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Paris 15 July 2004 JM

06/09/04

Re : Annual meeting of the OIE ad hoc Group on Non Tsetse Transmitted Animal Trypanosomoses (Paris , France , World Health Organisation for Animal Health HQ 23 May 2004)

Dear Dr.

Herewith , you will find the documents arising from this meeting : "Minutes" and relevant 20 Annexes submitted to your consideration .

Looking forward to hearing from you ,

With best regards ,

Dr. Louis Touratier
Secretary general of the Group

ENCLOSURES :

" Minutes of the meeting "

ANNEX I : Agenda
ANNEX II : List of participants
ANNEX III : Report May 2003
ANNEX IV : Progress rep.by Secret.
ANNEX V ZABLOTSKJI *et al.*
ANNEX VI : CLAES *et al.*
ANNEX VII : PUREVSUREN *et al.*
ANNEX VIII : CLAUSEN *et al.*
ANNEX IX : ILGEKBAYEVA *et al.*
ANNEX X : INOUE *et al.*
ANNEX XI : PATHAK
ANNEX XII : DAKKAK *et al.*
ANNEX XIII : Ali M.A. MAJID
ANNEX XIV : MONZON
ANNEX XV : GUTIERREZ *et al.*
ANNEX XVI : AHMED&ELMALIK (a)
ANNEX XVII :AHMED&ELMALIK (b)
ANNEX XVIII :HILALI *et al.*
ANNEX XIX :DAROSA&ELMALIK
ANNEX XX : FAO Communication:

7 July 2004

MINUTES (*) OF THE 25th ANNUAL MEETING OF THE O.I.E. (THE WORLD ORGANISATION FOR ANIMAL HEALTH) ad hoc GROUP ON NON TSETSE TRANSMITTED ANIMAL TRYPANOSOMOSES (NTTAT) HELD IN PARIS , FRANCE ON 23 MAY 2004 AT THE O.I.E. HEADQUARTERS ON THE OCCASION OF THE 72nd O.I.E. GENERAL SESSION

On the opening of the session , a welcome message from Dr. B. VALLAT , Director General of the O.I.E. was passed on to the participants by Dr. A. SCHUDEL , Head of the O.I.E. , scientific and technical department , to whom he wished a good and fruitful meeting according to the items of the agenda (**See : Annex I**) . He also apologised to be unable to attend the meeting due to his involvement in the final process of the 72nd OIE General Session . He then handed the floor to Dr. SOLOMON Haile Mariam , Chief Livestock Project Officer , AU/IBAR , Nairobi, Kenya , seconded by Dr. A.G. LUCKINS , CTVM , Edinburgh , Scotland , UK who accepted to help Dr. L. TOURATIER to sum up the discussion.

Several apologies for absence were presented e.g. from : Dr. C.A. MONZON , CEDIVEF , , Resistencia ,Rep.Argentina ; Dr. Fred POTGIETER , Acting Director , Onderstepoort Veterinary Institute ,South Africa ; Dr. M. DESQUESNES , CIRDES , Bobo Dioulasso , Burkina Faso ; Prof. Getachew ABEBE , Veterinary College , University of Addis Ababa , Ethiopia ; Dr. E. CAMUS , Director , EMVT/CIRAD , Montpellier , France ; Prof. T. BALTZ , Head , Functional genomics of Trypanosomatidae and Molecular Parasitology , Université Bordeaux 2 , France ; Dr. E. AUTHIE , EMVT/CIRAD and IRD , Montpellier , France ; Dr. P.H. CLAUSEN , Freie Universität , Berlin , Germany ; Dr. G. ILGEKBAYEVA , University of Almaty , Kazakhstan ; Prof. V.T. ZABLOTSKY , Director of the OIE Reference Laboratory for Dourine, VIEV ,Moscow ,Russia ; Dr. D. TUNTASUVAN , National Bureau, ACFS, Bangkok , Thailand.

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(*) Provisional wording submitted to the addressees : possible remarks , corrections, suggestions and/or amendments to be returned by 30 September 2004.

Then , Dr. SOLOMON opened the meeting on 9h 15.
A dossier was handed to each participant **(See: ANNEX II : List of participants)** containing the following documents :

- Agenda of the meeting
- Report of the meeting of the OIE ad hoc Group on NTTAT held in Paris , 18 May 2003 (Document 72 /SG/18). **(See : ANNEX III) ;**
- Progress report May 2003-May 2004 by the Secretary general **(See : ANNEX IV)**
- 16 other reports or written statements sent by the participants to be presented during the meeting and/or to introduce the discussion.

1. RELEVANT DATA PROVIDED BY NATIONAL VETERINARY SERVICES TO THE O.I.E. HEADQUARTERS UNTIL 5 MAY 2004.

(Reports sent to the O.I.E. reporting Service : 3 volumes with brief accounts of the zoo- sanitary situation and tabulated reported outbreaks of epizootic diseases 2003).

1.1. Dourine *(Trypanosoma equiperdum)*

Botswana : 3 outbreaks 3 cases
(The disease is enzootic in the country . Animal are screened for the disease at import/export)

Canada : (Dourine is "an immediately notifiable disease" under the Health of Animals Regulations)

Latvia : 3203 blood samples tested for Dourine with negative results

Lithuania : 725 horses tested for Dourine : all negative.

Namibia: 16 outbreaks 33 cases
Serological evidence detected during tests to meet export requirements

South Africa : 10 outbreaks 19 cases 2 deaths
Due to late reporting of information the totals do not correspond with the totals of our monthly reports.

Russia : 27 outbreaks 390 cases 390 slaughters

1.2. Surra *(Trypanosoma evansi)*

Argentine : Reported present . Limited to specific zones.

Canada : (Surra is "an immediately notifiable disease" under the Health of Animals Regulations)

Egypt : 379 cases in camelidae.

2.

Eritrea : Reported present

India : 1 outbreak 10 cases 2 deaths in camelidae

(Completing these data on the occasion of a conversation with the Chief of the Delegation of India to the OIE General Session and her scientific advisers , it was understood that Surra is no more considered as a dangerous concern for animal health in India . In particular it was not noticed that FMD and HS cases are more numerous and severe in enzootic *T. evansi* areas where animals could , however, be strongly immunodepressed by the presence of the parasite.)

Jordan : Reported present

Oman : Reported present

Philippines :	Equidae : 51 outbreaks	692 cases	72 deaths
	Cattle : 69 outbreaks	1,920 cases	69 deaths
	Buffaloes : 728 outbreaks	1,609 cases	665 deaths

The report adds : " Surra *Trypanosoma evansi)* : The number of cases of Trypanosomosis was generally lower in 2003 than in 2002 . The Mindanao Unified Surra Control Approach (MUSCA) has been very active in its efforts to combat the disease (most cases of Surra are reported in this region)

Tunisia : 8 outbreaks 10 cases no deaths in camelidae

United Arab Emirates : 4 outbreaks , 13 cases no deaths in camelidae

2. PROGRESS REPORT MAY 2003 – MAY 2004 BY THE SECRETARY GENERAL.

(See : ANNEX IV)

On the request of Dr. SOLOMON , Dr. TOURATIER briefly introduced his report particularly emphasising the work which has been carried out by the team of the Institute of Tropical Medicine, Antwerp , in cooperation with the University of Leuven , Belgium both in achieving a first part of the programme which has been defined by the ad hoc Group in its annual meeting of May 1999 (joint article published on the beginning of 2004 in the *Rev. sci.tech.Off.int.Epiz.)* .

(See : ANNEX V)

V.T.ZABLOTSKY , C. GEORGIU , Th. De WAAL , P.H. CLAUSEN , F. CLAES & L. TOURATIER :- The current challenges of dourine : difficulties in differentiating *T. equiperdum* within the subgenus *Trypanozoon. Rev. sci. tech. Off.int.Epiz.*2003 , **22** (3),1087-1096.

Consecutive studies were carried out both on Dourine (PhD thesis of F. CLAES , defended in June 2003) and on immunosuppression induced by *T. evansi* in buffaloes and in pigs (PhD thesis of W. Holland , defended in June 2003).

(Summaries and/or conclusions of these research works were already given in the "addendum" of the proceedings of the 24th annual meeting of this Group (18 May 2993) However, they can be sent again on request).
Dr. SOLOMON opened the discussion which develops mainly around the possible influence of immunosuppression caused by *T. evansi* either in worsening other diseases or in preventing the development of a good immunity after vaccination campaigns against common plagues encountered in Asia.
Prof. UPPAL considers that no effect of immunosuppression has been observed in *T. evansi* endemic zones of India where vaccination against Rinderpest has been applied . He also did not notice some breakdowns of vaccination after campaigns against FMD and the depressing role of *T. evansi* is similarly not clear even in the vaccination of Haemorrhagic Septicaemia (HS) in buffaloes. India has a wide experience in this respect.
Dr. SOLOMON agreed for the vaccination campaigns against Rinderpest as there is a considerable experience with the PARC (Pan African Rinderpest Campaign) in Trypanosomosis infected zones in Africa.
Dr. LUCKINS remarks that trypanosome species are not the same and reminds that trials were carried out about twenty years ago with FMD vaccines in infected African trypanosome areas . May be similar trials could be conducted in Asia taking account of the trypanosome species and of the diversity of the pathogenicity of Asian strains or isolates as it was reported at the preceding meeting on 18 May by Dr. REID *et al.*
Dr. REID agreed with Prof. UPPAL and Dr. SOLOMON that there is a degree of resistance between the immunity induced by Rinderpest vaccine and FMD vaccine which could explain the rather light influence of *T. evansi* infection in the first case. Moreover , he mentions that , sometimes, in the Philippines , there is an association *Fasciola* spp. / *T.evansi* in buffaloes But ,there is no strong association between HS and Surra . The same for goats if they have Surra clinical disease then exposed to HS.

As a provisional conclusion of this discussion it seems that there is a need for definitive experiments to really assess effects of *T. evansi* e.g.:
(i) challenge with FMD.;
(ii) effect on young animals when maternal antibodies are declining .

3. EPIDEMIOLOGY AND DIAGNOSTIC METHODS OF NTTAT

In the following part of the meeting the discussion anticipated some items of the agenda for current purposes , inducing some displacement in the presentation of written statements (ANNEXES).

Project for the cotrol of Surra in South East Asia and Pacific :- Dr. SOLOMON handed over the floor to Dr. Simon REID for the description of this project concerning most of the ASEAN of which he is the Project leader :
Philippines / Indonesia / PNG / Australia Project.
Surra is regarded as a potentially dangerous pathogen for Australia.
In the Philippines (Southern Islands) there are an infection rate of 20 % with a mortality of 6 % and even more (See above in "1.2." and Proceedings of the 23rd meeting, May 2002 and 24th meeting, May 2003 on NTTAT) .
4.

The project prescribes a transfer of technology to the partners after determining how useful are the various diagnostic tests . What is the role of the various tests ? e.g. If PCR is not viable in the Philippines how useful are clinical tests ?
Another aspect : what trypanocidal drugs should be used in Indonesia ? – It seems that : Quinapyramine and Diminazene di-aceturate will not cure all isolates even at high doses . Melarsomine would be useful but needs the establishment of an accurate dose for buffaloes .

In Mindanao :
Goat ownership increases likelihood of having positive animals. Farmers with good education are less likely to have infected animals . Further work will determine impact of *T. evansi* on farming systems.

Several questions can also be raised :

- do we appreciate the nature of a disease after checking signs and symptoms ?
- what sort of diagnostic test to use ?
- infection does not equal disease necessarily ?
- what sensitive methods to detect infection or pen-side tests ?
- It seems that MAECT can detect 1 parasite / 1 ml of blood
- But does parasite detection give any useful information ?
- Is serology considered useful for pen-side tests ?
- But how useful is it ?
- Is CATT/ELISA sensitive and specific ?
- What is the probability of tests being correct ?
- As prevalence decreases what is the probability of tests being positive ?

Serology is not good for individual diagnosis , but good for herd testing.
There is the need of a good diagnosis post mortem according to the pathology.
Clinical signs : Farmers can identify infected animals as there is the case in several countries with the urine smell for Surra in camels (Debab , M'Bori in Africa : Chad , Kenya ,Morocco, Sudan)
Prof. DAKKAK and Dr. EL MALIK agreed . Dr. EL MALIK briefly presents a paper about this aspect :

(See : ANNEX XIX)

DAROSA (A. ELHAG MUSA) & EL MALIK (K.H.):- Urine odour change for detection of camel Trypanosomosis.

In Indonesia and in the Philippines other clinical signs are : buffalo circling and horse oedema and recumbency .Anyway it needs always to take account of the experience and even the knowledge of farmers to determine the best adapted procedures .
Dr. EL MALIK said that , in Sudan , a positive CATT and a low PCV determine to treat camels .She asks what are the major vectors of *T. evansi* in the Philippines and in Indonesia ?

Then Dr. SOLOMON requests Dr. INOUE to introduce his communication :
INOUE (N.) , TUNTASUVAN (D.) & IGARASHI (I.) :- Evaluation of loop-mediated isothermal amplification (LAMP) for detection of *T. evansi* in experimentally infected pigs (in Thailand)

(ANNEX X)

Emphasising the fact that all the tests of experiments were conducted in accordance with the "OIE Manual of Standards for Diagnostic Tests and Vaccines" , Dr. INOUE gave the following tabulated comparative results obtained according to the diagnostic methods used :.

MI :	65 %
LAMP	42 %
PCR	31 %
MHCT	35 %
Thin film	22 %

For Dr. LUCKINS it appears that the results provided by LAMP , PCR and MHCT methods are not significantly different.
Dr. INOUE remarks that LAMP is a general method which has been used now for a number of pathogens : African trypanosomes , West Nile Virus , SARS , *Legionella* spp., *M. tuberculosis* and , so far , mainly used by Japanese workers.
Hopefully the LAMP method can be disseminated more widely , especially with NTTAT .
MI is time consuming.
PCR has difficulty in acquiring DNA..
LAMP has advantage as it is easier to carry out . Now there is work on development of new primers for *T. evansi* , DNA method being simplified for use at field level.
Dr. SOLOMON opens the discussion :
Prof. LUN noticed that Dr. INOUE used 0.5 ml in MI and 0.1 ml in extracting DNA for PCR and LAMP . However, he may not use all DNA for one PCR reaction . This may be one of the important reason why these two methods show similar sensitivity.
Prof. BÜSCHER considers that LAMP and PCR do not show good concordance. Why ? Sensitivity of LAMP is high.
Dr. REID noticed that LAMP can detect a single parasite in blood like MHCT . Questioning Dr. INOUE on the cost of an assay with LAMP , the answer is : 10 $. If so , why don't use MHCT which seems cheaper ?
Dr. ROBINSON asks : is finally extracted DNA the same ? The answer is : yes .
Dr. CLAES would like to know whether the extraction procedure is controlled .-
Dr. INOUE said that sensitivity is based on single animals .Moreover , there are plans to survey *T. evansi* in Asia with LAMP and various other diagnostic tests

Dr. SOLOMON remarks that two communications on the same item of the agenda were sent by colleagues who are members of the Group and who were prevented to attend . He proposes to join their texts to the set of documents :

(See : ANNEX XI).

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

(See : ANNEX XIV)

MONZON (C.M.) :- Evaluation of an antigen-detection enzyme immuno-assay test with monoclonal antibody (AbELISA) for diagnosis of *T. evansi* in horses (Argentine).

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Then , Dr. SOLOMON handed over the floor to Dr. GUTIERREZ for presentation of his communication :
(See : ANNEX XV)

GUTIERREZ (C.C.) , DORESTE (F.) & MORALES (M.) :- The trypanosomosis in the goat : Current review .

The behaviour of *T. evansi* seems different – as far as Canary islands are concerned – in small ruminants , sheep and goats , and in horses and camels . A good validation for *T. evansi* in goats is not done despite experimentally infected goats for the study of sub-clinical infection. The knowledge of vectors responsible for the transmission is uncertain. In both cases very little is known .
Prof. DAKKAK agreed and said that , in Morocco , where a survey gave a seroprevalence of 3.7 % , no clinical signs were recorded . There is a need to know more about the epidemiological situation in North Africa , especially where camels are herded together. He then introduces the following paper which is a summary of an article already published in *Vet. Parasitol.*,2003,**111**,277-286 :
(See : ANNEX XII)

DAKKAK (A.) , ATARHOUCHE (T.) , RAMI (M.) & BENDAHMAN (N.) :- Camel trypanosomosis in Morocco : Results of a first epidemiological survey .

As to the problem of trypanosomosis in goats in Sudan Dr. EL MALIK *et al.* carried out several experiments using Nubian goats experimentally infected with *T. evansi* isolates from camels
(See : ANNEX XVI and ANNEX XVII)

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

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

Goats were injected with an isolate of *T. evansi* then haematological parameters studied . Serology was conducted by ELISA and histopathological examination was carried out on various tissues.
The inoculum was 500,000 trypaosomes by intraveinous inoculation .Variable incubation period with fluctuating parasitaemia was observed between 5 and 45 days , reflecting the pathogenicity of isolates and resistance of the host.- Two out of 15 goats were refractory. An abortion occurred in a pregnant goat . Nervous signs were exhibited as well as a decrease in PCV .A marked pathology was found in liver and heart Goats could be used in further experimental studies.
Young rats were more susceptible to infection and good for propagation of strains.
Sheep and goats have low parasitaemia and field isolates differ in their virulence.
The other experimental work showed that pre-infection of Nubian goats with *H. contortus* did affect susceptibility to *T. evansi* as an indication of lowered immunity according to clinico-pathological changes observed.

From their side , Egyptian workers studied haematological and biochemical changes in *T.evansi* infected buffalo calves , in the following written statement :

(See : ANNEX XVIII)

HILALI (M.) , ABDEL-GAWAD (A.) , NASSAR (A.) & ABDEL-WAHAHA (A.):- Haematological and biochemical changes in buffalo-calves *(Bubalus bubalis)* infected with *T. evansi* . (quoted in absentia).

Then , Dr. SOLOMON requests Prof. Ali M.A. MAJID to introduce his communication :

(See : ANNEX XIII)

Ali M.A. MAJID :- The prevalence of *T. evansi* infection in camels in Sudan .

Description of the epidemiology of *T. evansi* in East and West Sudan : Kassala and Kordofan. In Sudan there are 4 million camels and sales of camels generate 30 % of livestock productivity earnings .

Prevalence in animals is low , but probably higher in true , nomadic animals. Highest prevalence is found in the "Arabi" type of camels. There is higher prevalence in Kordofan in the rainy season according to flies trapped there , but there is no accurate information on insect species involved

Infected camels are treated by owners who are semi-sedentary. Their animals receive regular treatment with Quinapyramine and there is evidence of chemoresistance in some cases.

Problems of differentiation *T. evansi / T. equiperdum* .

Following the preceding presentation of papers / communications on many aspects of problems linked to Surra in several animal species it needs to underline once more both the difficulty and consequences of an accurate diagnosis of these two trypanosome species mainly in countries practising an extensive breeding of horses and camels : :

- are horses affected with *T. evansi* or with *T. equiperdum* ?
- what is the impact of *T. evansi* in bactrian camels ?
-

Three papers were sent to the Secretariat to illustrate this concern :

(See : ANNEX VII)

PUREVSUREN BYARUUZANA & BYAMBAA BADARCH :- Sero-epidemiological survey for Trypanosomoses in Mongolian horses : a summary.

(See : ANNEX VIII)

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

(See : ANNEX IX)

ILGEKBAYEVA (G.) , SAIDOULDIN (T.S.) , BÜSCHER (Ph.) & CLAES (F.):- Comparison of serological tests for equine trypanosomes in naturally infected horses in Kazakhstan .

At the suggestion of Dr. SOLOMON , Dr. CLAES accepts to comment the preceding papers.

Already in May 2003 information was received from the same countries (See above : ANNEX III) and this follow-up shows the importance which is granted to this matter.

As to the paper by CLAUSEN *et al.* , the work on bactrian camel is devoted to a validation of control strategies thanks to cross-sectional and longitudinal epidemiological study . High risk areas are to be identified and sampling of stabilates to be achieved . Surveys in the same herds for up to 12 months are planned as well as the examination of positive animals and their treatment with Melarsomine. Measurement of productivity is also planned to be carried out in relation to the disease . This work might commence in June 2004 and would be partially funded by the "Yak and Camel Foundation" .
Treatment of animals will be based on their either parasitological or serological positivity and their effectiveness judged clinically and parasitologically . Their eventual impact on productivity will be estimated.

For horses the differentiation of Dourine and Surra is always stopped by the absence of a specific diagnostic method if there are no characteristic clinical symptoms of Dourine . On this occasion Dr. CLAES reminds special traits still to be carried out .

(See : ANNEX VI)
CLAES (F.) , GODDEERIS (B.) & BÜSCHER (Ph.):- *Trypanosoma equiperdum* : taking second base .

Only two isolates of *T. equiperdum* have been identified as "real" *T. equiperdum* .Other isolates available in most of the national diagnostic laboratories for research are misidentified *T. evansi* .- Does *T. equiperdum* exist as a separate species ? Full characterisation is required .
Studies are carried out in Kazakhstan where , in some parts of the country, a prevalence of 20 % has been estimated with the hope to obtain an isolate from a horse after intratesticular injection of suspected material to rabbits .
Similarly trials of isolation are being conducted in Mongolia and will also be carried out in Ethiopia on the occasion of a Dourine survey which is scheduled to screen about 400 horses in the provinces where the disease is a plague for the agricultural Ethiopian economy.
The two "real" isolates of *T. equiperdum* will be assessed in horses at the National Veterinary Laboratory (NVL) of the US , Ames,Iowa to know whether they can induce characteristic symptoms of Dourine and to study the full development of the experimental infection.
Another point is the study to compare RNA of *T. equiperdum* , *T. evansi* and *T. brucei* and especially to compare RNA from *T. evansi* from different hosts to know if it is possible to identify virulence markers.
For the diagnosis of *T. equiperdum* a competitive ELISA had been proposed by the NVL ,Ames in 1999.
Prof. UPPAL said that ,according to Indian workers who visited Mongolia , no clinical cases of *T. equiperdum* was seen in this country.
Dr. CLAES went on to the diagnosis of Dourine and said that CATT / *T. evansi* works well in the field (his own experience in Kazakhstan) and seems as good as CFT. Possibly it could replace this old procedure which is not standardised and for its achievement different antigens are used by different diagnostic laboratories . In comparison CATT is fully standardised !

4. FAO COMMUNICATION ON PAAT ACTIVITIES

The document prepared by FAO / AGAH was handed to each participant. It describes :

- The background of the programme
- PAAT and PATTEC
- FAO support to PATTEC
- Recent developments
- Conclusions

5. INVITATION BY THE ORGANISING COMMITTEE OF THE XIIth INTERNATIONAL CONGRESS OF PROTOZOOLOGY (ICOP XII) – Guangzhou , P.R. China , 11-16 July 2005 .

As Co-Secretary General of ICOP XII , Professor Z.R. LUN invites the O.I.E. ad hoc Group on NTTAT to hold its 3rd International Seminar (after Annecy, France, October 1992 and Obihiro, Hokkaido, Japan, August 1998) in Guangzhou just before the venue of ICOP XII : 9-10 July 2005 .
Conference room and conference facilities will be provided free as well as shuttles between hotels and conference room . Other details are to be discussed after definite and mutual acceptance of the invitation principle . All Group members present accepted the invitation with a great interest .

After thanking Prof. LUN and all the authors of communications , written statements and participants in the relevant discussions , Dr. SOLOMON closed the meeting on 1h p.m.

MEETING OF THE OIE AD HOC GROUP

ON NON-TSETSE TRANSMITTED ANIMAL TRYPANOSOMOSES (NTTAT)

PARIS , OIE Headquarters , 23 May 2004 9 a.m. – 1 p.m.

Agenda

1.- Progress report by the Secretary general (May 2003 – May 2004)

2.- Epidemiology of the NTTAT in the world (*Trypanosoma vivax , T. evansi , T.equiperdum)*

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3- Diagnostic methods and epidemiological surveys currently in development.

Serological methods
Molecular methods (e.g. PCR , :LAMP , other method(s).
Importance of their sensitivity for the international trade (biosecurity)

4- Differentiation *T.equiperdum / T.evansi*

Progress in the development of the ad hoc Group programme
Other research work

5.- Pathogenicity of *T.evansi* strains according to geographical zones and animal species.

6.– Basic studies on *T.vivax* in Africa and South America

7.- Control methods
Problems of chemoresistance
Updating of research work in development on new trypanocidal drugs
Progress in immunoprophylaxis

9.-Other questions

Information on PAAT (FAO)
Coming events.

ANNEX II

25th Meeting of the OIE ad hoc Group
on Non Tsetse Transmitted Animal Trypanosomoses (NTTAT)

PARIS , OIE Headquarters , 23rd May 2004 - 9h a.m. – 1h p.m.

LIST OF PARTICIPANTS

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ANNEX III

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

Paris, 18 May 2003

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 18 May 2003. 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 71st 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. J.T. Musiime, Acting Director of AU/IBAR[1]. 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. This was followed by wide-ranging discussions. Dr. Musiime opened the meeting before handing over to Dr. Touratier.

1. Interim report of the General Secretary (May 2002-May 2003)

1.1. Scientific meetings on trypanosomoses and means for controlling them

- **Tenth International Congress of Parasitology (ICOPA X)** (Vancouver, Canada, 4-9 August 2002)

The many papers and posters presented included:

- A study of cystein proteases in trypanosomatids by the Glasgow team.
- A study of alternative oxidase in trypanosomoses, applied to *T. vivax*, by two Japanese teams.
- The characterisation of *T. rangeli* strains using molecular biology; molecular biological study of trypanosome strains isolated from vampire bats and the immunohistochemical detection of *T. evansi* in the cerebrospinal fluid of experimentally infected rats in Brazil.
- An assessment of the CATT[2] for diagnosing surra in water buffaloes in Egypt.

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1 AU/IBAR: African Union/Interafrican Bureau for Animal Resources

- **Third Conference on equine diseases** (Moscow, Russia, 15-16 August 2002)

Below are three of the presentations made at this meeting held by the veterinary association of Russia:

- The physiopathology of trypanosomoses (K.I. Skryabin Veterinary Academy of Moscow).
- The evolution of antibodies in rabbits infected with *T. equiperdum* (VIEV, Moscow).
- The status of dourine in the district of Almaty in Kazakhstan.

- **World Veterinary Congress** (Tunis, Tunisia, 25-29 September 2002)

Three of the several hundred papers were specifically related to NTTATs:

- The principal parasitoses of the dromedary in Tunisia.
- The treatment of dromedary trypanosomosis caused by *Trypanosoma evansi* using Cymelarsan, by a researcher from CIRDES[3], Burkina Faso.
- The results of comparative trials of *T. equiperdum* antigens supplied by various countries to the dourine reference laboratory in Moscow.

