations.

1.6. New system for notifying animal diseases to the OIE

In his April 2004 editorial, Dr Bernard Vallat, Director General of the OIE, said that the OIE Regional Commissions had asked the Central Bureau to establish a single list for notifiable diseases of terrestrial animals. In his editorial, he stressed that: “The overriding criterion for a disease to be listed is its potential for international spread”.

In the case of NTTATs, only dourine and surra of horses are still in the OIE B list.

When establishing the single list, all animal trypanosomoses could be considered as meeting the above criterion of hazardousness and should be included in the list in alphabetical order. The proposal would be as follows:

- Tsetse transmitted animal trypanosomoses (TTAT)
- Non-tsetse transmitted animal trypanosomoses (NTTAT)
  - Dourine (*T. equiperdum*)
  - Surra of horses (*T. evansi*)
- Other animal trypanosomoses and other animal species

A subdivision within the NTTATs would still be necessary in order to continue to employ the two names “dourine” and “surra of horses”, which countries are accustomed to using for their declarations.

2. Information reported to the OIE by the Member Countries

The following figures on NTTATs have been drawn from numerical data provided to the 72nd General Session:
Dourine (*T. equiperdum*)

*South Africa* (10 outbreaks, 19 cases, 2 deaths. Due to late reporting, the totals do not tally with those of the monthly reports).

*Botswana* (3 outbreaks, 3 cases. The disease is enzootic in the country. Animals are subject to import/export controls).

*Canada* (dourine is an “immediately notifiable disease” according to animal health regulations).

*Latvia* (3,203 blood samples examined with negative results).

*Lithuania* (725 horses tested, all negative).

*Namibia* (16 outbreaks, 33 cases, serological test carried out at the same time as the compulsory tests for exportation).

*Russia* (27 outbreaks, 390 cases, 390 animals slaughtered).

**Surra** (*T. evansi*)

*Argentina* (disease present, limited to certain areas).

*Canada* (surra is an “immediately notifiable disease” according to animal health regulations)

*Egypt* (379 cases in camelidae).

*United Arab Emirates* (4 outbreaks, 13 cases, no deaths in camelidae).

*Eritrea* (presence of the disease)

*India* (1 outbreak, 10 cases, 2 deaths (camelidae)).

*Jordan* (presence of the disease).

*Oman* (presence of the disease).

*Philippines* (equidae: 51 outbreaks, 692 cases, 72 deaths; cattle: 69 outbreaks, 1,920 cases, 69 deaths; buffaloes: 728 outbreaks, 1,600 cases, 665 deaths). The number of trypanosomosis cases was generally lower in 2003 than in 2002. The Mindanao surra control organisation has been very active in its efforts to combat the disease, most cases of which have been found in Mindanao).

*Tunisia* (8 outbreaks, 10 cases, no deaths in camelidae).

### 3. Epidemiology of NTTATs worldwide and diagnostic methods

#### 3.1. Influence of immunosuppression caused by *Trypanosoma evansi*

After presenting the General Secretary’s Report, Dr Solomon opened the discussion and handed the floor to a number of speakers:

Prof. Uppal said than no immunosuppression effect has been observed in India in the enzootic *T. evansi* infection zones where rinderpest vaccination has been carried out. Neither was a breakdown of immunity observed during the FMD vaccination campaigns. In addition, the immunodepressive role of *T. evansi* was not very clear, even during vaccination against haemorrhagic septicaemia in buffaloes. India has very extensive experience in this field.

Dr Solomon agreed with Prof. Uppal with regard to rinderpest, for which a vast vaccination campaign was conducted throughout Africa thanks to the PARC’s programme, including in zones highly infected with trypanosomoses.

Dr Luckins said that African trypanosome species differ from Asian ones and added that tests had been carried out in Africa twenty or so years previously with a number of FMD vaccines. It would perhaps be appropriate to carry out comparable tests in Asia taking into account the trypanosome species and the differing pathogenicity of the strains, or even of the Asian isolates of *T. evansi*, as S. Reid *et al* had reported to the NTTAT Group meeting on 18 May 2003.

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8 PARC: Pan African Rinderpest Campaign
Dr Reid agreed with Prof. Uppal and Dr Solomon that the degree of immunity induced by the rinderpest vaccine varies from that of the FMD vaccine. This could explain the rather weak influence of the *T. evansi* infection in the former case.

In addition, Dr Reid stated that, in the Philippines, there is sometimes an association between species of *Fasciola* and *T. evansi* in buffaloes. However, there is no potentiation between haemorrhagic septicaemia and surra. The same applies to goats, which can be exposed to both surra and haemorrhagic septicaemia.

Dr Touratier pointed out that *T. evansi*-induced immunosuppression could not, however, be called into question because W. Holland’s PhD thesis was the subject of extensive studies on the buffalo and pig in Vietnam, the clarifications of which were endorsed by the members of his thesis committee, one of whom was the Director of the National Veterinary Research Laboratory in Vietnam. That is why it is both important and urgent to study the differential pathogenicity of *T. evansi* strains.

The provisional conclusion of the discussion was that testing is required to determine once and for all the effects of *T. evansi*:

- On FMD vaccination.
- On young animals after maternal antibodies decline.

### 3.2. Diagnosis

Dr Solomon asked Dr Reid to describe the project he is heading in South Eastern Asia and the Pacific, emphasising the importance of diagnosis.

Dr Reid apologised for not presenting a text and gave the following account:

- **Australian project for controlling surra in the Philippines, Indonesia and Papua New Guinea**

  The surra agent is considered to be a potentially dangerous pathogen for Australia.

  In the Philippines (southern islands) the infection rate is around 20%, with a mortality rate of 6% or more, as reported to the Group’s previous annual meeting in May 2003.

  The project prescribes technology transfer to partner countries after determining suitable diagnostic tests for screening.

  What is the role of the different tests?

  For example, if PCR is not viable in the Philippines, what is the use of clinical tests?

  What trypanocides should be used?

  Quinapyramine and diminazene aceturate do not always appear to be active, even in strong doses. Melarsomine would be useful, but a precise dose needs to be defined for the buffalo.

  In Mindanao: goat herds are probably becoming increasingly infected. Properly trained livestock producers are increasingly reluctant to keep infected animals. Tests are soon to be undertaken to determine the impact of *T. evansi* on the different livestock systems.

  A number of other questions also need to be answered:

  - Is it possible to assess the nature of a disease by noting signs and symptoms?
  - What sort of diagnostic test should be used?
  - Is infection the same as “disease”?
  - What sensitive methods of detection are there, or else what tests can be carried out directly on the animal?
- Is it true that the Miniature Anion-Exchange Centrifugation Technique (MAECT) can detect one parasite/1 ml of blood?
- But does detecting the parasite give any sort of useful information?
- Is serology considered to be useful for tests directly on the animal?
- If not, in what way is it useful?
- Are CATT and ELISA sensitive and specific?
- What is the probability that tests are correct?
- When prevalence diminishes, what is the probability for tests to be positive?
- Serology is not good for individual diagnosis, but it is good for a herd test.
- A good necropsic diagnosis needs to be made, in relation with the pathology observed.

Dr Solomon said that Dr Reid’s remarks also applied to other regions, as for example in the case of surra-infected camelidae in Africa (Debab and M’Bori in North and West Africa, as well as in Chad, Kenya, Morocco, Mali and Sudan), where urine odour is taken into account.

- **Other diagnostic methods**
  - **ADAM ELHAG MUSA DAROSA & KHITMA HASSAN EL MALIK – Urine odour change for detection of camel trypanosomosis**
    
    The tests carried out at the Preventive Medicine Department of the University of Khartoum on dromedaries experimentally infected with *T. evansi* have shown that the trypanosome induced notable modifications in the normal components of urine (electrolytes, creatinine, urea) and in abnormal components (ketone bodies, albuminuria, proteinuria). These modifications give the urine an odour that camel drivers refer to as “characteristic of surra”. Ketone bodies in particular are held to be responsible for this. Even though the presence of ketone bodies is not pathognomonic of surra, their detection does provide an aid to diagnosis.

    Drs Reid and Luckins in turn added that in Indonesia and the Philippines other signs can reveal surra: circling of buffaloes or oedema and permanent recumbency in equidae. In any case, it is always necessary to take into account experience and even the knowledge of livestock producers in determining the best attitude to adopt for both diagnosis and treatment.

    According to Dr El Malik, a positive CATT and a low PCV (hematocrit) should lead to treatment of camelidae.