- **Health Meeting 2003** (Phnom Penh, Vietnam, 24-28 February 2003)

- A review of surra in South East Asia by the General Secretary of the Group.

- **4th Biennial Meeting on Parasitology** (Blaise Pascal University, Clermont-Ferrand, France, 25-28 March 2003)

Of the hundred or so papers and/or posters, attention was drawn to the following:

- Distribution of non-LTR retrotransposons (INGI and RIME) in the genome of *T. brucei* by molecular parasitology researchers from the University of Bordeaux II.
- The identification of the trypanolytic factor in human serum and the mechanism of resistance to this factor in *T. rhodesiense* (Free University of Brussels, Belgium).
- The induction of arginase in the trypanosomes of the *brucei* group (University of Bordeaux II).

- **20th North African Veterinary Congress** (Fez, Morocco, 8-9 May 2003)

Five papers were highlighted:

- The application of Bayesian mathematics to a surra survey of pigs in Thailand and Vietnam (El Harrach veterinary school, Algeria).
- The influence of pathology on the reproduction of the Naga she-camel in Libya.
- Dromedary diseases in central and southern Tunisia (Sidi-Thabet National School of Veterinary Medicine, Tunisia).
- Dromedary trypanosomosis in Morocco: results of an epidemiological survey (Hassan II Institute of Agricultural and Veterinary Science, Rabat, Morocco).
- Overview of the goals and proceedings of the OIE's NTTAT Group by its General Secretary.

1.2. Bibliographic references

- **Articles dealing with NTTATs**

- Evolution of Australian trypanosomes (Great Britain).

- Antibodies revealed by ELISA[4] in horses infected with *T. evansi* after being treated using quinapyramine sulphate (Argentina).
- Treatment of surra of equidae using diminazene aceturate (Thailand).
- Assessment of antigens/antibodies by rapid detection tests of *T. evansi* in dromedaries in Kenya.
- Metabolism and distribution of phenanthridine trypanocides in the body (Great Britain).

▪ **Tests for differentiating *T. evansi/T. equiperdum***

- Three articles from the Tropical Medical Institute of Antwerp, Belgium, in collaboration with the Universities of Bordeaux II, Louvain, Lelystad in the Netherlands and Onderstepoort in South Africa.
- Group analysis of *T. equiperdum* strains using RAPD[5] and MEGA[6]: position of the species in the *Trypanozoon* subgenus.
- Expression of the VSG[7] of RoTat 1.2 in *T. evansi* and *T. equiperdum*.
- Characterisation of pathogenic species of *Trypanosoma* spp.: the role played by the *T. equiperdum* species (PhD, Louvain, June 2003).

1.3. Information sent by correspondence

There were numerous exchanges of correspondence throughout the year with specialists from a variety of countries, mainly concerning dourine diagnosis and *T. evansi/T. equiperdum* differentiation, in particular with the:

- Debre-Zeit Faculty of Veterinary Medicine, University of Addis-Ababa (Ethiopia) for the preparation of a serological study on equidae in areas where *T. evansi* and dourine infections coexist and where the presence of camelidae may help to sustain *T. evansi* infections.
- Veterinary Association of Horse and Camel Breeding at Almaty (Kazakhstan) where there is a very similar problem and where the exportation of saddle horses is being hindered by the coexistence of dourine and surra, which are impossible to distinguish using the complement fixation test (CFT).
- The management of the Veterinary Research Institute of Ulan Bator (Mongolia) and the Free University of Berlin (Germany) on the subject of serological and molecular studies on trypanosomoses of equidae and camelidae, which are being conducted in Mongolia for a number of years with encouraging results.
- The Department of Veterinary Science of the Gluck Equine Research Center in Lexington (United States of America), strongly advocating a more accurate dourine method than CFT and the USDA-APHIS National Veterinary Diagnosis Laboratory in Ames (United States of America) on the same subject.
- Shanghai Institute of Animal Parasitology and the Research Institute of the Guangzhou Faculty of Science (China) for problems concerning the molecular parasitology of trypanosomes of the *Trypanozoon* subgenus.
- Institute of Tropical Medicine of Antwerp (Belgium), which has provided, and continues to provide, valuable support to the NTTAT Ad Hoc Group's activities in all matters under study.

In spite of all these contacts and our correspondents' motivation, it has still not been possible to effect a recent isolation of a new strain of *T. equiperdum*. However, in view of the latest publications, in particular the conclusions of the above-mentioned doctoral thesis of Filip Claes, such an isolation would be very useful for comparison with the references strains from the national diagnosis laboratories, most of which are closely linked with *T. evansi*.

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4 ELISA: Enzyme-Linked Immunosorbent Assay
5 RAPD: random amplified polymorphic DNA
6 MEGA: multiplex endonuclease genotyping approach

2. Information reported to the OIE by Member Countries

The following figures on NTTATs have been drawn from numerical data provided to the 71st General Session:

Dourine (*T. equiperdum*)

South Africa (10 outbreaks, 24 cases, 2 deaths, 17 slaughtered).

Germany (1 case in a mare, slaughtered).

Ethiopia (1,141 blood samples, all negative).

Italy (diagnostic tests for breeding stallions).

Kirghizistan (1 outbreak, 61 cases, all slaughtered).

Latvia (presence of the disease suspected, serological survey: 3,213 blood samples, all negative).

Lithuania (807 horses tested with negative results).

Namibia (20 outbreaks, 85 cases, no deaths, numerous horses tested for exportation requirements).

Uzbekistan (presence of the disease, surveys).

Pakistan (presence of the disease).

Russia (15 outbreaks, 343 cases, 8 deaths, 335 horses slaughtered. Surveillance, precautionary measures at borders, slaughter, surveys).

Surra (*T. evansi*)

Argentina (presence of the disease in equidae).

Saudi Arabia (presence of the disease in camelidae, outbreaks reported, cases, deaths).

Brazil (1 outbreak, 3 cases in equidae).

Egypt (349 cases in camelidae).

United Arab Emirates (presence of the disease in equidae; 1 outbreak, 3 cases in camelidae).

India (1 outbreak, 1 case in equidae; 15 outbreaks, 19 cases in camelidae).

Jordan (trypanosomosis reported in cattle and camelidae: 355 cases).

Laos (surra of horses: a few cases reported in the province of Louang Namtha).

Malaysia (Sarawak) (presence in all susceptible species, surveys).

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

Uzbekistan (presence, surveys).

Pakistan (presence in all susceptible species).

Philippines (4,613 cases, 345 deaths in equidae).

Tunisia (4 outbreaks, 36 cases, no deaths in camelidae).

Vietnam (presence in cattle, buffaloes and equidae).

In South East Asian countries, such as Laos, the Philippines and Vietnam, we are seeing in parallel a high prevalence of cases of haemorrhagic septicaemia in cattle and buffaloes, with a high mortality rate. The same is true in India.

3. Epidemiology of *T. equiperdum* and *T. evansi* infections worldwide

3.1. *Trypanosoma equiperdum* (dourine)

At Dr. Musiime's request, Dr. Touratier introduced the paper sent by the President of the National Veterinary Association of Horse and Camel Breeding of Kazakhstan:

- KUMEKBAEVA ZH. ZH. – ***Current problems of Dourine and Surra (Su-auru) in horses and camels in Kazakhstan***

There are around one million horses and 100,000 camels in this vast country, with very large breeding centres: 40 for horses and 20 for camels. In addition to thoroughbred horses and Kostanai breed saddle horses, bred mainly for export, there is also the Kushum breed, which is valued for its meat and the Mugaljar breed, for its dairy qualities.

Dourine of horses and surra of camels co-exist and, since surra can also affect horses, as in other countries, it creates a great problem because no diagnosis method is able to distinguish between the two diseases. Long-term studies of the large equine herds have shown that dourine is rife but often goes undetected. In south-east and northern Kazakhstan, trypanosomes have been identified in between 1.6% and 21.1% of animals and in southern and central Kazakhstan, between 0.6% and 46.7%. In seven regions, the trypanosomal infection was found in an average of 13% of horses tested.

Research is being conducted at the Kazakh State Agricultural University. Professor M.S. Sabanshiev has published numerous works and demonstrated in particular that the best means of isolating field strains of *T. equiperdum* is to inoculate virulent matter into young donkeys and male rabbits. Other researchers, including T.S. Saiduldin and G.D. Ilgekbaeva, have contributed to the identification of trypanosomosis. The Saiduldin reaction test is a variant of the complement fixation test and is more sensitive than the customary method (CFT): (85% positive results in horses experimentally infected with *T. equiperdum* compared with only 32% using the customary method). It is used for diagnosing dourine and surra of camels.

The key aims of the research coincide with those of the OIE Ad Hoc Group on NTTATs:

- To isolate new strains of *T. equiperdum.*
- To prepare diagnostic kits for use in the field.
- To seek trypanocidal substances.
- To compare several methods of diagnosing dourine and surra of camelidae for use in the field: CFT, Saiduldin reaction (SR), CATT.

Following on from this presentation, Dr. G.D. Ilgekbaeva commented on her own paper:

- ILGEKBAEVA G.D. – ***Problems related to trypanosomosis in horses in Kazakhstan***

Formerly dourine was confined to Kirghizistan, central and southern Kazakhstan and southern Russia. It has now spread to other areas, which has prompted the Veterinary Services of these countries to impose an annual control diagnosis before authorising the export of equidae.

The tests carried out in Kazakhstan on experimentally infected colts, showed that: urethral lesions occur after 40 to 150 days; the parasites appear as from the 6th day in scrapings from the urethral mucosa and as from the 90th day in the blood, and start to disappear after 120 days. A microscopic examination does not always reveal the causal trypanosome in these scrapings from the urethral mucosa: in 9 experimentally infected colts, it was found only 41 times in 74 samples.

Serodiagnosis (CFT or SR) starts to give positive results between day 19 and day 34 after infection using CFT and between day 13 and day 26 using SR.

The highest titer of 1:24 occurs between day 161 and 189 after infection and declines sharply between day 203 and 216 using the SR method.

A limited serological survey of 296 horses showed: no clinically infected animals, but 24 positive cases using SR and 6 positives using CFT.

Dr. Musiime opened the discussion in which Drs. D. Robinson and Z.R. Lun talked about the identification of antigen/antibody complexes in the immune response detected using these diagnosis techniques. It appears, moreover, that inoculating rabbits with suspect material is a good means for isolating *T. equiperdum.*

In the absence of their authors, Dr. Touratier briefly presented two papers on the situation in Mongolia:

- CLAUSEN P.H., CHULUUN S., SADUOMDARJAA B., GREINER M., NOECKLER K., STAAK C., ZESSIN K.H. & SCHEIN E. – ***A field study to estimate the prevalence of*** T. **equiperdum** ***in Mongolian horses.*** Joint research: Free University of Berlin and Central Veterinary Laboratory of Mongolia

A study was conducted in Mongolia between May and July 2000 to assess the prevalence of *T. equiperdum* in the equine population of the central province of Tuv Aimag. An average of four herds were selected in each of the 29 territorial subdivisions (119 herds). In each herd, samples were taken from 10 horses, also taking into account the sex and age of the animals. Sera from 1,122 horses were analysed for research using CFT and ELISA. The gross results gave 7.6% positives using CFT and 6.7% using ELISA. In many cases, a deplorable clinical state was associated with these positive results for each test. Neither trypanosomes nor other hemoparasites were found during the microscopic examinations.

Examinations of 130 samples by PCR, using *T. brucei* primers, gave positive signs in 6.2% of the samples, which were also serologically positive. A single stallion, positive using PCR and ELISA, displayed pronounced weight loss, scrotal and preputial oedema and oedematous cutaneous plaques. All these factors combine to show that trypanosomal infection exists in Tuv Airmag province. However, since at present neither serology nor molecular techniques allow for specific identification in the *Trypanozoon* subgenus, it is impossible to say whether the herds examined are infected with *T. equiperdum* or *T. evansi*. However, a comparison of the clinical examinations, the negative parasitological results and the manifest concentration of seroprevalence in individual herds, would indicate the presence of dourine.

- PUREVSUREN BYARUUZANA, BYAMBAS BADARCH, VILJOEN G.J., ELLIS CH., POTTS A. & LOPEZ-REBOLLAR L.M. – ***Serological survey of animal trypanosomosis in Mongolia*** (this paper is the result of cooperation between the Central Veterinary Institute of Ulan Bator and the Onderstepoort Veterinary Institute of South Africa)

A wide-ranging serological survey of 1,023 horses was conducted in 24 areas in 8 provinces using the CFT, ELISA and CATT/*T. evansi* methods. Furthermore, the CATT test was used on 68 camels, taking all the samples at random. A few serum samples were also examined using the PCR method. Using the serological and molecular methods, trypanosomosis of horses was diagnosed in five provinces and of camels in one province.

In addition, blood samples from 30 camels and 1,183 horses were subjected to microscopic examination: all these examinations were negative.

Since none of these methods differentiate *T. equiperdum* from *T. evansi*, all these results were compared with clinical examinations and the epizootiological situation. Accordingly, the view is that:

- Surra of horses exists in the Hovd province where surra of camels has been diagnosed.
- Dourine exists in horses in the central region (Selenge, Hovsgol, Bulgan).

Further research must be carried out to clarify this situation.

Dr. Musiime then asked Dr. Trawford to present his short paper:

- TRAWFORD A. – ***Research sponsored or undertaken by the Donkey Sanctuary***

The Donkey Sanctuary is an animal care foundation, which started to expand its activities abroad in 1976. Its tropical disease control projects are based on mobile clinics and on sanctuaries for donkeys and mules.

Most of its projects, relying on cooperation and sponsorship from institutes and universities, concern parasitic diseases, especially in Kenya, Botswana, Mexico and Ethiopia.

With respect to dourine, two projects are in progress:

- The epidemiology of dourine in donkeys in Kenya, supervised by Dr. J.M. Ndung'u, Director of KETRI, Mugugu. Study of dourine in donkeys in Botswana by a team from the Royal Veterinary College of London.

- Draft study of dourine in donkeys in certain areas of Ethiopia.

In Kenya, a survey of 423 donkeys in the Kajiado district (western Kenya) detected 1% of subjects positive to *T. congolense* (microscopic examination of the centrifugation pellet). Twenty percent of the serological examinations were positive but it was not possible to define the species involved.

In Botswana, samples from 169 donkeys in five districts of the Maun region in northern Botswana showed a serological prevalence of 8% using CFT. The clinical examination detected 11 female donkeys with vaginal discharge and 4 male donkeys with oedema of the genitalia and abdomen.

3.2. *Trypanosoma evansi* (surra)

Professor Ph. Büscher briefly presented the following paper:

- HILALI M., ABDE-GAWAD A., NASSAR A., ABDEL-WAHAB A. & BÜSCHER PH. – ***Studies on* T. evansi *infecting water buffaloes in Egypt***

Blood samples were taken from 200 buffaloes for microscopic examinations. All these examinations proved negative. A serological examination of the same samples was then carried out using CATT/*T. evansi*.

This time, 24% positives were found, i.e. 48 animals with often-high levels of antibodies, 15 of which were level 8 and 18 were level 7.

The experimental intravenous infection of four six-month-old buffalo calves caused parasitaemia after four days and irregular hyperthermia, after which the clinical study confirmed the constants already described.

Furthermore, blood samples were taken from 280 equines (84 horses and 196 donkeys) for the parasitological search for trypanosomes, either by microhematocrit or mouse inoculation. *T. evansi* was found using mouse inoculation only once.

On the other hand, 93 donkeys and 7 horses tested positive (one of which was positive to a dilution of 1/32).

In the absence of Professor K.M.L. Pathak, Dr. Musiime asked Dr. Touratier to present his paper:

- PATHAK K.M.L. – ***Statement about epidemiology and diagnosis of cameline surra in India***

Between July and September 2002, 217 dromedary blood samples were examined using four methods: blood smear, stained slide smears, Ag-ELISA and PCR. PCR was the most sensitive method and confirmed 17% positives.

Another survey of 173 animals under 10 years of age gave an average positivity of 21.74% using PCR, with a maximum of 24.34% for she-camels.

Dr. Touratier then handed over to Dr. Tuntasuvan, who presented his paper:

- TUNTASUVAN D. – ***Is surra an important disease only for horses?***

In Thailand, surra was first detected in mules in 1949, after which numerous outbreaks were identified in horses, which led to surra of horses being declared a notifiable disease in 1956. Since that date, the disease has been acknowledged in numerous wild and domestic species: camelidae, cattle, buffaloes

Even though there has been no special study to measure the economic impact of the disease, its effects are most evident in small herds: abortion leading to the death of piglets and calves, deterioration of draught animals, preventing them from working, and declining meat and milk production.

For diagnosing surra, the ELISA method is acknowledged to be highly sensitive and specific. Recently, PCR was introduced and might make it possible to verify the efficacy of a trypanocidal treatment, especially by means of diminazene aceturate, which is the main substance used.

In conclusion, the economic impact of surra is underestimated and it is to be hoped that the next edition of the "Manual of Diagnosis Tests and Vaccines for Terrestrial Animals" will recommend a method for diagnosing this List B disease.

Dr. A.G. Luckins was pleased with the results obtained using the ELISA method in Thailand, which moreover confirmed what he had obtained in the countries in the region, as had been reported at the Ad Hoc Group's previous annual meeting in May 2002. However, since each laboratory developed and used its own diagnostic kit, the method needed to be validated.

3.3. Diagnosis

Dr. Inoue took the floor to describe this new diagnostic method proposed by the National Research Center for Protozoan Diseases in Japan:

- INOUE N. & IGARASHI I. – ***Loop-mediated isothermal amplification (LAMP) and its application for the detection of African trypanosomes***

Like PCR, this method allows the DNA to be amplified whilst observing it by spectrophotometry without the use of a stain. It is highly sensitive, specific and relies on the loop-mediated isothermal amplification technique, which makes it possible to detect parasites from the *brucei* group, including *T.b. brucei, T.b. gambiense, T.b. rhodesiense* and *T. evansi.* This LAMP method is proven to be 100 times more sensitive than *in vitro* PCR.

Since it does not require the use of a thermocycler and can be completed in 30 to 60 minutes at a temperature of 60°C to 65°C (that of a water bath) and the process leads to the formation of a white precipitate by-product corresponding to magnesium pyrophosphate, permitting a visual identification of the DNA amplification, it might be suitable for laboratory examinations more akin to conditions in the field.

After Dr. Inoue's presentation, Dr. Musiime opened the discussion.

Dr. Robinson said that Dr. Inoue had presented an abstract of the work that he himself is conducting on trypanosomes using this method. It is an isothermal method capable of detecting parasites in blood dried onto filter paper that requires no recycling equipment to produce the reaction. In addition, the reaction gives immediate positive or negative results, based on the turbidity inside the reaction tubes.

Dr. Lun asked why the dilution of the target DNA in the PCR titration tests gave uncustomary and non-linear results using gel analysis and why different fragments were formed when using the same protocol with the LAMP method.

Dr. Inoue replied that this happened in some cases but did not know why.

Dr. Lun asked which results Dr. Inoue really trusted: microscope, PCR or LAMP?

Dr. Inoue replied that he trusted the LAMP results.

Dr. Robinson asked how the primer-dimer artefacts were reduced in this reaction? If the PFR C (paraflagellar rod protein C) had been tested and if a protein specific to the parasite, which can differ slightly from one species to another, had been planned for, would that not be a starting point for identifying different trypanosome species, based on the specificity of the primers and genes used?

Could the polymerase used (*Bst*) be modified to act at a temperature of 20°C to 30°C, for example, which would allow for a field diagnosis in close proximity to the subjects (animals or humans) to be examined?

Dr. Inoue gave the following answers:

a) It was highly unlikely that a primer-dimer pair formation would be produced since the primers had been carefully chosen and the software was available for doing so.

b) He was familiar with the PFR C gene and knew that species-specific cytoskeletal proteins could be used in the diagnosis.

c) He did not know whether the polymerase used would act at the laboratory temperature but it was an interesting idea.

Dr. Luckins was not sure that a new diagnostic kit was needed.

Dr. Robinson summarised Dr Inoue's presentation, saying that he thought the LAMP method seemed an excellent idea. It appeared robust, accurate, easy to use and gave good results without the need for costly thermocyclers. It could be adapted to field diagnosis, particularly if other parasite-derived genes could be used. He felt that this protocol had the potential to identify various parasites.

In conclusion, Dr. Inoue felt that:

a) The LAMP method was a fast, easy and sensitive new method of DNA amplification; it was up to us to assess, improve and standardise it to include it as one of the methods for diagnosing NTTATs.

b) The genes of the PFR A elements of *T. brucei* and *T. evansi* that encode a single member of a cytoskeletal protein have been successfully amplified using LAMP. Further studies should be carried out to develop genes that allow several trypanosome species to be differentiated.

Dr. Robinson was very interested in the PFR A genes used by Dr. Inoue and felt that fruitful collaboration could be established between his own laboratory[8] and that of Dr. Inoue[9].

Then it was Dr. D. Verloo's turn to present his short paper:

- VERLOO D., MAGNUS E. & BÜSCHER PH. – ***Performance of the CATT/T. evansi in water buffaloes, dromedaries and camels***

The CATT/*T. evansi* test was used to compare the results of the microhematocrit centrifuge test (MHCT) on 729 dromedaries (Niger) and 267 water buffaloes (North Vietnam). In both groups of animals, the respective sensitivity and specificity were compared. The following results were found:

a) Sensitivity: 96.64% using the CATT and 25.38% using the MHCT in dromedaries
86.35% using the CATT and 68.68% using the MHCT in buffaloes.

b) Specificity: 97.32% using the CATT and 99.09% using the MHCT in dromedaries
96.40% using the CATT and 99.74% using the MHCT in buffaloes.

These results were discussed to estimate prevalence.

When the discussion opened, Dr. S. Reid said he agreed with the levels of sensitivity and specificity reported, which were in line with previously published material. Dr. Olaho-Mukani commented that mouse inoculation was still a good means for assessing sensitivity. Dr. Tuntasuvan said that the CATT has only low sensitivity when used on cattle and pigs.

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[8] Interaction mitochondrie-cytosquelette chez les trypanosomes [Mitochonrion/cytoskeleton interaction in trypanosomes], University of Bordeaux II, France.

In Dr. Luckins' view, the validation of diagnostic tests had to be based on their use in the field. Indeed, with regard to the CATT/*T. evansi*, Dr. Touratier pointed out that the Ad Hoc Group had recommended the use of the CATT for diagnosing *T. evansi* infection in the African dromedary following numerous field trials. This had already been the subject of a critical study and had given rise to the 1994 publication: O. Diall *et al.* (1994).- Evaluation of a direct serologic card agglutination test for the diagnosis of camel trypanosomoses caused by *T. evansi. Scientific and Technical Review of the Office International des Epizooties,* **13**, 793-800. Study of 1,093 dromedary sera.

The operating instructions for the CATT draw attention to the following points:

- CATT/*T. evansi* is an experimental direct card agglutination test for detecting *Trypanosoma* antibodies in the serum or plasma of infected animals.
- The test is done on a plastic card placed on a rotator turning at 60/70 revolutions/minutes for 5 minutes.
- The test is not strictly species specific, which can complicate the result in areas where other salivarian trypanosomes exist.
- If the test is used in species other than camelidae and buffaloes, for example in equidae, its conditions of use, i.e. the dilution threshold of the serum, as well as its sensitivity and specificity, must be determined in advance.

(A rotator is available that has been specially designed for performing the test under optimal conditions).

4. Special studies

4.1. Basic research into trypanosomes

In the absence of Dr. M. Desquesnes, Dr. Musiime asked for brief comments on the following two papers:

- DESQUESNES M. – ***Trypanosomes du bétail et leurs vecteurs en Amérique du Sud.*** EMVT Eds, 2004, 260 pp., Tables, diagrams, maps and illustrations.

Book reviewing the latest knowledge about *T. vivax* and *T. evansi* infections in South America.

- DESQUESNES M. & DIAI M.L. – **T. vivax:** ***mechanical transmission in cattle by one of the most common African tabanids:*** **Atylotus agrestis.** *Exp. Parasitol.*, 2003, **103**, 35-42.

This research shows once again that *T. vivax* infection by tabanids in Africa is possible and that it can play a significant role in the persistence of animal trypanosomosis in tsetse-free areas.

Dr. D. Robinson presented the abstract of the work he is conducting in his laboratory with the University of Bordeaux II research into basic trypanology:

- ROBINSON D. – ***Laboratory Interest and Research: Equipe ATIP (Interaction Microchondrie-cytosquelette ches les trypanosomes)***

He has identified new flagellar proteins by producing monoclonal antibodies on protein-free gel. These proteins appear to be specific to the parasite and are located in the flagellar basal body near the junction between the flagellum and the mitochondrial DNA. He is currently identifying the genes encoding these proteins, as well as new proteins specific to the parasite which could be targeted by trypanocidal drugs and at the same time carry great potential for a specific diagnosis.

Following this presentation, Dr. Touratier briefly summarised the short paper sent by researchers from the Institute of Animal Parasitology of the Chinese Academy of Agricultural Sciences in Shanghai:

- ZHOU JINLIN, ZHOU YONGZHI, SHEN JIE, BAI XIA & GONG HAIYAN – ***Comparison of different variable antigenic types (VAT) from a cloned* T. evansi**

Animal trypanosomosis in China is caused by *T. evansi*. It affects the following animal species: buffaloes, dairy and beef cattle, camels, horses, donkeys, cervidae, pigs, goats and tigers. It affects 20 provinces and can have a severe impact. For instance, during the last epizootic wave, in 1988, more than 120,000 cattle and buffaloes were infected in 8 provinces, with a mortality rate of 14.08%. The disease has now declined but is still enzootic.

At the Shanghai Institute (trypanology laboratory), 12 stocks of *T. evansi* from 10 provinces were set up. At the same time the laboratory holds *T. equiperdum*. Research is being conducted on all the topical issues, including trypanocide trials. ELISA, latex agglutination test (LAT) and PCR have all been tried as diagnostic methods. LAT was one of the first tests chosen for diagnosing trypanosomosis in China.

Numerous immunological and genetic studies have been carried out on VATs (Variable Antigenic Types), VSG genes (Variable Surface Glycoproteins) and PARP (Procyclic Acidic Repetitive Protein).

4.2. Pathogenicity and immunosuppression of *T. evansi* infections in South East Asia

Dr. Musiime asked the various speakers who had sent summaries of their papers to make their presentations one by one on these very important aspects of surra and their repercussions.