Dr Solomon handed over to Dr Inoue for a presentation of the following paper:

- **INOUE N., TUNTASUVAN D. & IGARASHI I. – Evaluation of loop-mediated isothermal amplification (LAMP) for detection of *T. evansi* in experimentally infected pigs in Thailand**

    Six surra-free piglets were infected with *T. evansi*. Detection of the infection was 65% positive using mouse inoculation, whereas the LAMP, PCR, microhematocrit centrifuge and blood smear methods gave 42%, 31%, 35% and 22% positives respectively. Apart from mouse inoculation, the most sensitive method for detecting *T. evansi* was LAMP.

    Commenting on these results, Dr Inoue stressed that all the diagnostic tests used in the experiments were carried out in accordance with the specifications of the OIE Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals.

    Dr Luckins remarked that the results obtained using LAMP, PCR and the microhematocrit centrifuge test method (MHCT) do not differ significantly.
Dr Inoue replied that LAMP is a general method that has now been used for diagnosing a great number of pathogenic elements: African trypanosomes, West Nile virus, SARS, *Legionella* spp., *M. tuberculosis*, up to now by Japanese researchers. However, it is hoped that the LAMP method will be disseminated more widely, particularly for diagnosing NTTATs because mouse inoculation is very time consuming and PCR poses the problem of obtaining DNA. The LAMP method is easier to implement. Work is currently being carried out to develop new primers for *T. evansi*, which will simplify the DNA amplification method for use close to the field.

When Dr Solomon declared the discussion open, Prof. Lun remarked that Dr Inoue was using 0.5 ml for mouse inoculation and 0.1 ml in the DNA extraction for PCR and LAMP. However, he should not use all the DNA for a single PCR reaction. This could be a major reason why the two methods display similar sensitivity.

According to Prof. Büscher, LAMP and PCR do not show good concordance. Why? LAMP is highly sensitive.

Dr Reid stated that, like MHCT, LAMP can detect a single parasite in the blood. Furthermore, when he asked Dr Inoue what a LAMP test cost, the reply was $10. If so, why does MHCT not seem cheaper?

Dr Robinson asked whether the DNA extracted in the end was the same. The answer was yes.

Dr Claes wanted to know whether the extraction process is controlled. Dr Inoue replied that the sensitivity relies on taking animals separately. In addition, it is planned to carry out epidemiological surveys on *T. evansi* infections in Asia using LAMP and several other diagnostic tests.

Dr Solomon then asked Dr Touratier to briefly present the subsequent papers. One of the papers concerned Asia:

- **PATHAK K.M.L. – Comparative evaluation of parasitological, serological and DNA amplification methods for diagnosis of natural *T. evansi* infection in camels (in India)**

A representative sample of 217 dromedaries (*Camelus dromedarius*) from different areas in the Indian State of Rajasthan was examined between July 2002 and May 2003 to search for *T. evansi* infections. Several tests were used: thick drop, stained blood smear, immunodiagnosis (double sandwich ELISA for detecting the antigen) and DNA amplification using PCR. These techniques were compared and PCR was considered the best, since the prevalence rates detected using PCR, Ag ELISA, blood smear and thick drop were 17.05%, 9.67%, 4.60% and 4.14% respectively with sensitivities of 100%, 56.75%, 27.02% and 24.32%. PCR revealed a specific band of 227 bp in the positive samples. The intensity of the PCR bands varied according to the different samples tested, and appeared to depend on the infection rate. The symptoms – intermittent fever, emaciation, oedema and poor general condition – correlated significantly with the positive results using ELISA, as well as with the detection of trypanosome DNA using PCR.

The other paper concerned South America:

- **MONZON C.M. – Evaluation of an enzyme-linked immunosorbent assay (Ab ELISA) for diagnosing the circulating *T. evansi* antigen in horses (Argentina)**

A double-sandwich-ELISA technique was first applied to a horse experimentally infected with *T. evansi*. The circulating antigens appeared after 16 days and the test remained positive even when the parasites could not be detected using the parasitological method.

The test was then evaluated on sera from 156 horses, four groups of which were infected with *T. evansi*. Of these, 86 were positive using the customary parasitological examinations. The circulating antigens were found in between 70% and 91% of them. 269 horses from a free zone used as control animals were tested for the presence of *T. evansi* antibodies and all were negative.

The results were evaluated and discussed statistically from the standpoint of the sensitivity and specificity of the test used. In conclusion it appears that using this ELISA test in combination with the customary parasitological method provides the best response to a diagnosis of surra of horses in the sub-tropical zone of Argentina.
Dr Gutierrez in turn presented the following paper:

- **GUTIERREZ C., DORESTE F. & MORALES M. – Trypanosomosis of goats. Progress report.**

According to the above authors, *T. evansi* plays a key role among small ruminants in the Canary Islands (sheep and goats), as well as among horses and camelidae. However, the clinical expression of the disease is very subtle and there is no clear understanding of the vectors responsible for its transmission. Studies are under way to clarify the situation and at the same time to verify whether goats act as trypanosome reservoirs in the Canary Islands.

Prof. Dakkak agreed and said that in Morocco an epidemiological survey on trypanosomosis of goats had given a seroprevalence of 3.7% without clinical signs. A better understanding was needed of the situation in North Africa, especially where there are mixed herds of dromedaries and goats. He went on to present the paper summarising an article published recently in *Veterinary Parasitology*.

- **DAKKAK A, ATAROUCHE T., RAMI N. & BENDAHMAN N. – Camel trypanosomosis in Morocco: Results of a first epidemiological survey**

The survey was carried out in 1999 and 2000. A total of 1,460 serological samples were collected and tested using CATT and Ab ELISA to search for *T. evansi* antibodies. Seroprevalence rates were 14.1% using CATT and 18.2% using Ab-ELISA. The infection rate increases with age. 79.7% of the seropositive animals had swollen lymph nodes and a very low red blood cell count compared with the seronegative animals, testifying to significant anaemia. The survey showed that the Saharan provinces of Morocco are particularly affected by trypanosomosis, Zagora and Merzouga being two hyperenzootic outbreak areas.

Since the problem of trypanosomosis of goats also exists in Sudan, Dr El Malik and her colleagues have carried out various experiments using Nubian goats which they had infected with *T. evansi* isolates.

The following two papers give an account of this work:

- **AHMED E.A. & EL MALIK K.H. – The effect of experimental infection of goats with *T. evansi***

The observation of six Nubian goats, inoculated intravenously with $7.5 \times 10^5$ *T. evansi* from a Sudanese strain revealed key clinical signs (hyperthermia, anaemia, neurological signs, dull unhealthy coat, abortion of pregnant females, without weight loss in well nourished animals, and so on). Anaemia is of the microcytic and normochromic type when there is a reduction in the hematocrit and haemoglobin counts. However, the number of red blood cells is increased. These results contradict the previous results reported, which concluded that the anaemia was of the normocytic type. There are a reduced number of leucocytes. Parasitaemia is low and the *T. evansi* strains differ in their virulence.

- **AHMED E.A. & EL MALIK K.H. – Clinico-pathological changes due to concurrent experimental infection of Nubian goats with *T. evansi* and *H. contortus***

After infection with *T. evansi*, then infestation with *Haemonchus contortus*, or the reverse, the test animals were observed in comparison with a control group for several weeks. Antibody levels were affected by the superinfection and retarded in the case of an acute infestation with *H. contortus*, declining in chronically infected animals. It was concluded that prior infestation with *H. contortus* affects susceptibility to *T. evansi* and lowers immunity.

Dr Touratier presented the following paper:

- **HILALI M., ABDEL-GAWAD A., NASSAR A. & ABDEL-WAHALA A. – Haematological and biochemical changes in buffalo calves (Bubalus bubalis) in Egypt infected with *T. evansi***

Since Egypt has a large buffalo population (around 3 million) and few studies have been made so far on the herd’s infection with *T. evansi*, it was useful to verify the behaviour of this trypanosome in buffalo in Egypt. However, the presence of *T. evansi* is frequently reported in dromedaries and cases of surra are regularly declared.
Experiments were conducted on four six-month-old buffalo calves for three months and involved the study of different parameters after infecting the animals with a camel strain of *T. evansi*.

The principal results gathered were as follows: significant reduction in the number of erythrocytes, haemoglobin and hematocrit; macrocytic and hypochromic anaemia; increase in the levels of serum AST and LDH enzymes, as well as blood proteins, globulins and bilirubin; reduction in creatinine and blood urea.