Dr. S. Reid (Murdoch University, Perth, Australia) presented the following paper which he had written with the participation of other colleagues from the region, in particular Dr. A. Dargantes from Mindanao in the Philippines and Dr. A. Husein from the National Laboratory of Bogor Balitvet, Indonesia:

- REID S.A., DARGANTES A., HUSEIN A. & CONSTANTINE C.C. – ***Geographic variation in the pathogenicity of* T. evansi**

Surra epidemiology varies considerably from one geographical region to another. In South East Asia, there are very pronounced differences between countries. For example, the Philippines are currently experiencing a series of severe surra outbreaks with high mortality and morbidity rates, whereas surra rarely causes mortality in Indonesia.

So we are seeing intra-specific variations in the pathogenicity of *T. evansi* strains, whereas genetic studies do not allow us to identify differences between the strains.

The experimental infection of mice using different strains of *T. evansi* showed that the Philippine strains are more pathogenic than those of Indonesia and that the Indonesian strains are more pathogenic than those of Sudan (the post-infection survival rate is taken as an indicator of pathogenicity).

However, tests to identify genetic variations in pathogenicity with priming techniques using ITS 1 (interspace 1) of the ribosomal region or else minisatellites according to Mc Leod and Biteau, failed to give conclusive results. Tests were then repeated using 11 microsatellites according to Biteau *et al.*

A wide-ranging discussion then began, involving Professor Büscher, as well as Drs. F. Van Gool, Musiime, Monzon, Luckins, Sidibé, Tuntasuvan and Halliwell. Several of their comments are worthy of mention.

Dr. Tuntasuvan agreed with Dr. Reid that a further study had to be carried out in severely or slightly affected animals, especially where there were nervous signs.

Dr. Sidibé felt that this study was very important for the epidemiology of trypanosomoses in Africa. More and more cases of surra were being described (Debab or M'Bori in Africa), but no clear distinction was made between *T. vivax* and *T. evansi* infections in tsetse-free areas. This seemed to be due to two factors:

i) All trypanosomal infections of horses and camelidae are labelled "*evansi*". with no consideration

ii) Any trypanosomes isolated are considered to be *T. congolense, T. vivax* or even *T. b. brucei* because *T. evansi* is not generally considered to be pathogenic for cattle, and since *T. evansi* is morphologically identical to *T. brucei,* people tend to consider it as a *T. b. brucei.*

Comparative studies by the authors of the paper of *T. vivax* should not only clarify their pathological scale, but also ascertain their real impact and help to assess the disease in equidae in tsetse-infested areas more effectively.

Dr. Halliwell fully agreed with these remarks. In addition, he thought that none of this basic research had immediate applications for the clinical and diagnostic aspects, nor for ability to treat the disease in the field. In this respect, we are constrained by a number of factors, including a lack of sampling and diagnostic equipment, drugs, means of transport, electricity and basic infrastructures. It had to be stressed that people living in the middle of nowhere often did not know where to get help. This was why such an Ad hoc Group ought to help set up a system for assisting farmers by providing them with effective means of diagnosis, advice, prevention and treatment.

Dr. Gutierrez presented his paper:

- GUTIERREZ C. CORBERA J.A. & BÜSCHER PH. – ***Performance of serological and parasite detection tests for* T. evansi *in experimentally inoculated goats***

T. evansi was diagnosed for the first time in the Canary Islands in 1997 in a dromedary imported from West Africa. The affected animals were treated and cured, but it is possible that the parasite exists in other host species, and goats in particular could play an important role as reservoirs.

The experimental infection of five goats with a local strain of *T. evansi* isolated from a dromedary took place over eight months. They were followed up using CATT/*T. evansi* and the Miniature Anion-Exchange Centrifugation Technique (mAECT) as serological and parasitic diagnoses.

All the animals remained positive to the CATT at a dilution of ¼ throughout the trial. Likewise, parasites could be detected using mAECT at a low power magnification (10x10), whereas this was not always the case using simple blood smears. From a clinical standpoint, the goats showed only a subacute evolution with signs of arthritis after six months.

Dr. Luckins found this detailed study on *T. evansi* infection of goats useful because the disease was common in South East Asia and little data was available.

Following on from Dr. Gutierrez, Dr. Holland successively presented two recently published papers on work he had conducted in South East Asia:

- HOLLAND W.G., MY L.N., DUNG T.V., THANH N.G., TAM P.T., VERCRUYSSE J. & GODDEERIS B.M. – ***The influence of* T. evansi *infection on the immunoresponsiveness of experimentally infected buffaloes.*** *Vet. Parasitol.*, 2001, **102**, 225-234.

- HOLLAND W.G., DO T.T., HUONG .N.T., DUNG T.V., THANH N.G., VERCRUYSSE J. & GODDEERIS B.M. – ***The effect of* T. evansi *infection on pig performance and vaccination against classical swine fever.*** *Vet. Parasitol.*, 2003, **111**, 115-123.

In the first publication, the experimental results showed that *T. evansi* infection seems to interfere with the development of the immunity conferred by a heterologous vaccination and could explain the low protection conferred by the vaccination of buffaloes against haemorrhagic septicaemia in areas of Vietnam where *T. evansi* is enzootic.

In the second publication, the immunosuppression induced by *T. evansi* seems to explain findings of low vaccine protection after classical swine fever vaccination of pigs living in areas where *T. evansi* is enzootic.

Right at the start of the discussion, Dr. Luckins and Dr. Tuntasuvan fully agreed with the harmful immunosuppression effects induced by *T. evansi.* Since Dr. Holland also had experience with surra of pigs in Thailand, Dr. Tuntasuvan remarked that the surra infection rate depended on the period of the year. She stressed that this rate was high during the rainy season when the biting insect populations were at their height and that it declined in summer. Moreover, north east Thailand was an area where surra was enzootic.

Dr. Touratier asked whether similar experiments on the influence of immunosuppression could be done in pigs in connection with FMD vaccination in areas where surra was enzootic.

Dr. Holland replied that tests on immunosuppression had already been done as a follow-up to the Vietnam/Belgium programme to study parasitic zoonoses. However, a new programme would be needed to study the potential effect of immunosuppression on FMD vaccinations, which would require additional international aid funding.

4.3. Progress with the development of the current dourine research programme (*T. evansi/T. equiperdum* diagnosis and differentiation, 3rd year)

Dr. Claes summarised the main phases of this programme, developed in his doctoral thesis (examined on 4 June 2003):

- CLAES F. – ***Characterisation of pathogenic* Trypanozoon *spp. How does* T. equiperdum *fit into the subgenus* Trypanozoon?**

Review of the principal laboratory work carried out, in response to the Ad Hoc Group's request (1999) and the proposal of the Institute of Tropical Medicine of Antwerp (Professor Ph. Büscher):

- To set up a collection of *T. equiperdum* strains with the kind cooperation of the national diagnostic laboratories.

- To examine these strains using common serological methods (including CFT) and various molecular techniques using PCR: ITS 1 , RAPD, MEGA, leading to the following conclusion:

  "Most of the *T. equiperdum* strains examined are similar, if not identical, to *T. evansi*, except two: BoTat1 and OVI, which are identical to *T. b. brucei.* It is interesting to note that these two particular strains of *T. equiperdum* lack the VSG gene of the RoTat 1.2 strain of *T. evansi.* Interpreting this data, it is possible to formulate a new hypothesis that the species *T. equiperdum* does not exist and that the BoTat1 and OVI strains are particular strains of *T. b. brucei*, whilst all the others now considered as *T. equiperdum* and containing the gene VSG RoTat 1.2. are in fact strains of wrongly identified *T. evansi.*

  To confirm this hypothesis, new strains should be isolated from horses with clinical signs of dourine in order to subject them to the same characterisation tests and to compare the results. Following that, experimental infection should be carried out on horses".

This last part of the research programme, also designed to result in a new dourine diagnostic test that is more sensitive and specific than CFT, is coming up against funding problems. To obtain new strains, contacts are being maintained with interested countries: South Africa, Ethiopia, Kazakhstan, Mongolia.

Two other papers were briefly presented by their authors:

- MONZON C.M. – ***Evaluation of monoclonal antibody for the differential diagnosis between* T. evansi *and* T. equiperdum**

The monoclonal antibody (Mab) 2-4 F_6 of *T. evansi* was tested to establish whether it could be used in the serodiagnosis of *T. evansi.*

Sera from 10 horses infected with *T. evansi* were compared using the indirect ELISA method with a

Subsequently, the antigens subjected to ultrasound were tested at different concentrations, which yielded differences between antigens from *T. evansi* and those from *T. equiperdum*, observed at the optical density (OD). Further tests were carried out with polyclonal antibodies and showed differences in captures of *T. evansi* and of *T. equiperdum* antigens, which, with Mab 2-4F$_6$, could make it possible to develop a specific diagnosis for *T. evansi.*

- ZHAO-RONG LUN, LI AN-SING, XIAO-GUANG CHEN, LI-XIN LU & ZHU- XING-QUAN – ***The genetic relationship among* T. brucei, T. evansi *and* T. equiperdum *stocks revealed by random amplified polymorphic DNA (RAPD) method***

The RAPD method was chosen because of its sensitivity, lower price and safety for examining 20 randomly-chosen primers to amplify the genomic DNA of 4 stocks of *T. brucei* (West and East African), 4 stocks of *T. evansi* (Africa, Asia, South America) and 1 stock of *T. equiperdum* from Asia.

The results showed that, in spite of the small number of each trypanosome species used in the tests, the stocks of *T. evansi* from China and Brazil were more closely related to the stocks of *T. equiperdum* from China and of *T. brucei* than to the stocks of *T. evansi* from Kenya, which suggested that these stocks of *T. evansi* from China and Brazil came from the same line.

In the absence of the authors, Dr. Touratier went on to introduce the following paper, sent by researchers from the Institute of Animal Parasitology of the Chinese Academy of Agricultural Sciences in Shanghai:

- JINLIN ZHOU, XIA BAI, YONGZHI ZHOU & HAIYAN GONG – ***Procyclic acidic repetitive protein (PARP) gene in* T. evansi *and* T. equiperdum**

This protein is a major VSG of the procyclic form of *T. b. brucei*, the function of which is not fully defined. It appears to play a role in protecting the parasite against the proteases present in the middle intestine of the vector insects.

This study made it possible to clone and sequence three PARP genes of the Sh Tat 1.3 clone of *T. evansi* (EP Glu-pro) and one gene of *T. equiperdum* (GPEET Gly-Pro-Glu-Glu-Tr).

A strong similarity emerged between the different PARP genes of *T. evansi* and *T. brucei*. The only GPEET gene of *T. equiperdum* was 100% identical to a gene of *T. brucei*. Further studies are in progress into *T. evansi* and *T. equiperdum.*

Dr. Robinson felt that this study should be continued, because if the previous data were confirmed, it would make it possible to clarify the role of the PARPs and the origins of this protein or family of proteins.

5. Control of trypanosomoses

5.1. Basic and applied research on trypanocidal drugs and candidate trypanocides

The following papers were presented in quick succession and were followed by only very brief discussions due to lack of time:

- BRUN R. – ***New diamidines as new drugs for African trypanosomoses including* T. evansi *infections***

After describing the establishment in 2000 of the trypanocide research consortium at the University of North Carolina (UNC) (United States of America), under the direction of Professor Tidwell, with the support of the Bill et Melinda Gates Foundation, Dr. R. Brun reviewed the work carried out at the Swiss Tropical Institute in Basel with the molecules supplied by the UNC and the University of Georgia (Department of Chemistry). The organisation of this research had been described at the OIE Ad Hoc Group meeting in 2001, document 69 SG/16).

At the request of the WHO, only the molecules derived from pentamidine with an activity when administered orally on *T. gambiense*, one of the trypanosomes of sleeping sickness, were chosen. However, the trypanocidal power of such molecules on *T. evansi* was also studied at the Institute of Basel (Switzerland)

A few of these diamidines tested *in vitro* also had a significant activity on *Plasmodium falciparum*, as well as on *P. berghei*, using the "mouse" model. In addition, eight diamidines were tested on a Chinese strain of *T. evansi* (strain STIB 806 in its kinetoplastic and akinetoplastic forms) with an excellent *in vitro* activity. Furthermore, one of these diamidines tested on mice was twice as active as didiminazene aceturate. The tests continue with other pentamidine derivatives and other strains of *T. evansi.*

Dr. Robinson remarked that although certain molecules were proven to be active on *Apicomplexa* and trypanosomes, this could be due to the high content of the adenine/thymine (AT) complex in the genome of these organisms and that some of these diamidines could be specific by inserting themselves into AT-rich DNA.

Dr. Touratier said that this remark was important, because if a new molecule possessed this double activity, as diminazene aceturate, both on *Apicomplexa* (agents of malaria and piroplasmosis) and on trypanosomes, the industry would be less reluctant to invest in developing such a derivative than in a purely trypanocidal molecule.

Dr. Brun agreed, saying he thought this was one of the numerous possibilities of this series of compounds, some of which are "pro-drugs". He also replied that he was only seeking activity on the members of the *Trypanozoon* subgenus, whereas Dr. Karambe was asking him whether the molecules studied were also active on *T. vivax.*

Dr. Van Gool then presented his abstract:

- VAN GOOL F. – ***An evaluation of the safety and efficacy of Melarsen Oxide cysteamine (Cymelarsan) in horses and cattle***

This trypanocide, which is active on *T. evansi,* was initially marketed only to treat surra of camelidae, due to the cost of setting up a registration dossier for each animal species.

However, the small number of active substances on the market and the emergence of chemoresistance to most of the trypanocides used in South America led to it being registered in this sub-region for the treatment of *T. evansi* infections in horses and cattle. The pre-trials had been conducted in Venezuela and Colombia to establish its efficacy in naturally and experimentally infected animals.

At present, new trials with African and Asian strains of *T. evansi* are in progress in Kenya (KETRI) and in Burkina Faso (CIRDES).

5.2. Paper from FAO[10] on the activities of the Programme Against African Animal Trypanosomoses (PAAT)

This 10 page document had been prepared by the Animal Health Service (FAO/AGAH), Animal Production and Health Division of FAO. It reviews the activities of the PAAT. A copy was handed to each participant.

5.3 GFAR[11] proposals to put into application effective measures for controlling trypanosomoses

Dr. E. Camus presented the following document:

- CAMUS E., DERMOTT J.MC. & LEFRANÇOIS T. – ***Global partnership programme: genetic resource management and biotechnology. Lutte contre les trypanosomoses***

  - Overview of the objectives and milestones of this global initiative and the reasons for choosing the control of trypanosomoses, which are imposing constraints on animal production in Africa, Asia and Latin America. List of founding organisations.

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[10] FAO: Food and Agriculture Organization of the United Nations

- Definition of the global priorities: exploitation of the genomics and biotechnology for developing and improving diagnostic methods, chemotherapy, the genetic resistance of animals, molecular epidemiology, immunological and therapeutic targets, etc., by taking into account risk analysis, risk prediction and mapping.

- Definitions of the regional priorities for each continent.

6. Any other business

The Secretariat had received the brochure "***Tsetse control: the next 100 years***" from the DFID.[12]

After this last announcement, Dr. Musiime thanked the participants and the meeting was closed at 13.15 hours.

.../Appendices

Appendix I

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

Paris, 18 May 2003

Agenda

1. **Interim report of the General Secretary (May 2002-May 2003)**

   1.1. Scientific meetings on trypanosomoses and means for controlling them
   1.2. Bibliographic references
   1.3. Information sent by correspondence

2. **Information reported to the OIE by Member Countries**

3. **Epidemiology of *T. equiperdum* and *T. evansi* infections worldwide**

   3.1. *Trypanosoma equiperdum* (dourine)
   3.2. *Trypanosoma evansi* (surra)
   3.3. Diagnosis

4. **Special studies**

   4.1. Basic research into trypanosomes
   4.2. Pathogenicity and immunosuppression of *T. evansi* infections in South East Asia
   4.3. Progress with the development of the current dourine research programme (*T. evansi/T. equiperdum* diagnosis and differentiation, 3rd year)

5. **Control of trypanosomoses**

   5.1. Basic and applied research on trypanocidal drugs candidate trypanocides
   5.2. Paper from FAO on the activities of the PAAT
   5.3 GFAR proposals to put into application effective measures for controlling trypanosomoses

6. **Any other business**

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

Paris, 18 May 2003

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LXXII OIE General Session
World Organisation for Animal Health

Paris , 23 – 28 May 2004

25 th Meeting of the ad hoc Group on Non Tsetse Transmitted

Animal Trypanosomoses (NTTAT)

(23 May 2004)

Progress Report 2003-2004
by Dr. Louis Touratier
Secretary General

Following the meeting of the ad hoc Group on 18 May 2003 an invitation was received from the Institute of Tropical Medicine ,Antwerp (Professor P. BÜSCHER) and from the Leuven University (Professor B. GODDEERIS) as well as from the Veterinary College ,Department of Parasitology (Professor J. VERCRUYSSE) , Gent , Belgium to attend the defences of the following PhD theses :.

Filip CLAES :- Characterisation of pathogenic trypanosomes – How does *T. equiperdum* fit into the *Trypanozoon* Group ?
Wicher HOLLAND :- The diagnosis of *T. evansi* and its immunosuppressive effect in water buffaloes and pigs

Among the directors and referees of these theses were members of the ad hoc Group (e.g. T. BALTZ , Ph. MAJIWA , A.G. LUCKINS) as well as Dr. TRUONG VAN DUNG , Director General , NIVR (National Institute for Veterinary Research , Hanoi , Vietnam) who raised interesting discussions and presented some data resulting from their research work in laboratory and/or in the field : A new expressed multigene family containing a hot spot insertion of retroelements is associated with polymorphic subtelomeric regions of *T. brucei* (T. BALTZ) ; Improvement in the detection of *T. evansi* infection (Ph. MAJIWA) ; Economic impact and cost of *T. evansi* infection (in Indonesia) (A.G. LUCKINS)..
In overall these PhD defences provided the occasion to draw – once more – the attention to two of the main concerns of the ad hoc Group :

- the need for the control of Dourine (PhD thesis : F. CLAES – See : ANNEX IV)
- a better understanding of *T. evansi* infections for their control and of Surra in particular in South East Asia (PhD thesis : W. HOLLAND – See : ANNEX V) .

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1 Dourine

If we are referring to the programme which was established at the annual meeting of this ad hoc Group in May 1999 , the PhD thesis of F. CLAES is one of its first steps . To sum up , this programme planned the following studies to try to clear up the situation resulting from discrepancies in results obtained by the use of CFT :

- isolation of new strain(s) of *T. equiperdum* from clinical dourine cases ;
- identification of specific markers for *T. equiperdum* which would make it possible to differentiate it from among the other species within the subgenus *Trypanozoon* ,
- the experimental infection of horses with newly isolated *T. equiperdum* strains to compare their pathogenicity with those currently used in national diagnostic laboratories and with that of *T. evansi* ;
- phylogenetic studies ;
- the proposal and validation of new internationally recognised diagnostic test(s) for dourine.

The following joint article – a comprehensive literature retrieval on the matter - ,written by specialists of the disease from Belgium, Germany, South Africa, Russia , in co-operation with the OIE Reference Centre for Dourine in Moscow , was published in February 2004 in the "*Rev. sci. techn. OIE*":

ZABLOTSKIJ V.T. , GEORGIU C. , DE WAAL T. , CLAUSEN P.H. , CLAES F. & TOURATIER L.:- The current challenges of dourine : difficulties in differentiating *T. equiperdum* within the subgenus *Trypanozoon. Rev. sci. Tech. Off. int. Epiz* , 2004 , **22** (3) ,1087 – 1096.

Moreover ,a written statement by F. CLAES will be presented in this meeting to mention the interest in his findings from the National Veterinary Diagnostic Laboratory , Ames , USA which could provide a valuable help for the experiments in horses.

In the field :

In Ethiopia : Thanks to a good co-operation between : The Veterinary Faculty, Debre Zeit , University of Addis Ababa , Ethiopia ; CIRAD/EMVT , Montpellier, France – ITM , Antwerp , Belgium – Freie Universität ,Berlin , Germany a serological epidemiological dourine survey will be initiated in the following months on 400 horses under the supervision of Professor ABEBE Getachew.
Similarly , with the support of the British Foundation " Donkey Sanctuary" an epidemiological survey will be tentatively launched in donkeys with the co-operation of Professor FESEAH Gebreab. (This important problem of dourine in donkeys in Ethiopia was evoked in Saint Louis, USA during the WAAVP Congress with Dr. Andrew TRAWFORD of the Foundation and Lord Professor L. SOULSBY , Honorary President).

In Kazakhstan , our colleague G. ILGEKBAYEVA – who described in the preceding annual meeting on 18 May 2003 the situation of NTTAT in her country - developed her screening to tentatively isolate new strain(s) of *T. equiperdum* in presence of some clinical cases of the disease. In the same time she is trying to differentiate *T. evansi* from *T. equiperdum* infections in horses which are extensively bred with camels in some parts of her country .

Thanks to a support from the Equine Association of French Practicioners (Association Vétérinaire Equine Française = AVEF) and on the kind invitation of Dr. Zh. KUMEKBAYEVA , President of the National Veterinary Association of Horses and Camel Breeders of Kazakhstan , F. CLAES could visit Kazakhstan in September 2003 for a short time . He and G. ILGEKBAYEVA visited the Agrarian Faculty , several camel and horse breeding farms and carried out numerous blood samples they examined with the Card Agglutination Trypanosome Test (CATT) to evaluate its practicability and reliability as a field test . They results were summed up in an article which will be published in French in the journal "Pratique Vétéerinaire Equine" (Equine Veterinary Practice) of which the summary is in the relevant set of documents provided for this meeting .

2. Differentiation *Trypanosoma equiperdum / T. evansi*

It seems that this concern of the Group is shared by several research workers in different countries . The following references with quotations are particularly interesting

GIBSON Wendy :- Species concepts for trypanosomes : from morphological to molecular definitions ? *Kinetoplastid Biology and Disease , 9 Feb. 2004,10 pp.,51 ref.*

The following extracts have to be quoted : ...."Neither *T. evansi* nor *T. equiperdum* is cyclically transmitted by tsetse (although tsetse potentially could transmit *T. evansi* mechanically), and indeed neither species is capable of cyclical development. *T. evanis* lacks a mitochondrial genome and its kinetoplast contains only a homogeneous set of minicircles. The few isolates of *T. equiperdum* examined also have missing kinetoplast DNA. One Chinese strain of *T. equiperdum* had maxicircles just over half the size of those of *T. brucei* and homogeneous minicircles like *T. evansi* Two other laboratory strains of *T. equiperdum* also had homogeneous minicircles ,one had full-size and one reduced size maxicircles. Examination of nuclear DNA polymorphism by isoenzymes, RFLP, karyotype, minisatellite or phylogenetic analysis has shown no obvious differences between *T. evansi* , *T. equiperdum* and *T. brucei.*
In a sense then, *T. evansi* and *T. equiperdum* can both be regarded as natural mutants of *T. brucei* . Do they deserve separate species status ? Arguably yes, because both satisfy the biological species definition above of non interbreeding populations. Since genetic exchange in *T. brucei* takes place during cyclical development in the tsetse fly , this excludes participation of *T. evansi* or *T. equiperdum.* "

Zhao-Rong LUN , An-Xing LI & Xiao-Guang CHEN :- Molecular profiles of *T. brucei* , *T. evansi* and *T. equiperdum* stocks revealed by the random amplified polymorphic DNA (RAPD) method. *Parasitol. Res.,* 2004 , **92** ,335-340 .

Two other articles , resulting from a co-operation between a South American laboratory well known for its research work on hemoparasites – including pathogenic trypanosomes isolated in the Region : *T. evansi* and *T. vivax* (Universidad Simon Bolivar ,Departamento de Biologia Celular , Caracas , Venezuela) – and a European Institute with a wide experience on the diagnosis of dourine (Istituto Zooprofilattico de Teramo, Italy) , were published about the differentiation :

GIARDINA S. , GIANSANDE D. , MERCANTE T. & PAGANICO G. :- Anticuerpos monoclonales producidos contra *T. equiperdum* y *T. evansi* reconocen ambas especies (Monoclonal antibodies produced against *T. equiperdum* and *T. evansi* recognise both species)*Revista Cientifica ,FCV-LUZ,* 2002 , **12** , 83 – 93.

GIARDINA S. , PAGANICO G. , URBANI G. & ROSSI M. :- A biochemical and immunological comparative study on *T. equiperdum* and *T. evansi. Vet. Res. Commun.* , 2003 , **27** , 289 – 309.

The authors conclude that their trials (monoclonal antibodies or biochemical and immunochemical studies) fail to differentiate both trypanosome species under study.

3. Some data on other NTTAT (*T. evansi* and *T. vivax*)

3.1. Immunosuppressive effects of *T. evansi* in South East Asia

The PhD thesis of W. HOLLAND : ' The diagnosis of *T. evansi* and its immunosuppressive effect in water buffaloes and pigs ' (See: ANNEX V) already quoted above brings very interesting findings about the effect of *T. evansi* infections in buffaloes and – even unapparent – in pigs in this subregion .
(i) **In buffaloes** ...." *T. evansi* infection could interfere with the development of protective immunity upon heterologous vaccinations and could explain the poor protection of *Pasteurella*-vaccinated buffaloes in *T. evansi* areas in Vietnam"

(ii) **In pigs** ...." This immunosuppression could explain the accounts of poor protection of CSF-vaccinated pigs reported in *T. evansi* endemic areas in Vietnam"

3.2. Data provided by the literature

HILALI M. , ABDEL – GAWAD A. , NASSER A. , MAGNUS E. & BÜSCHER Ph.:- Evaluation of the card agglutination test (CATT/*T. evansi*) for detection of *T. evansi* infection in water buffaloes (*Bubalus bubalis*) in Egypt:- *Vet. Parasitol.* 2004 , 7 May , **121** (1-2),45-51

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UZCANGA G. , MENDOZA M. , ASO P.M. & BUBIS J.:- Purification of a 64 kDa antigen from *T. evansi* that exhibits cross-reactivity with *T. vivax* . *Parasitology* , 2002, **124** , 287-200.

4.

BRETANA A. , NANEZ B. , CONTRERAS-BRETANA M. & GIARDINA S. :- Multiple infection in bovines from the tropics : observation of blood parasites by scanning and transmission electron microscope . *Parassitologia* , 2002 , **44** ,173-178.

VENTURA R.M. , PAIVA F. , SILVA R.A. , TAKEDA G.F. , BUCK G.A. & TEIXEIRA M.M. :- *T. vivax* : characterisation of the spliced-leader gene of a Brazilian stock and species-specific detection by PCR amplification of an intergenic spacer-sequence.*Exp. Parasitol.*, 2001, **99** , 37-48.

DAVILA A.M. , HERRERA H.M. , SCHLEBINGER T. , SOUZA S.S. & TRAUB-CSEKO Y.M. :- Using PCR for unraveling the cryptic epizootiology of livestock trypanosomosis in the Pantanal,Brazil. *Vet. Parasitol.*, 2003, **117** ,1-13.

GONZALEZ J.L. , TUDOR W. JONES , PICOZZI K. & RIBERA H.:- Evaluation of a polymerase chain reaction (PCR) assay for the diagnosis of bovine trypanosomosis and epidemiological surveillance in Bolivia . *Kinetoplastid Biology and Diseases* , 2003 , **2** , 1-16.

DESQUESNES M. , DIA M.L. , BOUYER J. & FATCHI M. :- Mechanical transmission of African *Trypanosoma spp.* by African Tabanids . (Paper to be presented on the IX European Multicolloquium of Parasitology , to be held in Valencia,Spain,18-23 July 2004).

DESQUESNES M. & DIA M.L.:- Mechanical transmission of *T. congolense* in cattle by the African tabanid *Atylotus agrestis* . *Experimental Parasitol.* , 2004 (*in press*).

LEJON V. , REBESKI D.E. , NDAO M. , BAELMANS R. , WINGER E.M. , FAYE D. , GEERTS S. & BÜSCHER Ph.:- Performance of enzyme-linked immunosorbent assays for detection of antibodies against *T. congolense* and *T. vivax* in goats . *Vet. Parasitol.*,2003 , **107** , 87-95.

BASTIAENSEN P. , DORNY P. , BATAWUI K. , BOUKAYA A. , NAPALA A. & HENDRICKS C.:- (Small ruminant parasitism in the suburban area of Sokode , Togo) (in French – comprehensive summary) I. Sheep:- *Rev. Elev. Méd. Vét .Pays trop.*,2003,**56** ,43-50.
(...it appeared that trypanosomoses (13 % of which 5.8% with *T. vivax*) remained the parasitosis with the highest impact...).

Second part of the study : sama authors , same title but : II Goats .*Rev. Elev. Méd. Vét. Pays trop.*, 2003 , **56** ,51-56.
(.... Trypanosomosis (8% of which 3.54% with *T. vivax*) was still the main parasitological constraint in terms of impact on health parameters , particularly on PCV).

3.2. Provided by the correspondence (South East Asia)

From this vast area where *T. evansi* infections are prevailing we interesting comments were received from two colleagues of the Philippines to whom we communicated the proceedings of the NTTAT meetings held in 2003 (Paris in May and Pretoria in September = round table on the occasion of the 27th ISCTRC meeting (see below).:
Dr. Gilbert BUENVIAJE , Dean , College of Veterinary Medicine , University of Southern Mindanao is conducting the surra research in the Philippines in partnership with the Mindanao Unified Surra Control Approach under the leadership of Dr. MERCADO . The research project is funded by ACIAR (Australian Centre for International Agricultural Research).

They outlined the importance of Surra in the Philippines , particularly in Mindanao, j where , in addition to horses , cattle, buffaloes (carabaos), goats, sheep, pigs and dogs are also affected The immunosuppression due to the trypanosome is evaluated in the risk assessment studies as follows : surra positive animals are 2 to 5 times more likely infected with *Pasteurella multocida* than the others.-

Moreover, it seems that *T. evansi* strains in the Philippines are more pathogenic than other strains in Asia and Indonesia because the increase of mortality and morbidity rate in cattle, buffaloes, goats and sheep.

lAs to the "new proposed diagnostic tests" Dr. MERCADO added that : " While PCR would do a lot in detection, as we are unable to set a laboratory because of the restrictive cost it would entail. A technical cooperation with corresponding fund support is a welcomè undertaking in our country. It is of our interest interest to establish genetic markers and compare our isolates with that of the other countries.

Control methods : We are confronted with the possibility o drug resistance mounted by *T. evansi* . The possibility is not far-fetched due to the fact that there are lots of factors that may influence it.: (I) Drug factor : We are using commercially available diminazene diaceturate : studies have shown that this drug does not have a 100% kill of trypanosomes. (ii) Human factor : Some field workers in our control areas are unable to perfectly administer the right dose of the drug (e.g. calculation of weight of the animals) under field conditions ; some workers may not beable to restrain the animals properly, hence the incorrect administration of drugs when animals struggle vigorously.

Impact of Surra :- " There are been no econoùic impact studies on Surra. During the pressing time we need a policy directive to address the problem on Surra. Unless we have the economic assessment data and information, we would not be able to persuade policy makers to advocate policies to address the Surra problem.
In the case of my region , in 1994, the estimates were based on the book value of the animals which were pegged at about 290 $ multiplied by the number of deaths incurred. The productivity losses were never considered such as those lpsses that maybe incurred throught the incapacity of the animals as draught power, other factors as low reproductive performers and many more. Farmers are forced to sell animals at 50% less than the actual book values even lower and the lost opportunity of a farmer to augment income through leasing of animals or work for rent. Again this an area where we should consider and give attention.
Moreover, we wish to determine the seasonality of the disease. The 1994 outbreak in Region XI taught us several things . One , Many of those animals dying are horses and carabaos which were used as draught animals. Larger number of those that have heavy parasitic load succumbed to death especially when nit treated with trypanocidal drugs.
The outbreak happened on extreme weather conditions. There were animals dying of the disease (diagnosed using direct microscopic examination) in the Northern part of the region where it rained heavily while on the Southern part , many died but on sunny days.

4. International specialised meetings

4.1. 19th International Conference pf the World Association for the Advancement of Veterinary Parasitology . New Orleans , USA , August 10 – 14 2003.

Only a few papers were devoted to pathogenic trypanosomes and animal trypanosomoses. :

AKSOY S. :- Control of tsetse flies and trypanosomes using molecular genetics
(This conference is published in : *Vet. Parasitol.*, **115** , 125-146.).

SVENDSEN E. & TRAWFORD A.F. :- Symposium donkeys : Hero or villain of the parasite world ?Past, present and future.

AULAKH G.S. , SINGLA L.D. , SOOD A.C. , KUMAR S. , PAUL H. SINGH J.:- Canine Trypanosomosis due to *T. evansi* (Punjab Agricultural University , Ludiana,India).

JEYABAL L. , CHAUDRY S.S. & DEVENDER CCS :- Detection of circulating immune complexes (cIC) of Trypanosomosis suspected cattle and buffaloes of Haryana (India) using sandwich-ELISA (Haryana Agricultural University , Hisar , India).

THOMPSON R.C.A.:- Advances in the diagnosis and systematics of parasites of veterinary importance : new and exciting prospects. (Western Australian Biomedical Research Institute , Division of Veterinary and Biomedical Sciences , Murdoch University , Western Australia).

These advances are described with reference to a range of parasites including *Cryptosporidium* , *Giardia* , *Toxoplasma* , *Trypanosoma* , *Echinococcus* and *Ancylostoma* . The future promises more exciting developments with the advent of proteomics and the prospect of adapting PCR-based diagnostics for use in remote sensors

CAI J. , WANG Z. & SHEN Y.:- Studies on the antigenicity of invariant surface protein of *T. evansi* . (Laboratory of Veterinary Parasitology , Institute of veterinary Science , Guangdong Academy of Agricultural Sciences , Guangzhou , 510640 , China).

4.2. 27th Meeting of the International Scientific Council for Trypanosomosis Research (ISCTRC). Pretoria , South Africa , 29 September – 3 October 2003.

An account of this very important Conference was already given on the occasion of the sending of the 'Minutes' and its annexes resulting from the Round Table on NTTAT which took place in Pretoria on 30 September 2003 thanks to the courtesy of the ISCTRC organising committee.
However, it is interesting to remind some points which are valid for all the countries in which NTTAT have some prevalence.

F. CLAES presented its communication on dourine :

CLAES F. , AGBO E. , BALTZ T. , GODDEERIS B.M. & BÜSCHER Ph.:- Relationship between *T. equiperdum* , *T. evansi* and *T.b.brucei* by molecular fingerprinting,

which allowed to introduce the discussion about the dourine in the African continent and the need to carry out epidemiological studies . This was reflected in a Recommendation in the plenary session of the Congress. The need for a specific and easy to perform diagnostic test was underlined by several participants . Dr. KHITMA EL MALIK added that infected animals should be attempted to be treated in limited trials to see whether the response is positive or not . On the othe hand, it is worth to be mentioned that , on historical records , a project is initiated in Nyala district , South Darfur State in Sudan to survey horses for *T. equiperdum.*h .

Cooperation between research workers of Africa, South America and Australia was illustrated with the following study
8. :

:

NJIRU Z.K. , DAVILA A.M.R. , GUYA G. & NDUNGU J.M.:- Detection of pathogenic trypanosomes using ITS1 based primers in a single PCR test in Kenya (Report n° 205).

The described technique which is resulting rom a cooperation between KETRI,Kikuyu,Kenya; Institute Oswaldo Cruz , Rio de Janeiro, Brazil and Murdoch University , Perth, Australia – allows to eliminate the non pathogenic trypanosomes *T. theileri* , *T. lewisi* which don't give PCR product. The authors conclude that : ..."The primers used seem promising in routine diagnosis and treatment purpose through a single PCR ".

The same method was applied in the articles quoted above in " **3** " .

Then there were wide exchanges of views about the transmission of *T. vivax* infections without tsetse flies (experiments in West Africa by M. DESQUESNES and M.L. DIA and observations outside the tsetse belt in Sudan (K. EL MALIK) and the diagnosis of *T. evansi* after the presentation of the following paper :

MAJIWA Phelix A.O. , URAKAWA T. & BÜSCHER Ph.:- Improvements in the detection of *<t; evansi* infections.

Other communication was presented on the same subject :

KHALIL KHALIL EL , ATIL ABDEL GADIR E; MUBARAK ABDEL RAHMAN , M. YASSIR MOHAMED O. , KHITMA EL MALIK & INTISSAR EL RAYAH :- Application of Card Agglutination Test for Trypanosomosis (CATT) and Card Indirect Agglutination Agglutination Antigen Test (CIATT) for detection of camel trypanosomosis in West Sudan.

New proposed diagnostic tests

Following the presentation of the above papers Prof. BÜSCHER and Dr. V. LEJON described the main modern techniques with the help of expressive CD they formerly processed.
All molecular techniques have high technical requirements which are seldom met in African diagnostic laboratories :

- PCR needs thermocycler , developing equipment (gel electrophoresis , ELISA system of hybridization equipment ;
- PNA – FISH needs hybridization block and UV microscope;
- (Peptic nucleic acid probe – Fluorescence *in situ* hybridization)
- LAMP : thermostable water-bath
- Proteomics : sophisticated approaches
-
- **Loop-mediated isothermal amplification (LAMP)**

This new diagnostic method has been presented at the 24th annual meeting of this Group on 18 May 2003 and was published by the end of 2003 :
KUBOKI T. , INOUE N. , SAKURAI T. , DI CELLO F. , GRAB D.J. , SUZUKI H. , SUGITOMO & IGARASHI I.:- Loop-mediated isothermal amplification for detection of African trypanosomes . *J. Clinical Microbiol.* 2003 , **41** , 5517-5524.

Prof. BÜSCHER describes the method as follows :
LAMP consists of auto-cycling strand displacement DNA synthesis at constant temperature by *Bst*DNA polymerase with two inner and two outer primers. A by-product of the reaction (magnesium pyrophosphate) precipitates In the reaction mixture thus allowing optical or spectrophotometrical reading of the result.
This technique looks promising for diagnosis of *Trypanosoma* infectio,s in laboratory condition where installation and maintenance of a thermocycler , as used for classical PCR , is not recoùùended.
Advantage over classical PCR is less technical demand but one should realise that LAMP is still a sophisticated technique , that developing the best primer is not vident and that the *Trypanosoma* specific LAMP system has not yet been exte,sively evaluated and thus remains far from validated.
Since this comment (November 2003) other articles were published on the LAMP showing its use to the diagnosis of Avian Influenza , Coccidioidomycosis , Legionellosis with rapidity , great sensitivity and specificity in comparison with PCR.- LAMP is developed by Eiken Chemical Co.,Ltd.,Tochigi,Japan.

Control methods (including trypanocidal drugs)

During the Conference , in Pretoria , several papers were presented about the laboratory and clinical trials which are in achievement with the metabolites of pentamidine , a drug discovered in the 1930's which was only active on the phase I of Human Sleeping Sickness (HSS). In particular , the derivative DB 289 which is a pro-drug , administered orally , gave encouraging results as well as its other derivatives against *T. gambiense*
The chemical research work is initially conducted at the University of North Carolina , USA by the consortium of Prof. TIDWELL , then in the Basel Tropical Institute and funded by the Bill & Melinda Gates Foundation.
The preliminary experiments were described in former reports of this OIE ad hoc Group on NTTAT (EU-COST meetings in Lisbon, Portugal,2001 and in London , UK , 2003). Moreover , during the 24th annual meeting of this Group in Paris on 18 May 2003 Dr. Reto BRUN gave valuable information about the progress of the research work aimed at synthesising new molecules active against the trypanosomes of the sub-genus *Trypanozoon* , including *T. evansi.*
Dr. BRUN added the following statement :
" In a pilot study carried out two years ago , 9 selected diamidines showed excellent *in vitro* activity against the Chinese *T. evansi* strain STIB 806 as kinetoplastic and as akinetoplastic forms. The dyskinetoplastic population showed a similar sensitivity as the kinetoplastic one.
Three compounds were also tested by the intraperitoneal route (ip) in the mouse model. All 3 compounds were able to cure mice infected with STIB 806 , the test compound DB 75 – which is the drug of the pro-drug DB 289 orally active (on *T. gambiense* infection – HSS) – was 8-times more effective than the standard drug diminazene aceturate and also than pentamidine. But as in animal one would not aim at applying per os (p.o.)therefore we did not test DB 289 orally against the same strain of *T. evansi* . This work will finally be continued in the form of a PhD programme.
Dr. BRUN plans to systematically screen diamidines *in vitro* , to confirm activity *in vivo* in his mouse model using different strains of *T. evansi* and to conduct experiments with selected candidates in larger animals.

4.3. Workshop on Cysteine Proteinases of Pathogens and their Roles in Host – Parasites Relationships .Université de Bordeaux II , 2 – 4 March 2004.

In this EU sponsored workshop with the participation of 48 fundamental research workers from 14 countries , 29 papers were presented of which 12 were devoted to animal and/or human Trypanosomoses .(Programme available on request).
Concerning especially NTTAT it is interesting to quote :

PERRONE T.M. , BREMO A. , GONZALEZ L. , TURCIOS L.M. , ASO P.M. , CAFFREY C.R. , McKERROW J.H. & GONZATTI M.I. :- Could Evansain be a pathogenic factor for *T. evansi* ?

BITEAU N. , LECUIX I. , LALMANACH G. , BOULANGER A. , AUTHIE E. & BALTZ T. :- Characterisaion of vivapains , *T. vivax* cysteine proteinases.

4.4. 1er Congrès International de Brazzaville sur la mouche tsetse et les Trypanosomoses (Brazzaville , Congo , 23-25 mars 2994).

Besides reports entirely devoted to Human Sleeping sickness , several papers dealing with animal trypanosomoses were announced :

MATTIOLI (R.):- La lutte contre les trypanosomoses animales , une voie pour la réduction de la pauvreté.

MELVILLE S.J. :- The *T. brucei* genome project : access to data analysis.

Dr. Sarah MELVILLE underlined the main advantages which will result from the very proximate knowledge (Summer 2004) of the nuclear genome of a single clone isolate of *T. brucei* . ..."The sequence data and associated bioinformatic analyses open resource, viewable via publically available web-accessible genome databases (or CD copy) . In the coming years these data will be further annotated by the research community with functional data derived from laboratory experiments. Therefore the ongoing curation of the genome sequence represents a centralised community-owned endeavour.
Genome sequencing is in essence an enabling technology : it enables researchers to refine hypotheses *in silico* before proceeding to wet bench experiments; it enables faster progress in many molecular biological experiments; and it enables whole genome analyses both *in silico* and using modern techniques of genome analysis in the bench. However, it is necessary to understand the format of the data, the underlying aims of the various databases and also the potential and limitations of *in silico* analysis for experimental design.
Of course, it is the fervent hope of all involved in the genome project that the data should subsequently serve to facilitate more rapid progress in research aimed at improving diagnostics and drug discovery. This presentation will aim to clarify what data will be available and how they should be accessed and interpreted, leading to discussion of how such data may contribute to fundamental research aimed at development of improved tools for management of trypanosomoses ".

HASSAN HAHAMAT :- Historique et actualité de la Trypanosomose animale .

MUSIIME J.T. & JANNIN J. :- Le projet FAO/OMS/ICIPE : Integrated control of tsetse and trypanosomosis in Southern Chad (ISCTRC).

4.5. International Symposium of Parasitology , Russian Academy of Sciences
Dedicated to 125 year anniversary of K.I. SKRYABIN , Moscow , 14-16 April 2004

On the invitation of the Institute of Parasitology (Russian Academy of Sciences) the following report was presented :
TOURATIER L.:- Current development of research and new perspectives in the knowledge of Non Tsetse Transmitted Animal Trypanosomoses.(NTTAT)

4.6. International meeting on camel husbandry in Central Asia - Ashgabat , Turkmenistan – 19-22 April 2004 .
The meeting – NATO sponsored – met specialists from various countries of Asia , Africa and Europe . Proceedings are being issued by CIRAD/EMVT..

5. Problems with chemoresistance to trypanocidal drugs.

Two relevant updated reprints were received on this important matter :
:
1) from Dr. P.H. CLAUSEN , Freie Universität , Berlin , Germany :

TEWELDE N. , ABEBE G. , EISLER M. , McDERMOTT J. , GREINER M. , AFEWORK , Y. , KYULE M. , MÜNSTERMANN S. , ZESSIN K-H. & CLAUSEN P.H. ;- Application of field methods to assess isometamidium resistance of trypanosomes in cattle in western Ethiopia . *Acta Tropica* , 2004 , **90** , 163-170.

2) From Fr. Oumar DIALL :
(Management of chemoresistance in the framework of the integrated control of Trypanosomosis in the cotton area of West Africa) . Gestion de la chimiorésistance dans le cadres de la lutte intégrée contre la Trypanosomose dans la zone cotonnière de l'Afrique de l'Ouest). Bulletin n°6 – Mars 2004 , 6 pp.
(Un projet financé par la coopération allemande ; BMZ/GTZ).

6. Sequencing of the genome of *T. brucei* (See also above *in* 4.4.)

According to a message from Tryplink the sequencing teams funding agencies are investigating the possibility of producing a CD containing the genome information.
Moreover , an international meeting is scheduled to be held in Seattle in September 2004 on the occasion of the next availability of the results of the sequencing project.

7. New animal disease notification system of the O.I.E. , the World Organisation for Animal Health.

According to the Editorial from the Director General , updated April 2004 , " the Regional Commissions have instructed the OIE Central Bureau to establish a single OIE list of notofiable terrestrial animal diseases ".....-
" The overriding criterion for a disease is to be listed if its potential for international spread".
Taking account of this unification , the Secretary general of this Group wrote to the Director General to ask the NTTAT be introduced in the new classification . Parasitic diseases are playing a role in the biosecurity and several historical examples can be quoted after severe infections or infestations into countries or even sub-regions where they did not formerly exist before undue imports of pathogens.

8. Coming events

8.1. IX European Multicolloquium of Parasitology (EMOP IX). Valencia , Spain , 18-23 July 2004

8.2. I Simposio Internacional : Hemoparasitos y sus Vectores . Universidad Simon Bolivar , Departamento de Biologia Celular , Caracas , Venezuela.
www.hemoparasitos.eventos.usb.ve..
The Secretary general of this ad hoc Group has been invited to give an opening lecture.

8.3. XIX Congreso Panamericano de Ciencias Veterinarias . Buenos Aires , Argentina , 24-28 October 2004.

Thanks to the courtesy of Dr. M. BULMAN , President of the Asociacion Argentina de Parasitologia Veterinaria (AAPAVET) we tried to organise a seminar or a round table on NTTAT within the time which was attributed to the AAVEPA but the lack of time within the general programme prevented this proposal. However, Dr. C.A. MONZON will be able to present posters on problems arising from *T. evansi* infections (Derrengadera and mal de caderas) in Argentina

8.3. STVM 8th Biennial Conference , Hanoi , Vietnam , June 26-July 1 2005.

Jointly organised by the Society of Tropical Veterinary Medicine ,USA and CIRAD , Montpellier, France this Conference will be centred on : " Impact of emerging zoonotic diseases in animal health ".
Website : www.stvmvietnam.org

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8.4. XIIth International Congress of Protozoology (XIIth ICOP) , Guangzhou , China, July 11-15 , 2005.

Proposal by the XIIth ICOP Organising Committee to hold and to support the 3rd NTTAT International Seminar (after Annecy , France , 1992 and Obihiro,Japan,1998) on July 9-10,2005 in Guangzhou just before ICOP XII.
Website : www.congress.com.cn.

ANNEX V

Rev. sci. tech. Off. int. Epiz., 2003, 22 (3), 1087-1096

The current challenges of dourine: difficulties in differentiating *Trypanosoma equiperdum* within the subgenus *Trypanozoon*

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Summary

During its 20th annual meeting in Paris in May 1999, the OIE (World organisation for animal health) Ad Hoc Group on Non-Tsetse Transmitted Animal Trypanosomoses expressed the following concerns about dourine:

– the discrepancies in some of the results of the complement fixation test (CFT), which is the only international diagnostic test officially recognised by the International Organisation for the Transportation of Equidae

– the persistence of suspected cases of dourine in some Asian, European and African countries

– the impossibility of differentiating *Trypanosoma equiperdum* from *Trypanosoma evansi* and of isolating new strains of *T. equiperdum* from clinical cases that have appeared in various parts of the world since 1982.

In the light of these concerns, it was decided, in agreement with the Directorate of the Federal Veterinary Services of Russia in Moscow, to perform comparative trials on the value of CFT/dourine at the OIE Reference Laboratory for dourine in Moscow (The All-Russian Research Institute of Experimental Veterinary Medicine) using reagents (antigens and sera) from seven countries with extensive experience in the field of dourine diagnosis, namely, South Africa, France, Italy, Germany, Russia, the United States of America and the People's Republic of China. It is thanks to the successful co-operation of these countries that the trials were made possible. Results showed an overall concordance and were submitted for consideration to the OIE Biological Standards Commission, the commission which is in charge of the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. These trials serve as a starting point for further study, particularly in the following areas:

– the isolation of new strains of *T. equiperdum* from clinical dourine cases

– the identification of specific markers for *T. equiperdum* which would make it possible to differentiate it from among the other species within the subgenus *Trypanozoon*

– the experimental infection of horses with newly isolated *T. equiperdum* strains to compare their pathogenicity with those currently used in national diagnostic

ANNEX VI

Trypanosoma equiperdum . taking second base.

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From my PhD research thesis it appeared that *T. equiperdum* is more heterogeneous than *T. evansi* and is more closely related to *T. brucei* than formerly thought.
Only two of the presently available *T. equiperdum* strains , *in casu* BoTat1.1 and OVI are considered to be "real" *T. (b) equiperdum* based on serodeme analysis and DNA fingerprinting (PCR RoTat1.2 , Random Amplified Polymorphoc DNA – RAPD – and Multiple-Endonuclease Genotyping Approach - - MEGA -). The rest of the strains are considered to be misidentified *T. evansi* .
For *T. evansi* we already identified a specific molecular marker : the RoTat1.2. variable surface glycoprotein (VSG).
However, to confirm the above hypothesis , two conditions are required : (i) isolation and characterisation of trypanosomes from confirmed Dourine cases and (ii) experimental infections in horses with the BoTat and OVI strains .
In the next stage , we will compare the differential gene expression of *T. equiperdum* , *T. evansi* and *T. brucei* using new molecular techniques such as microarrays. This will lead to new molecular markers for species characterisation , differences in transmission route and host spectrum.
Moreover, since the sequencing of the *T. brucei* genome is nearly finished , it will become relatively simple to identify the differentially suppressed genes and to compare them with the available sequences..

ANNEX VII

Sero-epidemiological survey for trypanosomiasis in Mongolian horses : a summary

Mongolia is a country of animal husbandry, it is very important for Mongolian economic and life of herdsmen.

In Mongolia, grazing animals play a major role in animal husbandry and these livestock populations are camels, horses, cattle, sheep and goats.

In the past years the number of livestock have decreased, because of various factors such as environmental and climatic (drought, blizzard) and diseases. According to statistical data, horse population was 2.7 million in 2000, 2.2 million 2001, 2 million in 2002.

Mongolian horses are used for transportation and horse riding. Also, they provide us with meat, milk, hides, and hair from tail and mane.

In Mongolia, Surra has been diagnosed in one province. In last years, horses in different provinces of Mongolia have been examined for trypanosomiasis.

Complement Fixation Test has been used for the examination for trypanosomiasis. Approximately 5000 horses were tested by this test, they were sampled from 14 provinces (aimag), 55 villages (soum).- German (Bgvv), South African (OVI), and Mongolian (SCVL) antigens were used for the horse trypanosomiasis which were prepared from *T.equiperdum* stabilates. Trypanosomiasis has been diagnosed in horses in 8 provinces of Mongolia with CFT, the prevalences were $0.41 \pm 0.20 - 18.0 \pm 3.84\%$.

The horses were examined using CATT (Ro Tat 1.2, a predominant Variable Antigen Type (VAT) of T.evansi), samples were collected from 6 provinces and some horses of these 6 provinces were positive ($37.2 \pm 2.98\%$).

Furthermore, some horses were tested with ELISA technique. The samples were collected from the same area in which Surra has been diagnosed in camel. Sonicated German and Mongolian antigens were used for the ELISA. The antigens were the same as those in use for CFT. Some horses were positive by this technique. Also, some other

tests such as PCR were used for the same horses, they also showed positive results. CATT was used for horse trypanosomiasis which was prepared from the same area where Surra has been diagnosed in camel and bordered area. Positive reaction has been shown from those horses by CATT. However, they gave negative results in CFT by *T.equiperdum's* antigen.

In other words, these area's horses were negative by CFT *(T.equiperdum)*, they were positive by CATT *(T.evansi)*, they also showed positive results by ELISA (sonicated *T. equiperdum* antigen) and PCR techniques.

Besides this, diagnostic kits were prepared for CFT and ELISA. Antigen was prepared from *T.equiperdum* stabilate (SVP), positive control sera were prepared from a field infected horse and hyperimmunised rabbits, negative control sera were prepared from non infected horses and non immunised rabbits by antigen of *Trypanosoma* ..