This study supplements the previous one which demonstrated the usefulness of CATT for detecting surra in water buffaloes in Egypt.

Dr Solomon handed over the floor to Prof. Ali M.A. Majid from Sudan for a presentation of his paper:

- **MAJID ALI M.A. – *The prevalence of T. evansi infections in camels in Sudan***

Description of the epidemiology of *T. evansi* in Western and Eastern Sudan: Kassala and Kordofan. There are around 4 million dromedaries in Sudan and 30% of the country’s animal production revenues come from trade in dromedaries.

The prevalence of surra is considered to be low, but in reality it is probably higher. The highest prevalence is found among “Arab” type dromedaries. In Kordofan, surra is more prevalent during the rainy season, which tallies with the large number of insects captured in ad hoc traps, even though there is no accurate information on the vector species. Infected dromedaries are treated by semi-sedentary farmers, whose animals are given regular treatments with quinapyramine. There are a few cases of chemo-resistance.

- **Problems in differentiating *T. evansi* from *T. equiperdum***

Following the presentation of the above papers and the ensuing discussions, Dr Solomon said that the Secretariat had received three other summaries mentioning the difficulty and consequences of accurately diagnosing the equine trypanosomoses currently rife in countries where cameldiae are reared together with equidae.

In such cases, one might wonder:

- Whether equidae are infected with *T. evansi* or *T. equiperdum*.
- What is the impact of *T. evansi* on Bactrian camels (*Camelus bactrianus*).

The three summaries are as follows:

- **PUREVSUREN BYARUUZANA & BYAMBAA BADARCH – *Sero-epidemiological survey for trypanosomoses in Mongolian horses: a summary***

In Mongolia, surra has been diagnosed by parasitological examination in only one province. In eight other provinces, with the technical cooperation of Germany and South Africa, blood samples from 5,000 horses were subjected to CFT (Mongolian antigen of *T. equiperdum*): prevalence rates varied from around 0.4% to 18%.

When CATT/*T. evansi* RoTaT1 was used, the samples collected in six provinces were around 37% positive.

- **CLAUSEN P.H. & RUURAGCHAS SODNOMDARJAA – *Control of surra (T. evansi) in the two-humped camel (Camelus bactrianus) population of Western Mongolia***

(Announcement of the finalisation of a project for treating Mongolian surra-infected two-humped camels with the specific trypanocide for *T. evansi*: melarsomine⁹. Follow-up study of the effects of this treatment on wool production).

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⁹ ®Cymelarsan
ILGEBAYEVA G.D., SAIDOULDIN T.S., BUSCHER P. & CLAES F. – Comparison of serological tests for equine trypanosomosis in naturally infected horses in Kazakhstan

In this study, the three diagnostic tests routinely used - complement fixation test (CFT); horse complement fixation test (HCFT) or Saidouldin test, employed primarily by Russian speaking authors; and CATT/T. evansi or the card agglutination trypanosome test, expressing the variable iso-antigens of the RoTat1.2 strain - were compared on horses in Kazakhstan.

Good concordance was found between these tests.

At the suggestion of Dr Solomon, Dr Claes made the following comments on the three summaries.

These texts are a follow-up to the information already given by the representatives of Kazakhstan and Mongolia in May 2003.

The summary on surra control in the two-humped camel is based firstly on an epidemiological study. High-risk zones are yet to be identified and a collection of isolates is planned. The surveys are due to take place over a 12-month period, and will include the examination of positive animals and their treatment with melarsomine. It is also planned to assess their productivity. This study is due to commence in June 2004 and will be partially financed by the Yak and Camel Foundation.

The treatment would be aimed at animals deemed to be affected on the basis of parasitological and serological examinations. Its effectiveness will be based on the same criteria.

For horses, the situation with dourine/surra differentiation is still the same due to the lack of a specific diagnostic method making it possible to distinguish each of the two species: T. evansi and T. equiperdum. An accurate diagnosis of dourine therefore requires, in addition to a positive serological test, the co-existence of clinical symptoms recognised as quasi specific.