Pathogenesis has been studied in spleen with *Trypanosoma* infected mouse ; lymphocytic and plasmocytic hyperplasia, macrophages and necrosis were detected.

Trypanosomiasis has been diagnosed by CFT *(T.equiperdum antigen)* in several provinces of Mongolia.

Note: Ogange provinces – Trypanosomiasis has been diagnosed

Trypanosomiasis has been diagnosed by CATT *(T.evansi* antigen*)* in several provinces of Mongolia.

Note: Green provinces – Trypanosomiasis has been diagnosed by CATT

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ANNEX VIII

Control of Surra (*Trypanosoma evansi*) in the two – humped camel (*Camelus bactrianus*) population of Western Mongolia.

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Unlike the dromedary , the bactrian camel so far has largely been neglected by scientists in the Western world. Therefore, the Yak and Camel Foundation has taken up intensive research activities in Mongolia on the subject of bactrian camels.
Surra caused by *T. evansi* was diagnosed for the first time in Mongolia in 1936 in bactrian camels in Zereg in North-Western Mongolia.

Our objective is the validation of control strategies against Surra in the camel population of the great lake depression of Western Mongolia.
Based on the results of a cross sectional study , a longitudinal study will be conducted to estimate the incidence of *T. evansi.* A random sample of the camel population at risk will be selected and divided in two groups : a treatment group and a non-intervention group. The treatment group will be treated with melarsomine (CYMELARSAN) at the recommended dose. The non-intervention group will be traditionally managed and not examined nor treated by the project prior to the completion of the study. Animal health and production data (PCV%, body condition score, yield and wool production) will be assessed and compared between both groups at the end of the study . A cost-benefit analysis will show whether the control strategy can be justified.

Comparison of serological tests for equine trypanosomoses in natural infected horses from Kazakhstan

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INTRODUCTION

Among the widespread protozoan illnesses bringing significant economic damage to the development of horse- and camel-breeding in the Republic of Kazakhstan, trypanosomoses occupy an important place. For mass screening of the horse population for equine trypanosomoses, serological methods of choice have been studied since the early 20th century (Luckins, 1994).

For dourine, the only diagnostic test recommended at this moment, by the World Animal Health Organisation (OIE), is the Complement Fixation Test (CFT) (Watson, 1915). CFT detects antibodies against *T. equiperdum* in the serum of the host. Using this test, *T. equiperdum* has been eradicated in the USA, Canada and the EU. More recently, a horse complement fixation test (HCFT) (Saidouldin, 1999) has been developed for the diagnosis of dourine. Ilgekbayeva et al. (1999) have demonstrated that in HCFT antibodies are found earlier, in high titers and during a longer time in comparison to CFT.

On the other hand, a diagnostic antibody detection test for surra (*T. evansi*) based on the RoTat 1.2 VAT has been developed, namely CATT/*T.evansi*, a direct card agglutination test (Bajyana Songa et al., 1988). It has been proven that most so-called *T. equiperdum* strains also express isoVATs of RoTat 1.2 and therefore the CATT/*T. evansi* might prove to be a good test for equine trypanosomoses, regardless from the fact whether the causative agent is *T. evansi* (surra) or *T. equiperdum* (dourine) (Claes et al., 2003).

In this study, we compared the classic CFT, HCFT and CATT/RoTat 1.2 for the diagnosis of equine trypanosomoses in Kazakhstan. We evaluated the concordance between tests, using the chi square test.

RESULTS

All sera from the naïve population (n=89) tested negative for all three tests (data not shown). For the field samples (n=132), cross comparisons from the three different tests are shown in tables 1 and 2. Table 1 compares CFT versus HCFT and CATT respectively; Table 2 compares the HCFT versus CATT. The Chi-square result from CFT versus HCFT is 46.2 ($p < 0.001$); CFT versus CATT also gives an identical Chi-square of 46.2 ($p < 0.001$) indicating a significant concordance between CFT and HCFT or CATT respectively. Comparison of HCFT versus CATT shows a lower but still significant Chi-square of 16.8 ($p < 0.001$) indicating also a good concordance between these two tests.

Table 1. Comparison of CFT results versus HCFT and CATT/*T. evansi*

CFT		Results																
end titer	No	HCFT											CATT						
		1:5-	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:4-	1:4	1:8	1:16	1:32	1:64	1:512
1:5-	113	107	1	4	1								107	2	4				
1:5	4	3		1									4						
1:10	10	2		2	1	2	1			1	1		3	1	3	1		1	1
1:20	3	2									1			1	1	1			
1:40	1			1										1					
1:640	1											1			1				
total	132	114	1	8	2	2	1	-	-	1	2	1	114	5	9	2	-	1	1

Table 2. Comparison of HCFT results versus CATT/T. evansi

HCFT		CATT					
titres	No	1:4-	1:4	1:8	1:16	1:64	1:512
1:5-	114	104	4	6			
1:5	1	1					
1:10	8	6	1			1	
1:20	2	1			1		
1:40	2	1		1			
1:80	1	1					
1:640	1						1
1:1280	2			1	1		
1:2560	1			1			
Total	132	114	5	9	2	1	1

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Evaluation of loop-mediated isothermal amplification (LAMP) for detection of *Trypanosoma evansi* in experimentally infected pigs

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Abstract

Six surra negative piglets were infected with *T. evansi.* There was 65 % of positive detection by mouse inoculation (MI), whereas, LAMP, PCR, microhematocrit centrifuge and thin blood smear detected 42 %, 31 %, 35 % and 22 %, respectively. Except for MI, LAMP detected *T. evansi* with the most sensitivity.

Trypanosoma evansi is a hemoflagellate protozoan parasite of veterinary importance that causes disease epidemics known as surra. It is the most widely distributed of the pathogenic trypanosomes, affecting domesticated livestock in Asia, Africa and South America (12, 23). *T. evansi* infects and produce a mild or subclinical form of the disease in buffaloes, llamas, cattle, donkeys, goats, sheep and pigs (19). Species of blood sucking insects belonging to the genera *Tabanus, Stomoxys, Atylotus* and *Lyperosia* transmit *T. evansi* among mammalian hosts (2, 13). Diagnosis of surra by simple parasitological and serological methods is limited by their lack of sensitivity, specificity and cross reactivity (4, 23). The polymerase chain reaction (PCR) is a sensitive and specific method that has been reported to be effective in detecting pathogenic microorganisms (3, 10, 20). However, it is not commonly used in developing countries since it requires delicate and expensive equipment.

Loop-mediated isothermal amplification (LAMP) is a novel method that can amplify few copies of DNA to 10^9 in less than an hour under isothermal conditions and it is simple and easy to perform as it requires 4 primers, *Bst* DNA polymerase, and a regular laboratory heat block or a water-bath for the reaction (6, 8, 16). Recently, the LAMP experiments conducted by Kuboki *et al.,* (11) successfully amplified African trypanosomes DNA and they reported that LAMP is a suitable diagnostic method that can be utilized in laboratory and field situations. This study evaluated the performance of different diagnostic methods including LAMP, PCR, microhematocrit centrifuge test (MHCT) and thin blood smear test (TBS) for the detection of *T. evansi* from experimentally infected pigs.

Experimental animals and sample collection

Six of the 8 piglets (6 weeks old) were infected intravenously with 6 x 10^5 *T. evansi* parasites (isolated from naturally infected horse in Khonkaen – Thailand) whilst 2 were kept as negative controls (National Institute of Animal Health – Thailand). Blood samples were collected weekly from each animal for a period of 12 weeks for microscopic examinations and DNA extraction using QIAamp® DNA blood kit (Qiagen, USA).

LAMP

We used 4 primers designed from paraflagella rod protein A (*PFR A*, GenBank Accession number X14819) by Kuboki *et al.,* (11) with the following nucleic acid sequences,

FIP: 5'-TCA GAA GCG TCG AGC TGG GAT TTT ATC GAC AAT GCC ATC GCC-3'; F3: 5'-TCA CAA CAA GAC TCG CAC G-3'; BIP: 5'-CGC AAG TTC CTG TGG CTG CAT TTT TTC CCA AGA AGA GCC GTC T 3'; B3: 5'-GGG CTT TGA TCT GCT CCT C-3'.

Reaction mixture (25 µl) contained: 2x reaction buffer (40 mM Tris- HCl (pH 8.8), 20 mM KCl, 16 mM $MgSO_4$, 20 mM $(NH_4)_2SO_4$, 0.2 % Tween 20, 1.6 M Betaine, 2.8 mM of each dNTP), primer mix (40 pmol of each FIP and BIP and 5 pmol of each F3 and B3), 2 µl of template DNA and 1 µl of *Bst* DNA polymerase. The reaction tube for each sample tested (total volume 25 µl) was incubated in a heat block (Dry Thermounit DTU 1B, TAITEC Co, Japan) at 63 °C for 1 hour then at 82 °C for 2 minutes to terminate the reaction. Thereafter, LAMP products were electrophoresed in 1.5 % TAE agarose gel and visualized by staining with ethidium bromide.

PCR

We used primers known as MP1 and MP2 (1, 5) that gave the product size of 362 bp with the following nucleic acid sequences,

MP1: 5'-CAA CGA CAA AGA GTC AGT-3'

MP2: 5'-ACG TGT TTT GTG TAT GGT-3'.

The PCR reaction mixture (50 µl) contained: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2 mM of each dNTP, 5 pmol of each primer, and 0.5 U of AmpliTaq Gold DNA polymerase (Applied Biosytems, Japan Ltd., Japan). The reaction mixture was heated at 94 °C for 1 min and cycled for 35 times by heating at 94 °C for 1 min, annealing at 55 °C for 2 min and elongation at 72 °C for 2 min. The PCR products were then electrophoresed in 1.5 % TAE agarose gel and visualized by staining with ethidium bromide.

Parasitological tests

The entire tests were conducted in accordance with OIE manual of standards for diagnostic tests and vaccines (17).

Out of a total of 72 DNA samples, 42 % (30/72) were positively detected by LAMP. The PCR, MHCT and TBS methods positively detected 31 % (22/72), 35 % (25/72) and 22 % (16/72), respectively (Table 1). It is evident from this study that LAMP has higher detection sensitivity than PCR and parasitological tests (MHCT and TBS) and it has been similarly reported by Kuboki *et al.*, (11). However, with statistical difference declared at ($p<0.05$),

($p>0.05$) in sensitivity between LAMP, PCR and MHCT (Table 2). There was a tendency of significant difference ($p=0.05$) in sensitivity between LAMP and TBS (Table 2). The MI method showed 65 % (43/66) positive detection and it was the most sensitive overall as compared to all diagnostic methods used in this study. However there was no significant difference ($p>0.05$) in detection sensitivity when MI was compared with LAMP (Table 2). Earlier studies showed that MI detected the greatest number of infections among various parasitological tests (21, 23), it is however, not practically applicable for diagnostic purposes as it is time consuming (22) and not economically viable. Hence, we used the test to confirm the presence of parasites in infected pigs and as a gold standard method.

In conclusion, the parasitological methods are widely used for diagnosis of trypanosomosis but are negated by their lack of sensitivity and specificity. The PCR requirements for infrastructure, equipment and technical skill remain as an obstacle to utilize the technique especially for many laboratories in underdeveloped countries where *T. evansi* is endemic (7, 9, 18). Therefore, LAMP offers better prospect for much sensitive diagnosis of surra with advantage of simplicity, high sensitivity and specificity. It also produces extremely large amounts of amplified products that enables simple detection methods such as by the visual judgement by the turbidity or fluorescence of the reaction mixture (14, 15, 16). LAMP has thus emerged as an alternative molecular diagnostic technique. With proper primer design, this method can be exploited as a useful diagnostic tool for trypanosome infections especially for epidemiological studies.

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Table 1: Detection performance of each method for *T. evansi* in pigs

Pig no.	Method	Week post-infection											
		1	2	3	4	5	6	7	8	9	10	11	12
A	LAMP	-	-	+	+	-	-	+	+	+	-	+	-
	PCR	-	-	+	+	-	-	+	+	-	-	-	-
	MHCT[a]	-	-	+	+	-	-	+	+	-	+	-	-
	TBS[b]	-	-	+	-	-	-	+	-	-	-	-	-
	MI[c]	nd	-	+	+	-	+	+	+	+	+	-	-
B	LAMP	-	-	+	-	+	+	+	+	+	+	-	+
	PCR	-	-	+	+	-	+	+	+	-	+	+	-
	MHCT	-	-	-	+	-	+	+	+	-	+	+	+
	TBS	-	-	-	+	-	+	+	+	-	+	+	+
	MI	nd	-	-	+	-	+	+	+	-	-	+	+
C	LAMP	-	-	-	-	-	-	-	+	+	-	-	-
	PCR	-	-	+	-	-	+	-	-	-	-	-	-
	MHCT	-	-	+	+	-	-	-	-	-	-	-	-
	TBS	-	-	-	-	-	-	-	-	-	-	-	-
	MI	nd	+	+	+	-	+	+	+	+	+	-	-
D	LAMP	-	+	-	+	-	+	-	+	-	-	-	-
	PCR	-	-	+	+	-	-	-	-	-	-	-	-
	MHCT	-	-	+	+	-	-	-	-	-	-	-	-
	TBS	-	-	+	+	-	-	-	-	-	-	-	-
	MI	nd	-	+	+	+	-	+	-	-	+	+	-
E	LAMP	-	-	+	+	+	+	-	+	-	-	+	-
	PCR	-	-	+	+	+	-	-	-	-	-	+	-
	MHCT	-	-	+	+	+	+	-	-	-	+	+	-
	TBS	-	-	+	-	+	-	-	-	-	+	-	-
	MI	nd	-	+	+	+	+	+	+	+	+	+	-
F	LAMP	-	+	+	-	-	+	+	-	-	-	-	-
	PCR	-	-	+	-	+	-	-	-	-	-	+	-
	MHCT	-	-	+	-	+	-	-	-	-	-	+	-
	TBS	-	-	-	+	+	-	-	-	-	-	-	-
	MI	nd	-	+	+	+	+	+	+	-	+	-	-
G*	LAMP	-	-	-	-	-	-	-	-	-	-	-	-
	PCR	-	-	-	-	-	-	-	-	-	-	-	-
	MHCT	-	-	-	-	-	-	-	-	-	-	-	-
	TBS	-	-	-	-	-	-	-	-	-	-	-	-
	MI	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
H*	LAMP	-	-	-	-	-	-	-	-	-	-	-	-
	PCR	-	-	-	-	-	-	-	-	-	-	-	-
	MHCT	-	-	-	-	-	-	-	-	-	-	-	-
	TBS	-	-	-	-	-	-	-	-	-	-	-	-
	MI												

This table indicates the performance of each method for detection of *T. evansi*, with (+) and (-) showing positive and negative detection, respectively. G* and H* are negative controls, [a]MHCT-microhematocrit centrifuge test, [b]TBS-thin blood smear and [c]MI-mouse inoculation test. The nd indicates not determined.

Table 2: Analysis of variance for method sensitivity

Contrasts	p-value
LAMP vs PCR	0.2505
LAMP vs MHCT	0.4690
LAMP vs TBS	0.0501
LAMP vs MI	0.0674
SEM*	0.8013

Analysis of variance between LAMP and other methods used in this study (Statistical Analysis System – general linear models procedure).
SEM* represents standard error of the mean.

Comparative evaluation of parasitological, serological and DNA amplification methods for diagnosis of natural *Trypanosoma evansi* infection in camels

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Abstract

A representative number of two hundred and seventeen camels (*Camelus dromedarius*) from different areas of western Rajasthan State, India were examined from July 2002 to May 2003 for *Trypanosoma evansi* infection. The tests used were parasitological (wet blood film-WBF, stained thin blood smear-TBS), immunodiagnostic (double antibody sandwich enzyme linked immunosorbent assay for antigen detection- Ag-ELISA), and DNA amplification by polymerase chain reaction (PCR). These techniques were compared and the best efficiency was found for the last named (PCR). A prevalence of *T. evansi* infection was detected in 17.05, 9.67, 4.60 and 4.14 per cent by PCR, Ag-ELISA, TBS and WBF with a sensitivity of 100, 56.75, 27.02 and 24.32 per cent, respectively. PCR revealed a specific 227bp band in positive samples. The intensity of PCR bands was variable in different test samples depending upon the level of infection in the test samples. The history of intermittent fever, emaciation, oedema, poor body condition significantly correlated with positive serological status in ELISA as well as trypanosome DNA detection by PCR.

Key words: *Trypanosoma evansi*; camels; diagnosis; Ag-ELISA; PCR

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1. Introduction

Trypanosomosis, caused by *Trypanosoma evansi*, a blood protozoan, is one of the most important diseases of camels (Leese, 1927; Gatt-Rutter, 1967; Higgins, 1983; Luckins, 1992) resulting in high morbidity in countries where the dromedary inhabits. India has about 1.03 millions camel population (FAO, 2002) and ranks third in the world after Somalia and Sudan. The Indian camel population is mostly confined to the Western part of India particularly in Western Districts (55.7 % of total Indian camel population) of Rajasthan state (Khanna *et al.*, 1990). In India, the full impact of the disease in camels is presently difficult to assess accurately largely because there are no suitable diagnostic tools for determination of the extent of its prevalence, incidence and morbidity (Pathak and Khanna, 1995).

Definitive diagnosis of a current infection with *T. evansi* relies on the demonstration of the parasites in the blood or tissue fluids of infected animals. However, in camel, parasite detection techniques are not always

(Nantulya, 1989; Olaho-Mukani *et al.*, 1993). The tests themselves have inherent weaknesses that limit their sensitivity and specificity (Desquesnes, 1996). With the introduction of molecular diagnostic technology, assays based on the detection of trypanosomal DNA by polymerase chain reaction (PCR) have been developed but not yet completely validated (Wuyts *et al.*, 1995 and Omanwar *et al.*, 1999a).

This paper reports the application of currently available parasitological techniques i.e. the wet blood film examination, thin stained blood smear examination, double antibody sandwich enzyme linked immunosorbent assay for antigen detection

(Ag-ELISA) vis-a-vis PCR, which is still in the process of development, for diagnosis of camel trypanosomosis.

2. Materials and Methods

In this study, realized from July 2002 to May 2003, a total number of 1161 camels (about 1% of camel population) of different age, sex were randomly examined for surra belonging to different villages (n=7) and towns (n=2) in Bikaner and Churu districts of Western Rajasthan. In these places camel husbandry systems are followed as pastoralists and camel owners frequently use quinapyramine methyle-sulphate chloride to rid their animals of the surra. However, cases of chemoresistance have bean reported from these areas (Pathak *et al.* 1997). Out of 1161 camels, 217 camels (102 males and 115 females) showing signs of intermittent fever, emaciation, oedema and poor body condition were reckoned clinically suspected of surra .Out of the 217 samples, 173 were collected from rural areas and 44 from urban area. Animals were grouped in three age groups viz., up to 5 years (23), 5 to 10 years (168) and > 10 years (26). Blood from 50 camels of National Research Centre on Camel, Bikaner (an organised camel farm) was also collected.

From each camel two blood samples were collected by the jugular venipuncture, one in a tube containing the sodium salt of ethylene diamine tetra-acetic acid (EDTA) and the second in the tube without the anticoagulant for subsequent serum collection. The blood samples collected in EDTA were used for extraction of trypanosome DNA for PCR amplification. Other samples of blood were used for separation of sera and subsequent detection of antigen using double antibody sandwich enzyme linked immunosorbent (Ag-ELISA) technique. The sera samples were stored at -20° C. A total of 0.2 ml of blood from each camel was used for preparing wet blood films and Giemsa stained smears (Godfrey and Killick-Kendrick,

1961) and examined under a microscope of 40x and 100x oil magnification for detection of trypanosomes.

2.1. Antigen

Whole cell lysate antigen was prepared from trypanosome pellets through ultrasonic cell disruption treatment using an ultrasonic processor (Soniprep-

Chandigarh, India). The protein content was adjusted to 1.0 mg/ml with PBS, pH 7.2.

2.2. Preparation of hyperimmune serum (HIS) against whole cell lysate antigen

The hyperimmune serum was raised in two New Zealand White rabbits and two guinea pigs against whole cell lysate antigen. Before the start of hyperimmunization blood from each rabbit and guinea pig was collected directly by heart puncture and sera were separated. The immunization schedule followed is given in Table 1. Rabbits and guinea pigs were bled after 5 days of the last injection through cardiac puncture. The sera were collected, inactivated, merthiolate was added as a preservative to a final concentration of 1:50,000 and stored aliquots in screw capped test tubes at -20° C.

2.3. Antigen Enzyme Linked Immunosorbent Assay (Ag-ELISA)

The double-antibody sandwich ELISA, for the detection of antigens of T. *evansi* in the test sera, was used as per the method described by Bidwell and Voller (1981) and Rae *et al.* (1989). Anti-trypanosome hyperimmune sera (HIS) were raised in guinea pigs and rabbits. Guinea pig anti-trypanosome HIS was used for coating of ELISA plates and rabbit anti-trypanosome HIS was used as tracing antibodies for antigen detection attached with the coating antibody. Controls, consisting of wells reacted with substrate only, negative control wells using serum from uninfected healthy camels (declared negative on mouse inoculation) and positive controls using serum from uninfected animals to which antigen of *T. evansi* was added, were included with each plate. Controls, consisting of wells reacted with substrate only, negative control wells using serum from uninfected healthy camels (declared negative on mouse subinoculation) and positive controls using serum from uninfected animals to which antigen of *T. evansi* was added, were with each plate. For calculation of titre of various test sera samples, relative percentage of positivity (RPP) according to the relation of O.D. with the negative control and positive control samples deposited on the same microtitre plate was determined using the following formula (Desquesnes, 1998 and 1999).

RPP of a sample = (OD of the sample- OD of negative control)/ (OD of positive control- OD of negative control) x 100.

The RPP of the samples was calculated for every plate. The cut off value for the positivity of the Ag-ELISA for all the samples was selected and a RPP value of 10 was considered the threshold between serological positive and negative results.

2.4. Isolation of *T. evansi* DNA

DNA extraction was carried out for extraction of genomic DNA from pellet of *T. evansi* as described by Sambrook *et al.* (1989) and Jithendran *et al.* (1998).

2.5. Preparation of field samples for PCR

A Triton lysis procedure with minor modifications was used for the extraction of DNA. This involved mixing of 1 ml of each blood sample with 1 ml of solution 1 (10 mM Tris, 10 mM KCl, 10 mM $MgCl_2$) and 25 μ l of Triton X followed by centrifugation at 2000 g for 10 min. To the pellet, 500 μ l of NET buffer (0.5 M EDTA, 1M Tris HCl, 5M NaCl), 500 μ l of lysis buffer (TES buffer, SDS) and 5 μ l of Proteinase K (100 μ g/ml) was added and incubated at 48-50° C for 3 h. Thereafter, the standard phenol: chloroform: isoamyl alcohol extraction procedure was followed after transfer of the contents in microfuge tube. The recovered DNA was precipitated by adding 95% cold ethanol and keeping it overnight at -20° C. It was then spun at 10000g for 30 min at 4° C. Pellet was then washed twice in 70% chilled ethanol and air dried. The DNA was then resuspended in 50 μ l of TE (pH 8.0) buffer and kept at 4° C. One μ l of this extract was used as a template for PCR amplification.

2.6. PCR amplification

The PCR amplification of all 267 samples was performed in 25 μ l in microcentrifuge tubes using a set of oligonucleotide primers constructed from repetitive sequence probe pMUTec 6.258 described by Wuyts *et al.* (1994). A 21mer sense primer (5`-TGCAGACGACCTGACGCTACT-3`) and a 22mer antisense primer (5`-CTCCTAGAAGCTTCGGTGTCCT-3`) synthesized by Bangalore Genei Pvt. Ltd., Bangalore, India were used. The reaction mixture contained 200 mM of each dATP, dTTP, dCTP and dGTP, 2.5 μ l reaction mixture buffer, one unit of Taq DNA polymerase, 0.125 μ M of each primer and one μ l template (extracted DNA from one ml of test blood sample dissolved in 50 μ l of TE buffer). The reaction mixture was placed in a thermal cycler (Eppendorf Master Cycler Gradient, Hamburg, Germany) and pre-incubated at 95° C for 5 min to completely denature the DNA. This was followed by 25 cycles of 1 min at 94° C (to denature), 1 min at 60° C (to anneal) and 1 min at 72° C (to extend) and one extensive polymerization at 72° C for 5 min. At the end of thermal cycling, the products cooled to 4° C. Ten microliters of PCR products were electrophorized on a 1.4% agarose gel with a 100bp marker as size marker. The gels were stained with ethidium bromide (0.5 μ g/ml) and analysed on a UV transilluminator (UVP-GDS-7600 Gel Documentation System (UVP International, Upland, U.S.A.) and appropriate photographs were exposed.

3. Results

The results obtained using parasitological, serological and PCR methods showed a significant difference in the prevalence of cameline surra (Table 2). The parasitological prevalence was 4.14% by wet blood film examination and 4.60% by thin blood smear examination. The overall prevalence based on Ag-ELISA reached 9.67%. However, there were 5 camels in which sera from parasitologically proven infection (WBF and TBS) did not show detectable

samples from the National Research Centre on Camel, Bikaner was found positive by any of the tests. PCR detected all those cases, which had been found positive by parasitological and serological tests, and, in addition, detected trypanosomal DNA in 10 camels over and above detected by other tests. The intensity of PCR bands was variable in different test samples depending upon the level of infection in the test sample (Fig. 1).

The positivity of PCR in rural and urban camels was 20.80% and 2.27% respectively, while its per cent positivity in different age groups i.e. up to 5 years, 5 years to 10 years and >10 years, was 21.74%, 17.26% and 11.53% respectively, indicating the highest incidence in camels up to 5 years of age. The higher incidence of *T. evansi* infection was observed in female camels (24.34%) than males (8.82%). PCR was found most sensitive test so assumed as 100% sensitive test. The relative sensitivity of other tests viz. Ag-ELISA, TBS, WBF was 56.75%, 27.02%, 24.32%, respectively (Fig. 2).

4. Discussion

Notwithstanding a wide array of diagnostic tests for trypanosomosis in camels, the search for a technique combining a high degree of accuracy and sensitivity is continuing. In recent years DNA based technologies including PCR had been used for diagnosis of trypanosomosis. These techniques were found specific and sensitive for large scale analysis of trypanosome samples (Hide and Tait, 1991). In the present study, the PCR conducted on DNAs extracted from blood samples of naturally infected camels showed a detection rate that is about two times higher than with the Ag-ELISA and four times higher than with the parasitological techniques i.e. wet blood film (4.14%) and thin blood smear examination (4.60%). The results are in accord with the findings of Clausen *et al.* (1998) and Solano *et al.* (1999), who had found PCR assay sensitivity more than double compared to conventional parasitological techniques. A significantly higher prevalence detected with PCR was due to the higher sensitivity of the molecular technique, which is able to detect less than one trypanosome per ml under experimental conditions. Such difference of prevalence would have a significant impact on the strategy selected for the control of the disease in the affected areas. Apart from identifying and confirming infections detected by parasitological and serological methods, PCR detected infections in 37 out of 217 field camels. In fact, PCR detected 27 (13%) camels out of 207 camels which, based on parasitological methods, were deemed negative.