Dr Claes also presented the following paper:

CLAES F., GODDEERIS B. & BUSCHER P. – T. equiperdum: taking second base

Only two isolates of T. equiperdum have been identified as “genuine” T. equiperdum. Other isolates, available in most national diagnostic laboratories, are wrongly identified as T. evansi. Does T. equiperdum exist as a separate species? A characterisation is required. Studies are under way in Kazakhstan where, in certain parts of the country, there is an estimated prevalence of 20%, in the hope of obtaining an isolate from an infected horse by intratesticular injection of suspect material into rabbits.

Similar isolation tests are taking place in Mongolia and will also be conducted in Ethiopia, during a serological survey on dourine covering 400 equidae in the provinces most affected by the disease. In fact dourine is a scourge of equidae in Ethiopia, which has the largest equine population of any African country.

The two “genuine” isolates of T. equiperdum – OVI and BoTat1 – will be tested on horses in the National Veterinary Services Laboratory (NVSL) of the United States of America, at Ames, Iowa, to find out if they can induce the characteristic symptoms of dourine via the genital route, and to study the complete development of the experimental infection, by comparison with the strains traditionally used at NVSL to prepare the antigens used for the “dourine” CFT.

A study of the RNA of T. equiperdum, T. evansi and T. brucei is also being carried out, in particular of the RNA of T. evansi from different hosts, in order to find out if it is possible to identify virulence markers.

At the group’s annual meeting in May 1999, NVSL (J. Katz) proposed using a competitive ELISA test to diagnose T. equiperdum.
Dr Claes remarked that CATT/T. evansi RoTat1.2 works well for diagnosing dourine, as he himself had experienced in Kazakhstan, and it seems to be just as satisfactory as CFT. Perhaps it could replace this relatively old technique which, unlike CATT, is not standardised.

Prof. Uppal said that Indian experts visiting Mongolia saw no cases of dourine in the country.

- **FAO paper on the activities of the Programme Against African Trypanosomiasis (PAAT)**

  A copy of this FAO/AGAH document was given to each participant. The document details and also summarises the activities deployed against African trypanosomoses under the following chapters:
  
  - General background on the creation and aims of the PAAT.
  - PAAT and the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC).
  - FAO/PAAT support for PATTEC.
  - Recent developments.
  - Conclusion.

4. **Invitation by the organising committee of the Twelfth International Congress of Protozoology (ICOP XII), Guangzhou, China, 10-15 July 2005**

As Joint General Secretary of ICOP XII, Prof. Z.R. Lun invited the OIE Ad Hoc Group on NTTATs to hold its third international seminar at Guangzhou just before ICOP XII opens, i.e. on 9 and 10 July 2005.

The conference hall and proper facilities would be provided free of charge, as well as shuttle buses between the participants’ hotels and the conference hall. All the Group members present warmly accepted the invitation.

The web site of the NTTAT seminar figures at the top left-hand side of the home page of the portal of the ICOP XII site: http://www.congress.com.cn.

After thanking Prof. Lun for his offer, and the speakers for their active participation in the meeting, Dr Solomon closed the meeting at 13:00 hours.

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.../Appendices
MEETING OF THE OIE AD HOC GROUP
ON NON-TSETSE TRANSMITTED ANIMAL TRYPANOSOMOSES

Paris, 23 May 2004

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Agenda

1. Adoption of the agenda
2. Interim report of the General Secretary (May 2003- May 2004)
3. Information reported to the OIE by the Member Countries
4. Epidemiology of NTTATs worldwide (T. vivax, T. evansi, T. equiperdum)
   - Serological methods
   - Molecular methods (PCR, LAMP, other methods)
   - Importance of their sensitivity for international trade (biosafety)
5. T. equiperdum/T. evansi differentiation
   - Progress with the programme proposed by the Ad Hoc Group
   - Other research
6. Pathogenicity of T. evansi strains in relation with geographic zones and animal species
7. Basic research into T. vivax in Africa and South America
8. Control methods
   - Chemoresistance
   - Progress with the search for new trypanocidal compounds
   - Progress with immunoprophylaxis
9. Any other business
   - Information on the PAAT (FAO)
   - 3rd International Symposium on NTTATs (9-10 July 2005) as a satellite meeting of the 12th International Congress of Protozoology (Guangzhou, China, 10-15 July 2005)
MEETING OF THE OIE AD HOC GROUP
ON NON-TSETSE TRANSMITTED ANIMAL TRYPANOSOMOSES

Paris, 23 May 2004

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