When calculated on the basis of the parasitological data, the diagnostic specificity is highest for PCR. The fact that the diagnostic sensitivity of PCR assay is apparently 100%, and the test can detect presence of even very low parasitaemia showed that the primers used in this study permitted the identification of parasite levels far below that detectable by microscopic examination as also reported by some of the earlier investigators (Wuyts *et al.*, 1994; Basagoudanavar *et al.*, 1998; Omanwar *et al.*, 1999a and b).

Results obtained with different age groups showed that the highest incidence was in camels up to 5 yrs of age (21.74%). The possible explanation may be

the urban camels may be due to better awareness towards chemoprophylaxis, better feeding and management, availability of medicines and related advice, less animal holdings and dependence of camel keepers on them as their sole source of livelihood. The sensitivity of females was higher (24.34%) than of males (8.82%). This may possibly be related to the stress of lactations and successive pregnancies in females.

PCR has some major advantages over the parasitological technique as the sample processing does not have to be done within a short period after collection and can be delayed thus enabling testing under controlled laboratory conditions. The technique will especially be useful when large number of animals need to be sampled during field surveys for remote testing where *T. evansi* is endemic. Further, the PCR could be an important tool for evaluating the efficacy of chemotherapy, the only reliable means of controlling trypanosomosis.

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Table 1. Immunization schedule to raise hyperimmune sera against whole cell lysate antigen

Injection	Day	Inoculum	Dose	Route of inoculation
Ist	1	1.0 ml antigen + 1.0 ml Freund`s Complete Adjuvant (FCA)	2.0 ml	Intramuscular at various sites in hind quarter
2nd	14	1.0 ml antigen+1.0 ml Freund`s Incomplete Adjuvant (FIA)	2.0 ml	Subcutaneous
3rd	28	1.0 ml antigen+1.0 ml FIA	2.0 ml	Subcutaneous
4th	35	1.0 ml antigen only	1.0 ml	Intra venous in ear vein
5th	42	1.0 ml antigen only	1.0 ml	Intra venous in ear vein
6th	49	1.0 ml antigen only	1.0 ml	Intra venous in ear vein

Table 2. Prevalence of *Trypanosoma evansi* infection in camels.

S. No.	Criteria	Camel		
		Examined	Positive on basis of PCR	
			Number	Per cent
1	**Test wise**	217	9	4.14
	(i) WBF	217	10	4.60
	(ii) TBF	217	21	9.67
	(iii) Ag –ELISA	217	37	17.05
	(iv) PCR			
2	**Area wise**	173	36	20.80
	(i) Rural	44	1	2.27
	(ii) Urban			
3	**Age wise**	23	05	21.74
	(i) Up to 5 yrs	168	29	17.26
	(ii) >5 to 10 yrs	26	03	11.53
	(iii) >10 yrs			
4	**Sex wise**	102	9	8.82
	(i) Male	115	28	24.34
	(ii) Female			
	Total	217	37	17.05

Fig. 2. Relative Sensitivity of different tests (assuming PCR 100%

Camel Trypanosomosis in Morocco: Results of a First Epidemiological Survey

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Abstract

An epidemiological survey of camel trypanosomosis was conducted for the first time in Morocco in 1999 and 2000. Five provinces located in the South and Southeast of the High Atlas mountain chain were included in this study. A total number of 1460 serum samples were collected and tested by a card agglutination test for trypanosomosis (CATT) and enzyme-linked immunosorbant assay (Ab-ELISA) to detect anti-*Trypanosoma evansi* antibodies. The overall seroprevalence was 14.1% by CATT and 18.2% by Ab-ELISA. Two very active foci were identified in Zagora and Merzouga with high Ab-ELISA prevalences of 43.3 % and 35.4 %, respectively for each area. The concordance between the CATT and Ab-ELISA was 94.1%. The age-related distribution of seroprevalence showed a tendency for the infection rate to increase with age up to a maximum in the 7-10 year-old group. Clinical examination revealed that enlargement of lymph nodes was the most frequent sign in seropositive animals (79.7%). The comparison of haematocrit values of seropositive and seronegative animals showed a significant difference that indicated severe anaemia in infected animals. Isolation of the parasite by inoculation into mice allowed us to collect 15 *Trypanosoma evansi* isolates from Zagora and 3 from Merzouga. The study showed that the Saharan provinces of Morocco are affected by camel trypanosomosis. Zagora and Merzouga were the seats of hyperendemic foci, that constituted a source of contamination for the surrounding regions.

Key words: *Trypanosoma evansi,* dromedary, epidemiology, Morocco, CATT, Ab-ELISA.

* Conférencier

THE PREVALENCE OF *TRYPANOSOMA EVANSI* INFECTIONS IN CAMELS IN SUDAN

Country Report

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INTRODUCTION

Sudan ranks second to Somalia in camel population worldwide (estimated at 4 million heads). It also ranks second to sheep in foreign currency earnings and contributes by 30% of the total foreign earning of the animal wealth in the country .In rural areas camels are still used for ploughing, traction, oil milling, pack and transportation as well as recreation.

Most importantly, it is the only sustainable agricultural activity for millions of nomads and pastoralists in the arid and semi-arid zones. They rely on the adaptability of camels to dry environments. They are further important because they represent personal wealth. In Sudan the majority of these animals are found in the western and eastern regions of the country.

Further development and exploitation of this animal is constrained by the prevalence of trypanosomosis (Surra). Although its economic impact is not fully evaluated, it is known as a chronic wasting disease causing anaemia, weight loss, reduced milk yield and abortion. It usually ends fatally if left untreated. In Sudan it is commonly known as "Guffar"(a chronic wasting disease).

Biting flies belonging to the genera of *Tabanus* and *Stomoxys* are incriminated to be responsible for the spread of the disease during interrupted feeding The transmission is mechanical and does not require cyclical development in the vector. Infection increases during the rainy season (high fly activity) especially towards its end.

Surra is known in Sudan and has been recognized as an important disease of camels since the beginning of the last century. Intermittent and scattered surveys were conducted from time to time to evaluate disease prevalence and epidemiological situations. All surveys confirmed the high prevalence of the disease. A prevalence of 10% has been recorded during the dry season, while a prevalence of more than 30% was recorded

during the rainy season. Serological tests also indicated high antibody titres

The present study was conducted to update our records on disease prevalence in camels in two of the most important areas in Sudan namely: the eastern Sudan and Kordofan areas. Serum samples and isolates of *T.evansi* stocks were also collected for further immunological and molecular studies.

MATERIALS AND METHODS

The Area (Fig. 1)

a) Parts of the eastern area (Kassala and Gedarif States). A total of 440 semi resident camels were examined for parasitaemia during the rainy season.
b) Parts of the northern Kordofan State. A total of 140 camels were examined for trypanosomosis during the dry season.

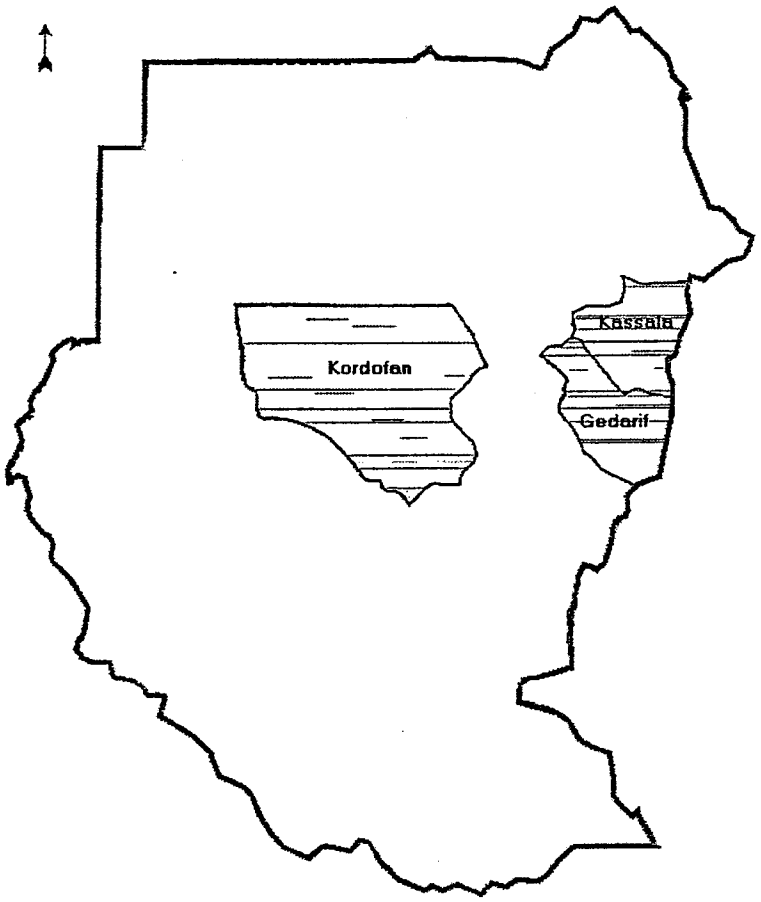

Figure 1: Study areas

Parasitological survey

Single visit prevalence (cross-sectional) survey was conducted in the above selected areas. Data regarding the following parameters were collected:

Parasitaemia:

The presence of parasite in the blood was examined by the buffy-coat method .

Degree of anaemia:

The pack cell volume (PCV) for each individual animal was recorded before examining the parasitaemia

Levels of anti-trypanosome antibodies:

Blood from jugular vein was collected into plain vacutainers, allowed to clot and serum was separated into ampules and stored at $-20C_0$ until needed. Levels of anti-trypanosome antibodies will be detected by ELISA

.

Molecular studies:

a) Approximately 50_{Ul} of blood was spotted into filter paper (Whatman No. 41), allowed to dry (about 8 samples per paper), placed in plastic bags, tied closely and stored at -20_c until need. DNA amplification (PCR) will be used to detect the presence of the parasite antigen .

b) Stocks of T.evansi were isolated from infected animals and transported to Khartoum either in Liquid Nitrogen ($L.N_2$.) or in albino rats. They were then propagated in rats and at the peak of parasitaemia, rats were sacrified and the stocks were stabilated in $L.N_2$. until used for biological studies.

Biting flies abundance:

Improved F3 traps were used to catch biting flies. They were deployed for 24 hours at the sites where animals were bled. Fly catches were expressed as the number of flies caught per trap per day.

RESULTS

In this present study only the parasitological data will be presented. However, the serological and molecular data will be communicated later.

Trypanosomosis Prevalence in the Eastern States

Out of the 400 camels examined, only 6 camels were found infected (1.5%) by buffy-coat technique. The table below shows the distribution of infection within the different localities in the region.

Table I: Prevalence of *T evansi* in Eastern Sudan

Locality	No. examined	No. positive	Prevalence (%)
Fashaga (Showak)	144	3	2.1
Kassala town	79	2	2.5
Hamoshkoraib	177	1	0.6
Total	**440**	**6**	**1.5**

Table 2: the distribution of infection according to camel type

Camel type	No. examined	No. positive	Prevalence (%)
Arabi	214	5	2.34
Bishari	183	1	0.6
Annafi	2	0	0.0
Kinani	1	0	0.0
Total	440	6	1.5

Biting flies Abundance:
The flies caught were so mutilated that it was too difficult to count them.

Trypanosomosis Prevalence in Kordofan
Out of the 140 camels examined in different localities, 10 animals were found infected with an overall infection rate of 7.1%

Table 3: the distribution of infection within the localities

Locality	Total No. examined	Total No. positive	Prevalence (%)
El Saisaban	20	1	5.0
El Khowai	27	2	7.4
El Rahad	32	4	12.5
Tendelti	61	3	4.9
Total	140	10	7.1

Biting Flies Abundance:
Fly catches in the different localities were as follows:
El Saisaban 10 flies per trap per day.
El Khowai 5 flies per trap per day
El Rahad 20 flies per trap per day
Tendelti 9 flies per trap per day

The present survey was conducted with the aim of updating the epidemiology of *T. evansi* in the most camel populated areas of the Sudan. If compared to earlier reports in the same areas, the prevalence of the disease in the present survey may be considered low. This may be due to the fact that most of the animals surveyed in the present study were from semi-sedentary areas and were receiving regular treatment by camel herders.

Further surveys of larger numbers and different management systems in the wet and dry seasons are underway. Immunological and molecular diagnosis (ELISA, PCR) are also in process and may provide different prevalence rates, especially in non-parasitaemic animals.

"Evaluation of an Antigen-Detection Enzyme Immunoassays Test with Monoclonal Antibody for Diagnosis of *Trypanosoma Evansi* in Horses in Argentina"

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A double antibody sandwich immunosorbent assay (ELISA) technique has been developed to diagnose ***Trypanosoma evansi*** infection in horses (Surra), based on circulating antigen detection. The assay uses as capture reagent the immunoglobulins fractions obtained from a goat immunised with ***T.evansi***, while the detection system was prepared from of a monoclonal antibody (Mab) directed against internal ***T.evansi*** antigen and secondarily a peroxidase conjugate anti-mouse IgM (μ-chain specific), antibody developed in goat. Previous results indicated that the Mab used in the test did not react with ***Trypanosoma cruzi***, ***Babesia equi*** and ***B.caballi*** the main horse's parasites protozoan in the sub-tropical area of Argentina.

In an experimentally ***T.evansi*** infected horse circulating antigens were first detected by Ag-ELISA 16 days after infection, thereafter antigens levels showed a progressive increase, the test being also positive even when parasites could not be detected. Diminazene aceturate failed to detain the disease and ELISA-Ag remained positive; contrarily, antigens were cleared from serum 30 days after a successful Suramin drug treatment (Figure 1).

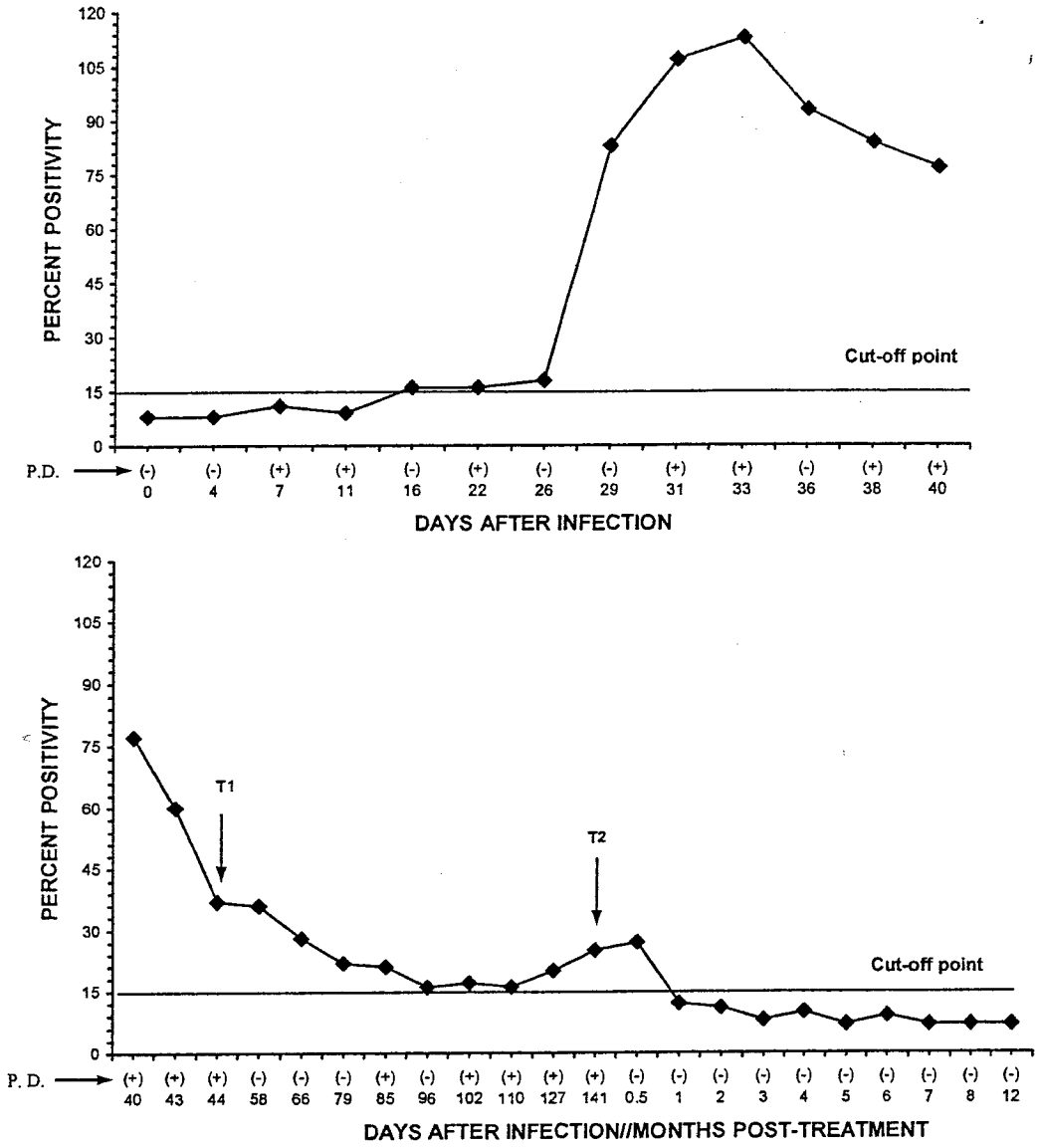
The test was evaluated with sera samples of 156 horses from four groups infected with ***T.evansi***; of which 86 horses were positive using standard parasite detection methods (SPDM). The Ag- ELISA sensitivity rate varied between 70-91% for the different groups (Table 1). As negative controls, 269 horses from the free area of this parasite were used which were all negative for ***T.evansi*** antibodies.

ELISA–Ag results were expressed in terms of percent positivity (PP) comparative to a reference control serum (CP+++ 100%). The difference between a positive and a negative test was based in the histogram of the frequency distribution of the results, using sera from infected and non-infected horses (Figure 2). A 15PP gave a sensitivity of 81% for confidence interval (CI), of 95% between 71% to 88% and specificity of 98% for a CI between 95.5% and 99.3%. There was a positive association between infected animals and positive ELISA-Ag test (Kappa = 0.83 IC 95%: 0.76-0.90; (P=0.000).

The inter-assay coefficient of variation (CV) expressed as PP, for the CP++, CP+ and CN was 5.4%, 10.6% and 43%, while de intra-assay CV for each of the above sera was 1.6 %, 0.97 % and 0.36 respectively. Positive and negative predictive values were determined for a 50% prevalence rate at 0.01%.

In the four horse groups with ***T.evansi*** Ag-ELISA was positive in 70 of 86 (81%) parasite-positive horses by SPDM. Interestingly also was positive in 18 of 70 (25 %) parasite-negative horses, while a combination of SPDM and ELISA-Ag tests detected as infected 104 animals, indicating that these methods constitute a good combination to increase test sensitivity.

Results obtained represent the first evaluation of this ELISA-Ag and the preliminary information generated so far indicate its usefulness for diagnosing ***T.evansi*** in horses in



P.D.: Parasitological Diagnoses; **(+):** Positive; **(-):** Negative.
T1: Treated with diminazene; **T2:** Treated with suramin

FIGURE 1: Following-up of Circulating Trypanosoma Antigen Detected by ELISA in an Experimentally *Trypanosoma evansi*-Infected horse

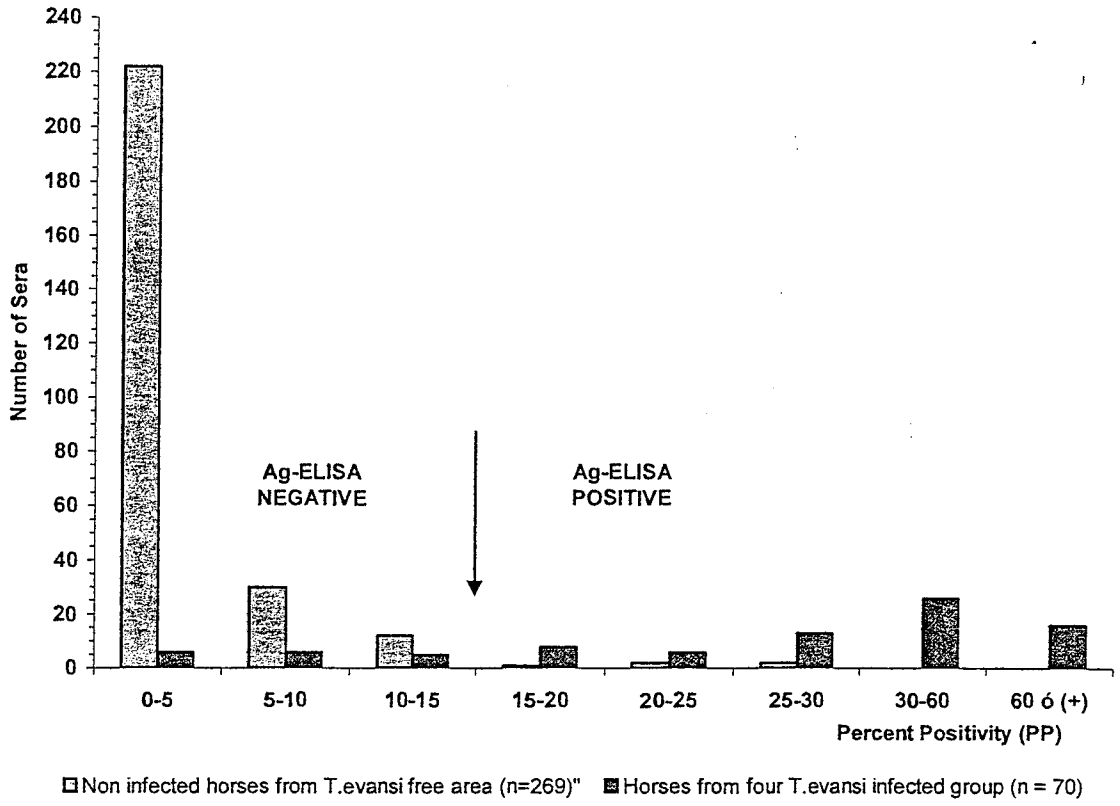


FIGURE 2: Frecuency Distribution of Antigen ELISA Test for Detecting *Trypanosoma evansi* in Horse Sera

TABLE 1

Results of Antigen-Elisa Test in Four Groups Horses Infected with *Trypanosoma evansi*

	Group N°	Horses	Antigen-E L I S A Test	
			Positive (%)	Negative (%)
Parasite Positive	1	11	10 (91)	1 (9)
	2	10	7 (70)	3 (30)
	3	13	11 (84.6)	2 (16.4)
	4	52	42 (80.7)	10 (19.2)
Parasite Negative	1	37	6 (16)	31 (84)
	2	5	2 (40)	3 (60)
	3	11	3 (27)	8 (73)
	4	17	7 (41)	10 (59)

The trypanosomosis in the goat. Current status

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Background

Trypanosomosis is a major constraint on ruminant livestock production in Africa, including goat production. The impact of South American trypanosomosis on goats is largely unexplored and is only briefly discussed. Current knowledge on the important animal and human trypanosomes related to their pathogenicity for goats is summarized in Table 1.

Etiology

In Africa, cyclic transmission of the parasite to mammalian hosts occurs via numerous species of tsetse flies (*Glossina* spp.) during feeding by the flies. Elsewhere in the world, mechanical transmission by other species of biting flies is the primary mode of infection.
T. congolense is the most common trypanosome of goats in Africa. *T. vivax* is the second most common. Natural infection of goats with *T. brucei* is also sporadically reported. Goats are susceptible to *T. uniforme*, in Uganda and Zaire, but only mild infections occur. *T. simiae*, a trypanosome of swine and camels is transmissible to goats by either *Glossina* spp. or biting flies but causes mostly mild or subclinical disease (Smith and Sherman, 1994).

Goats and other domestic animals are relatively resistant to *T. brucei gambiense*. When infection does occur, the clinical course is chronic. *T. brucei rhodesiense* is an uncommon cause of caprine disease. A nonpathogenic trypanosome, *T. theodori*, was found incidentally in goats in Israel. It is transmitted by a hippoboscid fly, *Lipoptena caprina*. This organism is morphologically similar to the common, nonpathogenic sheep trypanosome, *T. melophagium*.
Information on the pathogenicity of the trypanosomes that occur outside of Africa, primarily in South and Central America is limited. *T. cruzi*, which is cyclically transmitted by reduviid bugs, as well as *T. evansi* and *T. equiperdum*, which are mechanically transmitted, cause disease in humans, camels and horses respectively (Smith and Sherman, 1994). Their infectivity for goats is presumed to be low. Kids infected experimentally with *T. cruzi* showed no clinical signs of disease and carried the infection for 38 days (Diamon and Rubin, 1958). The goat is a natural host for *T. evansi*, but reports of the disease, surra, in goats , are lacking (Levine, 1973). Only one non-African trypanosome, *T. vivax*, is primarily pathogenic for ruminants, especially in cattle. The pathogenicity for goats is not well investigated.

Epidemiology

Trypanosomosis in Africa follows the distribution and intensity of the various species of the tsetse fly. Approximately 10 million km^2 or 37% of the African continent is tsetse-infested. This area includes 38 countries. Various estimates suggest that the livestock-carrying capacity of such areas in West and Central Africa could be increased five- to seven-fold by eliminating or controlling animal trypanosomosis (Griffin, 1978).

There are approximately 200 million goats in Africa with as many as 50 million in the tsetse-infested regions of the continent. Natural infections with *T. congolense*, *T. vivax*, or *T. brucei* resulting in clinical disease have been known in African goats since the turn of the

century. Until recently, however, the perception has persisted that goats are highly resistant to infection, that caprine trypanosomosis is only sporadic, and that the disease in goats is of little economic consequence (Griffin, 1978). This opinion is currently undergoing a critical reappraisal. Regional differences do exist in the prevalence of caprine trypanosomosis, but it can be high in some areas (Griffin and Allonby, 1979; Kramer, 1966). In general, caprine trypanosomosis is more common in East than West Africa. This is attributed to differences in feeding preferences between riverine species of *Glossina* and savannah species; the latter are more inclined to feed on goats (Smith and Sherman, 1994).

Goats may serve as a reservoir of trypanosome infection for other species. In the Sudan, goats infected with *T. congolense* developed a chronic form of disease from which many spontaneously recovered. When the organism was passaged from goats into calves however, acute fatal bovine trypanosomosis occurred (Mahmoud and Elmalik, 1977). Goats also have been implicated as a reservoir of *T. brucei rhodesiense*, transmissible to man (Robson and Rickman, 1973).

The economic impact of trypanosomosis on goat production is beginning to be studied. A Kenyan analysis demonstrated that goats receiving monthly chemoprophylaxis against trypanosomosis had significantly decreased mortality rates, increased weight gains, and improved reproductive performance compared to untreated control goats. Differences in performance were also noted between breeds in the study with indigenous breeds performing better than non-indigenous cross breeds (Kanyari et al., 1983).

The existence of inherent trypanotolerance in certain goat breeds is controversial. It is generally accepted that trypanotolerant breeds of cattle exist, particularly the N'dama of West Africa and the West African Shorthorn. This inherent ability to control parasitemia and minimize disease has not been clearly demonstrated for specific goat breeds, despite the general observation that some breeds of goats will readily survive in tsetse infested areas. Dwarf West African goats have been considered inherently trypanotolerant, yet they can be readily infected experimentally (Murray et al., 1982). While earlier studies suggested that indigenous goat breeds of East Africa may show inherent trypanotolerance, no evidence of genetic resistance was observed in a subsequent study with either natural or experimental challenge in East African, Galla, or East African goats cross bred with Toggenburg, Nubian, or Galla breeds (Whitelaw et al., 1985). One factor contributing to the perceived trypanotolerance of various goat breeds under field conditions may be the feeding preferences of *Glossina* spp. Flies may select other livestock over goats when mixed animal populations are present (Murray et al., 1984). The existence of true trypanotolerance in goats deserves additional careful investigation.

Pathogenesis

Trypanosomes fall into two groups regarding their ability to produce disease. The hematic group, which includes *T. congolense* and *T. vivax*, remain confined to the circulation after introduction into the bloodstream by feeding *Glossina* spp. The disease produced in these infections is characterized by anemia. The humoral group, which includes *T. brucei*, is more invasive, with trypanosomes found in intercellular tissue and body cavity fluids after initial infection. Anemia in these cases is overshadowed by marked inflammatory, degenerative, and necrotic changes.

Anemia in trypanosomosis may be due to extravascular hemolysis and erythrophagocytosis, and also decreased erythropoiesis in chronic infections (Kaya et al., 1977). The destruction of red blood cells may result from both non-immune and immune-mediated mechanisms. Hemorrhage secondary to disseminated intravascular coagulation (DIC) may also contribute to anemia. Thrombocytopenia, microthrombus formation, and hemorrhage suggestive of DIC have been observed in caprine trypanosomiasis due to *T. vivax*

(Van der Ingh et al., 1976; Veenendaal et al., 1976). Anemia may be exaggerated by hemodilution because of expansion of blood and plasma volumes, which increased, respectively, 29% and 44% in goats with subacute *T. vivax* infection (Anosa and Isoun, 1976). The pathogenesis of inflammation and tissue damage by humoral trypanosomes such as *T. brucei* is complex and have been reviewed elsewhere (Soulsby, 1982). Immunosuppression can occur in trypanosomosis. *T. vivax* and *T. brucei* infection of goats resulted in depressed responses to mitogen stimulation in lymphocyte transformation tests (Diesing et al., 1983; van Dam, 1981). T. evansi Impaired immune function may aggravate the severity of concurrent infections. This was suggested by evidence of higher mortality rates and parasite loads in goats concurrently infected with *T. congolense* and *Haemonchus contortus* than in goats infected with only one or the other parasite (Griffin et al., 1981a; Griffin et al., 1981b).

One of the most notable features of trypanosomosis is the successive waves of parasitemia that occur every few days in animals that survive initial infection. Each wave of parasitemia is followed by an increase in circulating antibody that temporarily reduces the parasitemia. The effect is only temporary, however, because cyclically transmitted trypanosomes are capable of repeatedly altering their surface antigens (surface coat glycoproteins) and thereby evade the host immune system sufficiently to avoid total elimination of infection.

Clinical signs

Main clinical pictures produced by the different trypanosomes are summarized in Table 1.

Diagnosis

Anemia and emaciation in goats from endemic areas suggest the diagnosis of trypanosomosis. Definitive diagnosis is based on identification of trypanosomes in blood smears or tissues. Common parasitological detection tests for *Trypanosoma* spp. are also used in the goat. Wet film, blood smears examination (morphologic identification is better performed on thin smears), buffy coat, lymph node aspirates or mouse inoculation are commonly used. Serologic tests developed for the diagnosis of trypanosomosis include indirect hemagglutination test, a complement fixation test, an indirect fluorescent antibody test, ELISA and direct card agglutination test (CATT/*T. evansi*) and the indirect card agglutination test (LATEX/*T. evansi*).

Treatment

A variety of trypanocidal compounds are available for treatment, but no new drugs have been marketed for quite some time. Subsequently, drug resistance has become a significant problem. Compounds and dosages are formulated for single-dose use and treatment is usually on a herdwide basis because serial treatments on individual animals are difficult to carry out in the semi-nomadic livestock farming systems prevalent in endemic areas. Several of the drugs are locally irritating so subcutaneous injections should be given in areas of loose skin and intramuscular injections given deeply, avoiding vessels and nerves. Curative doses used in cattle are also appropriate for goats and sheep (Ilemobade, 1986).
Diminazene aceturate is given intramuscularly as a 7% cold water solution at a dose of 3.5 mg/kg and is considered effective against the three major trypanosomes, as is quinapyramine dimethyl sulfate given intramuscularly as a 10% cold water solution at a dose of 10.0 mg/kg. Relapse of infection has been reported in goats treated with diminazene aceturate, presumably because of re-emergence of trypanasomes from the central nervous system where they were inaccessible to the drug during earlier treatment (Whitelaw et al., 1985).

Homidium chloride or homidium bromide are given in a 2% cold water solution at a dose of 1.0 mg/kg intramuscularly and are effective against *T. vivax* and *T. congolense*. Isometamidium chloride is also effective against the hematic trypanosomes when given at a dose of 0.25 to 0.75 mg/kg intramuscularly as a 1 or 2% cold water solution. This drug was shown to produce signs of shock or death in goats if given intravenously at doses greater than or equal to 0.5 mg/kg (Schillinger et al., 1985). Salicylhydroxamic acid with glycerol was found to be an unsatisfactory treatment for *T. vivax* in goats due to difficulties in administration and potential toxicity (van der Meer et al., 1980).

Control

Numerous constraints on control exist including reservoirs of infection in wild animal populations, the ability of trypanosomes to continuously alter their antigenic character thus confounding the development of suitable vaccines, a limited availability of effective drugs, the development of resistance to existing trypanocidal drugs, the difficult logistics of widespread tsetse control, lack of economic resources, poorly developed animal disease control programs, limited technical training programs, lack of international cooperation, and political instability (Murray and Gray, 1984; Doyle et al., 1984).

Currently, the major fronts in trypanosomosis control are reduction or elimination of tsetse populations and chemoprophylaxis of livestock. Tsetse fly control is accomplished by several methods, alone or in combination, including ground or aerial application of insecticides, such as chlorinated hydrocarbons and synthetic pyrethroids, tsetse trapping, and gamma-irradiated sterile fly release (Smith and Sherman, 1994).

Both isometamidium chloride and pyrithidium bromide will protect against infection with the three major goat trypanosomes for 2 to 4 months. The prophylactic dose of isometamidium is 1.0 mg/kg administered intramuscularly in a 1 or 2% cold water solution. Pyrithidium is given at an intramuscular dose of 2.0 mg/kg in a 2% solution of water that must be boiled. Quinapyramine chloride given subcutaneously in a 16.6% cold water solution at a dose of 7.4 mg/kg is prophylactic against *T. brucei* infection.

Despite intensive research, no effective vaccine is likely in the future because of the continuing problem of antigenic variation in trypanosomes. Given the obstacles to vaccination, there is a keen interest in the identification and promotion of tryanotolerant breeds of livestock in endemic areas, as discussed above in the section on epidemiology.

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ble 1. Trypanosomosis in goats. Adapted from Smith and Sherman, 1994.

:s	Major species affected	Geographic distribution	Vectors involved	Natural infection in goats	Experimental infection in goats	Clinical manifestation
ıria						
ax	Domestic ruminants, camels, horses, antelope	Widespread in tropical Africa and South America	*Glossina* spp.	Common	Readily	Acute and chronic forms, mild to fatal
forme	Domestic ruminants, antelope	Zaire, Uganda	*Glossina* spp.	Yes	Not reported	Non-pathogenic or subclinical infection
ıgolense	All domestic animals, wild game	Widespread in Tropical Africa	*Glossina* spp.	Common	Readily	Acute, subacute, and chronic forms, mild to fatal outcome
ıiae	Domestic pigs, camels, wild warthogs	Widespread in tropical Africa	*Glossina* spp. and *Stomoxys, Tabanus* flies	Uncommon	Not reported	mainly subclinical or mild clinical disease
ɑnsi	Camels, equines, dogs, water buffaloes	India, Far East, Near East, Philippines, North Africa, Central and South America	various biting flies	yes	yes	Subclinical, moderate or acute disease.
ucei	Domestic ruminants, horses, dogs and cats	Widespread in tropical Africa	*Glossina* spp.	Common but with strain variation	Yes, with strain variation	Noninfective to fatal outcomes
gambiense (West ɔan sleeping ıess	Humans	Tropical West and Central Africa	*Glossina* spp. and various biting flies	Uncommon Goats resistant	Very difficult	Noninfective or a chronic form leading to death or spontaneous recovery
rhodesiense (East can sleeping ıess)	Humans	East and Southern Africa	*Glossina* spp.	Uncommon	Yes	Experimental infections subacute and fatal
ɣuiperdum	Horses	Southern Africa (Namibia, Botswana ,South Africa) , Ethiopia, Central Asia, , Middle East, Russia	Venereal reported	Not reported	Not reported	Not reported
ɾcoraria						
cruzi (Chagas ɜase)	Humans	South and Central America, sporadic in USA	Reduviid blood-sucking bugs	Not reported	Yes	No

ANNEX XVI

بسم الله الرحمن الرحيم

The effect of experimental infection of goats with *Trypanosoma evansi*

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Abstract :

Six Nubian goats were inoculated intravenously with 7.5 x 10^5 *Trypanosoma evansi*. Daily clinical examination and a weekly haemogramme were done. They were slaughtered after infection for gross and histopathological examinations. Elevated body temperatures, anaemia, depression, nervous signs, weakness, rough coat appearance and abortion of pregnant females were recorded during the course of infection , packed cell volume and Hb mean values were lower than those of the uninfected goats, while the mean value of the red blood cells count was higher than that of the control goats. The mean value of the leuococytes count did not show deviation from normal control values.

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Introduction :

Studies on trypanosomiasis as a cause of losses in small ruminants had been investigated by many workers. Induced reproductive disorders were reviewed by Gunn, (1983) and Ikede, *et.al*, (1988). Other different species of Trypanosomes were found in investigations done in Africa by ,(Ngeranwa, *et.al*,1991, Ngeranwa , *et.al*, 1993, Okeleng , 1996.) and occur whenever the vector is found.The clinical signs of disease caused by these parasites vary according to the trypanosome species and the infected host species.

This study is meant to investigate the pathogenic effect of induced *T.evansi* infection in goats. It is an attempt to reveal sings due to this parasite which might not be correctly diagnosed under field conditions. It worth mentioning that in Sudan , goats graze in the same natural pastures in mixed herds with camels, the definitive host of this parasite.

Materials and Methods

Animals :

Eight goats of both sexes, aged 6-9 months were used. They were brought from Elhufra village on the Eastern bank of the Blue Nile. They were screened for natural infection and received the proper treatment. None was found to be infected with *T.evansi* as they were regularly examined throughout the one month adaptation period.The animals were kept in well ventilated, naturally illuminated, insect proof pens during the experimental period.

Preparation of the inoculum:

T.evansi used in this study was isolated from a male camel from Northern Kordofan State. They were then propagated in inbred Albino mice. The infected blood of these mice was used to infect goats used in this study.

Experimental Design:

The goats were divided randomly into two groups. Each of six goats were infected with $7.5x10^5$ *T. evansi* 1ml of infected blood intravenously (Elmalik, 1983) and named as group (I). The remaining two goats were used as uninfected control and named as group (II) .

Follow Up:

This was done until the end of the study, were the survived goats were slaughtered and it included the following :-

I- Daily clinical observations:

This included measurement of body temperature and record of parasitaemia. Other general observations were also recorded.

II- Weekly clinical observations:

This included the following:

i- *Haematological examinations*:

It included estimation of PCV, total red blood cells count, total white blood cells count, and Hb concentration.

ii- *Body weight measurement.*

III- Histopathological examinations :

Five of the infected goats which survived at the end of the study were slaughtered. Tissues from the brain were immediately fixed by perfusion with 10% formalin and then immersed in the same fixative. Samples from liver,

spleen, heart, lungs, and kidneys were also fixed by immersion in 10% fromalin.

Representative sections of 7 *um* thickness were cut and embedded in paraffin wax by routine method described by (Druppy , et al, 1967) and stained with haematoxylene and eosin (HE).

Results :

I- Daily clinical observations:

i- *Body temperature* :

There was a slight elevation in the mean value of group (I) from the control group(II) and the difference was significant ($P < 0.05$) see table (1).

ii- *Parasitaemia* :

The parasitaemia was fluctuating and usually one parasite/field was observed during the whole period of the experiment. The incubation period ranged between day 11 and 20. One of the six goats (16.7%) died of the disease within 36 days post infection. The rest of the animals suffered from the disease but survived up to the end of the experiment, where they were slaughtered.

iii- *Body Weight* :

The mean value recorded in group (I) was slightly higher than the control group (II).

vi- *General observations* :

a. Infected goats developed rough coats. They were weak and prefered the lying position (Fig. 1). Nervous signs were indicated by twisted necks, (Fig. 1). Loss of appettite and depression were also frequent signs. Any pregnant female also aborted.

II- Weekly Clinical Observation :-

i- *Haematological examination* :

There was a decrease in the mean values of PCV, Hb and while blood cells count of group (I), ($P<0.05$) compared to the uninfected control group (II), see table (I). The mean value of red blood cells count was elevated and differed significantly from group (II), ($P<0.05$).

III- Histopathological Findings :-

Liver :

The marked features were hepatocellular swellings and vacuolations with collapsed sinusoids. Other prominent changes were compression of hepatic plates and kupffer cell proliferation. (fig. 2,3). Also there was disfiguration and proliferation of bile duct epithelium. Some areas showed fibrosis and mononuclear cell infilteration composed mainly of lymphocytes, (fig. 4).

Spleen:

Marked changes included follicular lymphoid hyperplasia with prominent germinal centres and haemosiderosis, (fig. 5,6).

Kidney:

There was obvious dilitation in the Bowman's space and hypercellularity of glomerular tufft ,(fig. 7). There was proliferation of glomerular epithelium and some of the glomeruli presented amorphous pink material in the Bowman's space which is mostly proteinaceous, (fig. 8). There was degeneration and disfiguration of the tubular epithelium, (fig. 9).The tubular epithelial cells were swollen with granular cytoplasm ,(fig. 10). Foci of interstitial cell infilteration composed of lymphocytes were also observed.

Heart:

There were mononuclear cell infilteration of mainly lymphocytes and haemorrhages in the myocardium and epicardium, (fig,11).

Lungs:

Alveolar septa were thickened by fibrous tissues. There were also mononuclear cell infiltration and marked peribronchial lymphoid hyperplasia. The lungs also showed oedema and areas of scarring, (fig,12).

Discussion :

The report of this work reveals that the sequential pathology of Surra in domestic animals is indicated by the same general pattern of trypanosomiasis. Haemosiderosis and follicular lymphoid hyperplasia were the prominent changes in the spleen, this agrees with (Naylor, 1971, Losos and Ikede, 1972, A. Gadir and Shomain, 1977, Mohammed, 1988 and Elhadi, 1999). In the liver there was marked hepatocytes destruction, proliferation of Kupffer cells and fibrosis of the portal areas.These results agrees with Mohammed, (1988). The changes in the lungs were similar to those reported by Losos and Ikede, (1972) in cattle infected by *T. congolense*, where there was oedema, interstitial pneumonia, emphysema and mononuclear cell infilteration. Oedema of the lungs might be due to vascular damage or the direct action of the parasites on the interstitial connective tissues (Moulton and Solod, 1976). The kidneys in the present study showed obvious dilitation in Bowman's capsule with presence of pink material there(mostly proteinaecous). There was also degeneration and desquamation of tubular epithelium . The same findings were detected by Mohammed, (1988) .Changes in the Brain included vacuolation in the white matter of the cerebellum and some neurons were surrounded by satellite cells. This was reflected by nervous signs of the infected goats.In the Heart there was mononuclear cell infilteration in the Myocardium and epicardium. These agrees with Moulton and Sollod, 1976 and Damyanati , *et. al*, 1994).

The clinical and general observation recorded during the period of the study were all common features of *T. evansi* infection as seen in other species, (Losos, 1980, Dieleman, 1986). There was loss of appettite, depression, weakness. Rough coat, and abortion of pregnant females. The decrease in body weight was not observable, this is attributed to the effect of initial weight of the goats at the start of the experiment which was higher than that of the control. This reflects that loss of weight due to infection may be signifficantly reduced if animals were in a good nutritional status.

There was elevation in body temperature of group (I). This is in agreement with (Mohammed, 1988, Mutayoba, *et. al*, 1989 and Okeleng 1996).

The type of anaemia produced in the infected animals can be classified as microcytic, normochromic where there was a decrease in the PCV and Hb while the RBCs count was increased. These findings contradicts with (Mohammed, 1988, Okeleng, 1996 and Goosens, *et. al*, 1998) who found normocytic anaemia in goats infected with *T. evansi*, *T. vivax* and *T. congolense*, respectively. The leucocyte count was decreased.

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Clinico-pathological changes due to concurrent experimental infection of Nubian goats with *Trypanosoma evansi* and *Haemonchus contortus*

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Introduction:

Spread of pathogenic parasites in animals is very well documented .Also multiple infection with more than one parasite occurs in nature , the fact which may reflect the immunosuppressive action of parasitism. Infection with one parasite species can make the animal more susceptible to infection with another species .

In this respect if we consider the case of parasites which live in and on the blood like *Trypanosoma spp* and *Haemonchus spp* and apart from the depletion of blood cells , their interaction in an animal host was known to cause severe pathological changes ,(Elhadi,1999) .Although helminthiasis and trypanosomiasis are both endemic in tropical Africa ,most studies and surveys have examined these cases as separate entities ,ignoring the fact that they frequently occur as concurrent infection ,(Chiejina ,1987,Fakae and Chiejina,1993).

This study had been designed to record changes due to either of these parasites when the animal was superinfected with the other. These are measured by clinicopathological changes , weight changes prepatent period of the superinoculated parasite and mortality due to the mixed infection.

Experimental procedure :

Fourteen Nubian goats at an average age of six months were obtained from local markets and screened for freedom from trypanosomes and internal parasites. Accordingly, they were given the appropriate treatment at the recommended dosages. They were penned in a well ventilated and naturally illuminated housing unit which had concrete floor ,at the Department of Preventive Medicine and Public Health , Faculty of Veterinary Science . All animals were given the same quality of food, and water was allowed *ad .libidum* . Before the start of the experiment ,the animals were allowed to acclimatize to the housing unit for a period of two weeks.

Two groups were infected with either *T.evansi* or *H .contortus*. After patency, the alternate group was superinfected with *T.evansi* or *H .contortus* . The consequent groups were as follows:

Group I : six goats infected with *T.evansi* and superinfected with *H .contortus*.
Group II : five goats infected with *H .contortus* and superinfected with *T.evansi.*
GroupIII : three uninfected control goats.

Inocula :
A) *T.evansi* : the strain was isolated from a naturally infected camel in Northern Kordofan State . Each animal in group(I) was inoculated with 7.5×10^5 *T.evansi* parasite. Animals of group (II) were superinfected with the same dose of *T.evansi* (7.5×10^5) /animal.
B) *H .contortus* :
Adult worms were collectd from slaughtered sheep at Omdurman Slaughter House . Infective 3rd stage larvae were obtained by the Baermann technique (Angus ,1978). Each goat in group (I) was superinfected with 1390 L_3 / animal by the oral route .

Animal of group (II) received L_3 suspension at variable doses i.e.
Three animals had 1390 infective larvae / animal .
Two animals had 700 infective larvae / animal .
The initial *T.evansi* inoculum in group (I) or the superinfection in group (II) was 7.5×10^5 parasite /anima

Follow Up :

The values recorded before and after experimental inoculation included :

1. Body daily temperature : this was recorded daily.
2. Weekly record of body weight in (kgs)
3. Weekly haemogramme determination included the following:
   -Total RBCs/mm^3 of blood .
   -Total WBCs/mm^3 of blood .
   -PCV% .
   -Hb. concentration .
4. Weekly Changes in Urea Concentration , Creatinie ,Albumin and Globulin values.

5. Weekly Antibody titre in sera was detected using the ELISA technique.

Results:

1- **Body temperature** :
There was a decrease in the mean value of group (I) and (II) compared to the control group (III) and the differences were not significant ($p>0.05$).
The mean value of the control was (38.68±0.37)°C while that of group (I)

was (38.40±0.49) °C. In group (II), animals with lower dose of L_3 (700/ animal) showed lower mean value (37.83±1.65) °C than the value of the animals that received a higher L_3 dose (1390 /animal) where it was (38.64±0.38) °C ,(table,1).

2- BODY WEIGHT:

In group (I), the mean value recorded during infection was lower than that of the control group (III) and the difference was significant ($p<0.05$) .(table,1).Animals in group (II) which received low dose of *H.contortus* L_3 (700/animal) recorded a mean value of (13.60±1.62) kg ,while those that received a higher dose of (1390 L_3/animal) showed a mean value of (11.67±1.26)kg. Both values were higher than that of the control group (III) and the differences was not significant ($p>0.05$),(table.1.)

3- Total RBCs count:

There was a marked elevation in the mean value of group (I) where it was (25.40±6.05)x10^6/ml and the that of the control group(III) (15.25±4.20)x10^6ml. The difference was significant ($p<0.05$),(table ,1). In group (II), animals with a higher *H.contortus* L_3 (1390 / animal) showed the least mean value in all the three groups ,where it recorded (4.23±0.89)x10^6/ml. Those that received a lower dose of *H.contortus* L_3 (700/animal) recorded (9.24±4.08)x10^6/ml and the differences were significant ($p<0.05$) (table,1).

Total WBCs count:

The mean value of group (1) showed a marked elevation companed tto the control were it was (11.56±0.60)10^3 ml and that of the control was (3.15±0.42)10^3ml difference was significant (pc.0.05) In group animal with the lower dose of L_3 (700/ animal) showed the lowest elevation in both group 1&2 where it was (7.39±1.38)10^3/ml and that of the higher L_3 dose (1390L_3)10^3/ml recorded (8.254±.15)10^3 /ml compared to the control group (1.1) .(tabl.1) .

Packed cell volume :

There was a marked decrease in group (1) mean value where it was (12.192±.48)% .Animals in group (II) which received a lower dose of l_3 suspension (700/ animal) showed alight decrease in the mean value of the pcv compared to that of the control , when its value was (23±.645.4)%

. Goats that record a higher dose of L_3 suspension showed a marked decease where its mean value was (11.50± 0.50) % and differences in both group significant $p<0.05$.(table.1.)

Haemoglobin concentration :

The marked decrease in the mean value of Hb. concentration was in animals of group (I) where it recorded (4.13±1.20)gm/dl and the value of the control was (7.30±2.21) gm/dl. Group (II) animals which received a lower dose of *H.contortus* L_3 suspension showed a slight decrease and the difference was significant ($p<0.05$),while animals in the same group and received a higher dose of *H.contortus* L_3 suspension showed a marked decrease in the mean value and the difference was significant ($p< 0.05$) where the value was (5.74±1.82)gm/dl (table,1).

Total Protein , albumin and globulin:

Compared to mean values of the control group (III) the marked decrease in the above parameters was in group (II) animals that received a higher dose of L_3suspension (1390/animal) ,followed by animal of group (I) ,and the differences were significant ($p<0.05$) for the T.protein and albumin and not significant for the globulin ,($p>0.05$) (table ,2.)

Creatinine ana Urea concentration:
Animals in both groups showed a decrease in creatinine concentration,but the least value was that of group (II) animals which received a higher dose of *H.contotus* larval suspension and the difference was significant (p,0.05)(table,3).Animals of group (I) showed a marked elevation in urea concentration and the difference was significant ($p<0.05$).In group (II) Animals which received a higher dose of *H.contortus* larval suspension showed a decrease in the mean value of the urea concentration,while those that received a lower larval suspension showed an elevation,but the difference was not significant ($p>0.05$)(table,3).

Table (1):

Mean temperature ,body weight,total RBCs count , total WBCs count,PCV , &standard deviation of goats infected with multiple infection .

Group	Parameter / Treatment	Temperature °C	Body weight (Kgs)	Total RBCs CountX10^6/ ml	Total WBCs CountX10^3/ml	Haemoglobin gm/dl	PCV%
1	T+H	$(38.40\pm0.49)^a$	$(7.53\pm1.15)^a$	$(25.4\pm6.05)^a$	$(11.56\pm0.60)^c$	$(4.13\pm1.20)^{cd}$	$(12.2\pm2.5($
2	H+T	$(38.64\pm0.38)^a$	$(11.67\pm1.26)^b$	$(4.23\pm0.89)^c$	$(8.25\pm4.15)^b$	$(5.74\pm1.82)^c$	$(11.33\pm0.5$
	H+T*	$(37.83\pm1.65)^a$	$(13.60\pm1.62)^b$	$(9.24\pm4.08)^b$	$(7.39\pm1.38)^b$	$(6.81\pm1.90)^d$	$(23.61\pm5.3($
3	CN	$(38.6\pm0.37)^a$	$(10.04\pm0.63)^b$	$(15.25\pm4.20)^a$	$(3.15\pm0.42)^a$	$(7.30\pm2.21)^c$	$(24.00\pm5.5$

N.B:-

Means within the same column with different subscripts are significantly different.

CN : Uninfected control group .

T+H: This group of animals was infected with *T.evansi* and super infected with *H.contortus* .

H+T : This group of animals was infected with *H.contortus*($1390L_3$ / animal) super-infected with *T.evansi* .

H+T*: This group of animals was infected with *H.contortus* ($700L_3$/animal) & super-infected with *T.evansi.*

Table (2):

Mean weekly total protein ,albumin ,globulin & standard deviation of goats with multiple infections.

Group	Parameter / Treatment	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)
1	T+H	$(5.41\pm0.58)^{a}$	$(2.41\pm1.13)^{a}$	$(3.00\pm1.14)^{a}$
2	H+T	$(4.35\pm1.98)^{a}$	$(1.28\pm1.23)^{a}$	$(3.08\pm1.24)^{a}$
	H+T*	$(6.02\pm0.88)^{a}$	$(2.68\pm8.13)^{b}$	$(3.33\pm1.16)^{a}$
3	CN	$(6.13\pm1.44)^{b}$	$(2.71\pm1.69)^{b}$	$(3.16\pm1.20)^{a}$

N.B:-

Means within the same column with different subscripts are significantly different.

CN : Uninfected control group .

T+H: This group of animals was infected with *T.evansi* and super infected with *H.contortus* .

H+T : This group of animals was infected with *H.contortus*($1390L_3$ / animal) super-infected with *T.evansi* .

H+T*: This group of animals was infected with *H.contortus* ($700L_3$/animal) & super-infected with *T.evansi.*

Table (3):

Mean weekly Creatinie , Urea & standard deviation of goats with multiple infection .

Group	Parameter / Treatment	Creatinie (gm/dl)	Urea (gm/dl)
1	T+H	$(0.64\pm0.26)^{a}$	$(49.53\pm21.36)^{b}$
2	H+T	$(0.58\pm0.14)^{a}$	$(27.17\pm16.77)^{a}$
	H+T*	$(0.83\pm0.41)^{a}$	$(37.79\pm18.26)^{a}$
3	CN	$(0.93\pm0.45)^{b}$	$(34.84\pm3.08)^{a}$

N.B:-

Means within the same column with different subscripts are significantly different.

CN : Uninfected control group .

T+H: This group of animals was infected with *T.evansi* and super infected with *H.contortus* .

H+T : This group of animals was infected with *H.contortus*($1390L_3$ / animal) super-infected with *T.evansi* .

H+T*: This group of animals was infected with *H.contortus* ($700L_3$/animal) & super-infected with *T.evansi.*

Discussion :-

As shown from the results in table (1) there was no significant change in the mean values of temperature in both of the infected groups compared to the control group (III).This did not show the expected close relation to simultaneous infection with *T.evansi* and *H.contortus*.

The haematological value as measured by PCV and Hb results in group (I) agreed with Griffin.*et.al* (1981) ,Elhadi ,(1999) Kauffman (1992), and Okeleng,(1996),where the type of anaemia was microcytic indicated by increase in total RBCs count and decrease in PCV,and hypochromic as indicated by decrease in Hb.In group(II) the type of enaemia was normocytic where there was a decrease in RBCs count and PCV due to haemolytic process resulting from infection with *T.evansi* .

Parasitic infection is generally expected to raise the WBCs count due to cellular immunity , accordingly there was a marked increase in the mean values of both infected groups.

Measurements of total protein and its fractions revealed a decrease in the mean values of total plasma protein and albumin in both of the infected groups as compared to the control group (III).In group (II) the animals which were infected with higher dose of *H.contortus* showed the largest decrease in both parameters, since the animals suffered from chronic *H.contortus* and super infection this result with *T.evansi* .This result agree with Arsuni ,*et.al*,(1989) ,Ostie,*et .al* (1991) and Damayanati *et.al*,(1994).Elamin ,(1980)described this decrease by reduction in protein synthesis due to destruction in hepatocytes . Wellde *et.al* ,(1974) attributed this change due to disturbance in metabolism and increased protein catabolism .

Results of Urea and Creatinine concentrations in this work agrees with Anosa, (1988),Dam ,*et.al*(1998) and Elhadi ,(1999) .The Creatinine values decreased in the infected groups.There was an increase in the Urea mean values in group (1)and in the animals of group (II) which received lower *H.contortus* dose ,while those which were infected with a higher dose of *H.contortus* showed a decrease in the Urea mean values. According to Varley *et.al* ,(1980), the severe liver condition might result in the decrease of Urea level , while Anosa (1988) attributed these changes due to impairment of renal function.

Antibody titre results in animals with acute *H.contortus* and super infected with *T.evansi.* showed that positive results began three weeks after infection .

Animal with a higher dose of *H.contortus* and super infected with *T.evansi* showed a slight decrease in the mean value of antibody titre.In animals of group (1) which were infected with *T.evansi* and superinfected

with *H.contortus*, the positive results began two weeks after infection. Thus, the above immunological response pattern indicates that antibody titre and time for their appearance was affected by superinfection .It was delayed in the case of acute infection with *H.contortus* and reduced in chronically infected animals .

It is concluded than pre-infection with *H.contortus* did affect susceptibility to *T.evansi* as indication of lowered immunity.

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Haematological and biochemical changes in buffalo-calves (*Bubalus bubalis*) infected with *Trypanosoma evansi*

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Summary

In a previous study in Egypt (Hilali et al., 2004) (*), *T.evansi* was detected serologically in experimentally inoculated water buffaloes by using the card agglutination test (CATT). The test was found to be sensitive and specific and the parasite induced general emaciation and reduced weight gain.

Since information on surra in water buffaloes from Egypt; with about 3 million heads; is scanty, this investigation was initiated to study the haematological and biochemical changes in experimentally inoculated buffalo-calves with *T. evansi.*

Four water buffalo calves (age 6 months) were inoculated each with 10^6 *T. evansi* strain isolated (Egypt CD1) from naturally infected camel. A fifth non-infected calf was kept as a control. Two blood samples were obtained (one on heparin and one plain blood) from each calf every 4 days during the first month and then once weekly until the end of the experiment (day 88 post–infection [PI]). The heparinized blood was used for measurement of the haematological parameters including total erythrocytic count (RBCs), total leucocytic count (TLCs), packed cell volume (PCV), haemoglobin concentration (Hb%) and differential leucocytic count.

The serum alkalin phosphatase (AP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT) and gamma glutamyle aminotransferase (GGT/γGT) were measured using commercial kits supplied by Stanbio laboratory, USA and Quimica Clinica Aplicada(QCA).

The serum total proteins, serum albumin, globulins, A/G ratio, total and conjugated (direct) bilirubin, serum glucose, blood urea nitrogen and creatinine were measured by using the same commercial kits.

Our results showed a significant decrease in RBCs count, Hb, PCV and MCHC while MCV was increased. These values indicate that the

experimental calves suffered from macrocytic and hypochromic anaemia, due to injury of the RB

Cs and decreased levels of vitamin B12 and folic acid. The values of TLC, lymphocytes and monocytes were increased. Lymphocytosis may be attributed to polyclonal activation of circulating B-cells to clear the parasite.

The serum enzymes AST and LDH increased significantly during the experimental period. This increase could be due to mild hepatic disturbance, muscular dysfunction, renal congestion and/ or increased number of lymphocytes.

The total proteins and globulins were elevated especially at the termination of the experiment. This increase could be due to elevation in the gamma globulin, which was secreted as an immunological response against *T. evansi.*

The total bilirubin was increased as a result of enhanced erythrocytic destruction due to hemolysin and membrane injury.

The decrease in serum creatinine and urea could be related to tubular degeneration, interstitial nephritis and mononuclear infiltration of the renal golmeruli.

(*) Hilali, M. , Abdel-Gawad A. , Nasser A. , Abdel-Wahad A. , Magnus E. , Büscher Ph. :- (2004) Evaluation of the card agglutination test(CATT/*T. evansi*) for detection of *Trypanosoma evansi* in water buffaloes (*Bubalus bubalis*) in Egypt, *Veterinary Parasitol.* , 2004 May 7,**121** , (1-2), 45-51.

Urine Odour Change For Detection of Camel Trypanosomosis

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Khitma Hassan Elmalik (*)

Introduction

The diagnosis of camel trypanosomosis (Surra) by detection of characteristic odour is commonly practiced by camel owning societies. This was mentioned by Stephen (1986), Leese (1927) in South East Punjab, North Africa, Somalia, Kenya and Sudan (Hunter, 1986; Hussein, 1993).

An experiment was designed to test:

1. The validity of the urine odour change in camel trypanosomosis as suggested by traditional healers and its coincidence with parasite detection.
2. The clinco-pathological changes in urine which may be responsible for the characteristic odour changers in infected camel.

Materials and Methods

Four male camels and one female 2 – 4 years old camels were used in this experiment. They were purchased from North West Khartoum State, brought to the Department of Preventive Medicine, Faculty of Veterinary Medicine, University of Khartoum, where they were kept in fly proof double screened pens. The animals were allowed a two weeks period for acclimatization prior to parasite inoculation, during this period their freedom from blood parasite, internal and external parasites was confirmed.

Urine samples were also taken for electrolytes, protein and non-protein nitrogenous compounds to set the base line data. All through the experimental

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period the animal were fed on concentrate cakes and dura straw, drinking water was mixed with molase.

Experimental design

Three animals (number 1, 3 and 4) were chosen randomly for experimental infection, the other two were left as uninfected control animals (2 and 5).

Parasite strain

An isolate of *Trypanosoma evansi* was obtained from a naturally infected camel at Kassla State, the *Trypanosoma* species being determined morphologically. Jugular blood from the infected camels was subinoculated into rats. After 2 to 3 days incubation period the rats developed paracetaemia. One of the highly paracetaemic rats was bled and the blood was collected in test tube containing an equal volume of phosphate-glucose buffered saline (PSG).

Camels inoculation

Camels number 1, 3 and 4 were inoculated by *trypanosoma evansi* receiving ($7x10^6$) trypanosomes each.

Examination procedures

Daily record on the general clinical picture was made of all camels. Urine was collected and analyzed for odour (Darosa, 2000), albumin, sugar and bile pigment (King, 1958 and 1960). Ketone bodies (Varly, 1970) sodium and potassium (Mosher *et al.*, 1947) Calcium (Tinder, 1960) Magnesium (Orange, 1951) Creatine and Creatinine (Jaffe, 1886). Blood was microscopically examined for presence of the *Trypanosomes.*

A complete haemogram was done where Hb concentration (Jain, 1989), RBCs, WBCs counts and PVC values were recorded. Serum was separated and analyzed for total protein (Weichselbaum, 1946). Serum albumin (Bartholomens and Delany, 1966), Serum urea and SGOT and SGPT

(Reitmant, 1927). Also using Surratex reagent (Nantulya, 1994) urine and serum of the experimentally infected and control camels were tested.

Karraf test (Shail method)

Two traditional healers experienced (Karrafs) in the diagnosis of surra by inhalation of the infected camel urine were allowed to give their judgment versus those obtained by the microscope and this continued for two months. Also Ketostick test was carried and results recorded for the same period (Darosa, 2000).

Chemotherapy

After the seventh week the infected camels were treated using Antrycide (Quinapyramine sulphated) at the recommended dose.

Statistical analysis was carried for the result obtained.

Results

Clinical manifestation

The inoculated camels showed emmuciation general debility, rough coat (Plate 1) and alopecia symptoms (Plate 2) after the 5th week of inoculation.

Parasitological examination

Incubation period was 7 – 9 days. Wet blood examination was positive in 73.5% examinations carried, while Buffy count (BC) was positive in 90.3% of the examination done.

Urine analysis results

Urine odour result

The urine started to change as detected by Karraf seven days post-inoculation which continued up to two days post-treatment. Urine odour did not change in control camels throughout the experimental period. Urine electrolytes (Ca, Mg, Na and K) of infected camels showed increased values versus the control (Table 1). Protein and non-protein nitrogenous ingredients of the infected camels urine were increased compared to control (Table 2). Creatinine was increased in infected camels versus that of control camels (Table 2). Ketone bodies were abundant in the urine and serum of infected camels detected by the ketostick strips.

Plate 1a: Rough coat (infected animal)

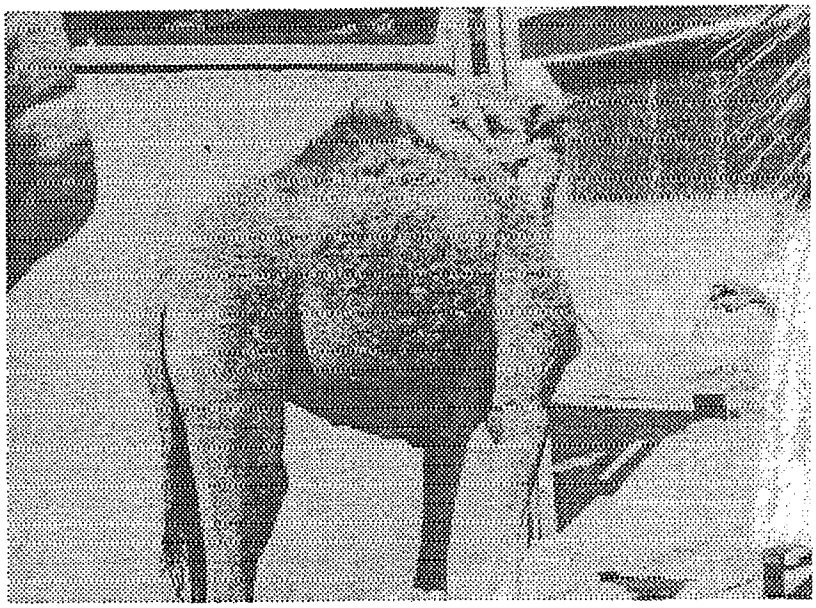


Plate 1b: Smooth coat (non-infected animal)

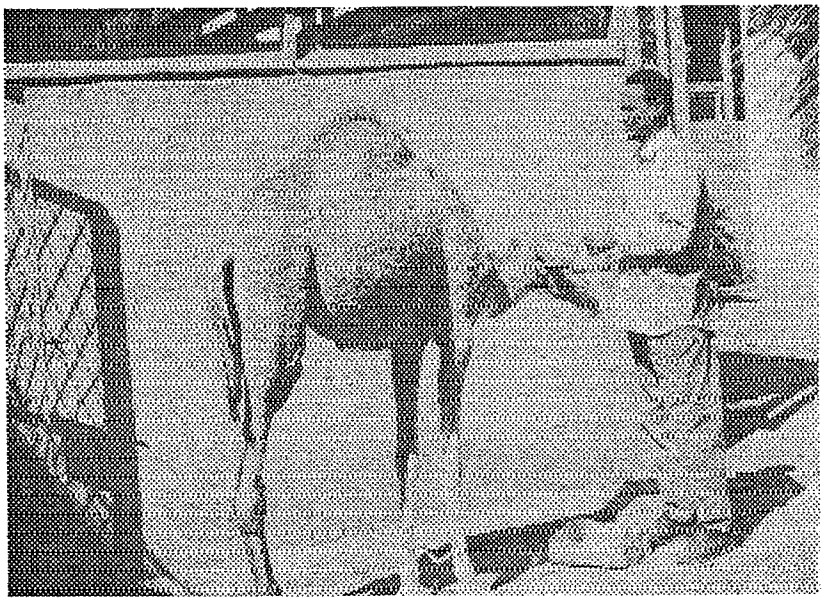


Plate 2: Alopicia

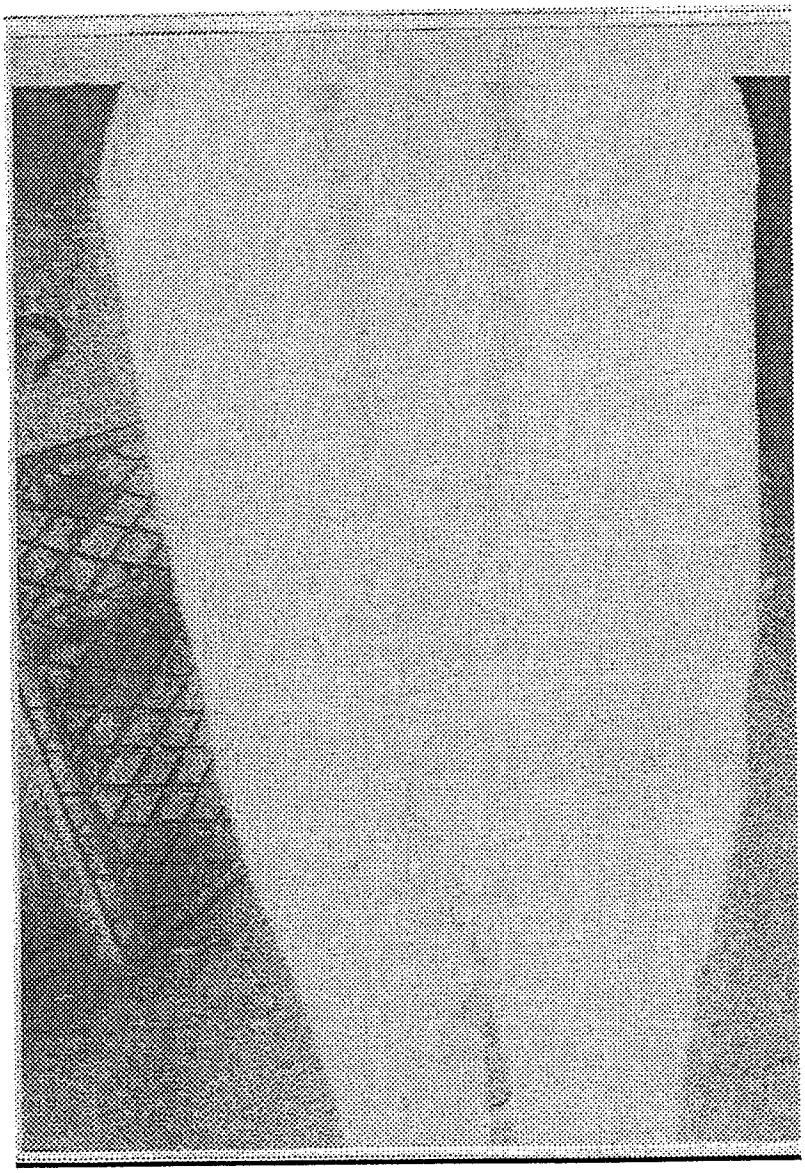


Table 1: Comparison of mean electrolytes values in urine from the experimentally infected camels versus negative control

Electrolyte	Control Camels	Infected Camels	L.S
Ca^{2+} (mg/l)	8.19±1.27[b]	10.02±7.02[a]	**
Mg^{2+} (mg/l)	145.54±9.67[b]	171.92±13.61[a]	***
Na^{+} (mmol/l)	136.87±21.09[b]	167.85±23.76[a]	***
K^{+} (mmol/l)	132.70±17.72[b]	156.13±23.55[a]	***

Means within the raw the same letter are not significantly different at $p<0.05$

* Significant at $P<0.05$

** Significant at $P<0.01$

*** Significant at $P<0.001$

Table 2: Comparison of mean values of protein and non-protein nitrogenous compounds in urine of experimentally infected camels versus negative control

Ingredient	Control Camels	Infected Camels	L.S
T.P (g/l)	7.16±0.81^{b}	9.16±1.95^{a}	***
Alb (g/l)	6.02±0.77^{b}	8.18±1.89^{a}	***
Uric acid (mg/l)	175.02±21.80^{b}	221.67±57.15^{a}	***
Creatine (mg/l)	17.22±2.93^{b}	22.73±5.97^{a}	***
Creatinine (mg/l)	191.30±52.51^{b}	228.13±82.39^{a}	***
Urea (mg/l)	10.32±0.77^{b}	13.32±2.19^{a}	***
Nitrogen %	15.33±13.00^{b}	17.68±1.92^{a}	***

Means within the raw the same letter are not significantly different at $p<0.05$

* Significant at $P<0.05$

** Significant at $P<0.01$

*** Significant at $P<0.001$

Serum results

Serum total protein (Albumin and globulin) was increased in the infected camels.

Haematological results

Haemaglobin concentration, packed cell volume and RBCs counts were decreased in the infected camels, while the WBCs increased (Table 3).

Rates of infection detection by the different methods

1. Microscopic method

84 out 93 readings were found positive which gave a percentage of 90.3%

2. Urine odour change method (by inhalation)

78 out of 93 readings were detecting to be positive which gave a percentage of 83.3%.

3. ketostick method

42 out of 93 readings were found to be positive which gave a percentage of 50.5% (Fig. 1)

The coincidence between the different methods in detection of infection

Microscopic and urine odour change methods coincidence rate was 86.0%, while microscopic and ketostick methods gave 53.8% rate of coincidence. On the other hand, urine odour change and ketostick gave 64.5% rate of coincidence.

Table 3: Comparison of blood mean values of experimentally infected camels versus negative control

Item	Control Camels	Infected Camels	L.S
Temp ©	36.67±0.41^{b}	37.14±0.87^{a}	***
P.C.V %	23.94±2.30^{a}	20.74±3.02^{b}	***
RBCs (counts/cm^{3})	6899750±398470.41^{a}	5751222±745026.00^{b}	***
WBCs (counts/cm^{3})	12788.2±2568.10^{b}	13989.20±2813.63^{a}	***
HB %	68.708±3.21^{a}	64.264±4.41^{b}	***

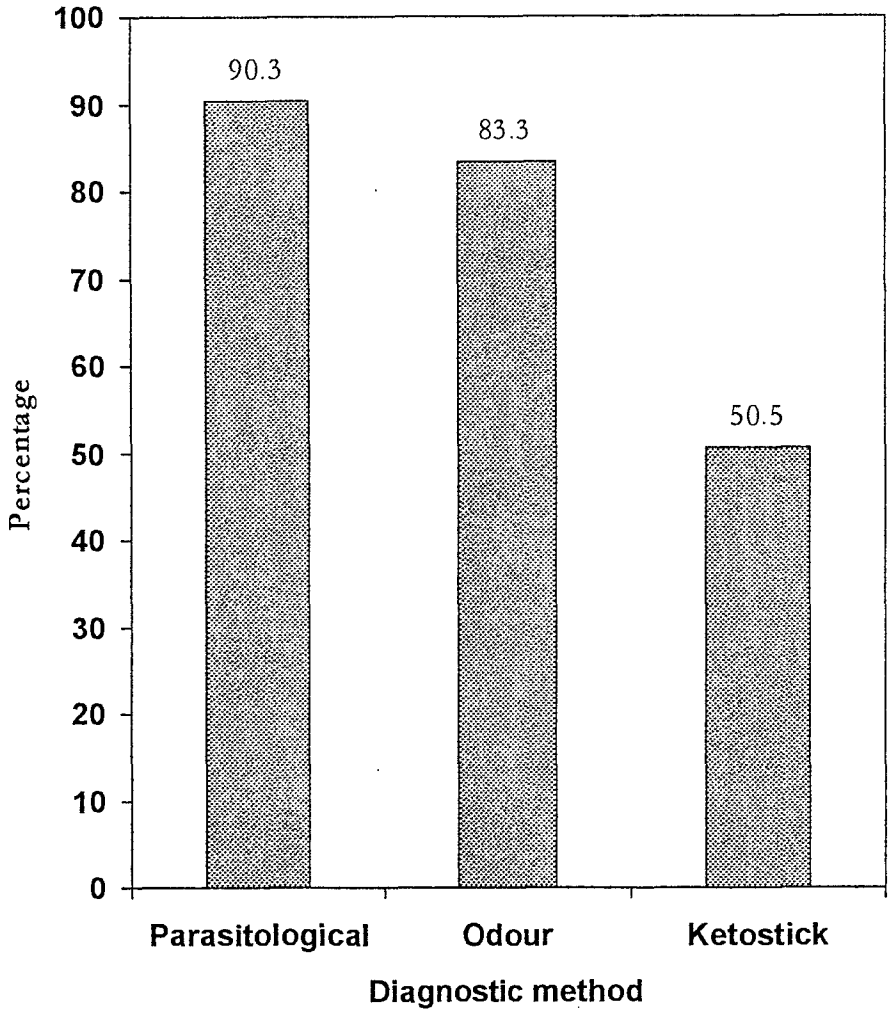
Means within the raw the same letter are not significantly different at $p<0.05$

* Significant at $P<0.05$

** Significant at $P<0.01$

*** Significant at $P<0.001$

Fig. 1: Rates of infection detection by the different methods



Discussion and Conclusions

It worth mentioning that there is a positive correlation and high coincidence rate between the microscopic examination of blood and urine odour change (shail) method of diagnosis. Due to metabolic disturbances and pathological change particularly in the kidney of *T. evansi* infected camels, there is increased urine electrolytes, urine creatinine, urine urea, ketouria, albuminuria and proteinuria. One or more of these ingredients may give rise to the characteristics odour in urine of the *T. evansi* infected camels.

The disappearance of parasite from blood and the characteristics odour from urine coincided with the disappearance of the ketone bodies from serum and urine post-treatment inspite of continued presence of the other ingredients. This indicates that the ketone bodies could be the main probable source of the characteristics odour in question.

Ketone bodies levels in serum or other body fluids, are known to be elevated in diseases as toxaemia, ketosis and starvation as well as in pregnancy. Comparison must be done between the ketone bodies exerted in the above mentioned diseases and that in the case of trypanosomiasis qualitatively and quantitatively (Darosa, 2000), this differentiation is necessary to determine whether Elshail method or ketostich techniques is specific for trypanosomosis diagnosis in camel.

It could be concluded that from all the findings obtained subsequent studies could be planned for:

1. Investigation on whether this odour change of urine is particular for camel infected with *T. evansi* or common for other animal species and other *Trypanosomes* infection.
2. To hold a training course for those experts (Krrafs) with the aim of unifying their traditional practices and exchange of knowledge.

3. It is suggested that shail method and ketosticks be tried in the field to validate their use in improving their diagnostic value.

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ANNEX XIX

18 March 2004

FAO Communication on PAAT Activities

Document prepared by FAO/AGAH

for

RÉUNION DU GROUP AD HOC DE L'OIE SUR LES TRYPANOSOMOSES ANIMALES NON TRANSMISES PAR LES GLOSSINES

Paris (Siège de l'OIE), 23 mai 2004

FAO Communication on PAAT Activities

1. Background

1.1. Trypanosomiasis has a severe economic impact on African agriculture. In affected countries, the disease causes an estimated annual loss of US$4.75 billion and greatly constrains socio-economic development, limits land use, optimal utilization of natural resources, and causes poverty and food insecurity.

1.2. The reinforcement of agriculture is a key element in the fight against poverty in most of tsetse and trypanosomiasis (T&T) affected countries, and livestock provides important contributions to livelihood and markets in more than 20 countries where the disease occurs. Trypanosomiasis is therefore a constant and serious threat to food insecurity in large areas of sub-Saharan Africa. The control and ultimate removal of the disease would significantly contribute to increased productivity of land and livestock and to reduce rural poverty in Africa.

1.3. Given the sub-continental scale of the tsetse fly problem and considering its complex and dynamic medical, veterinary, agricultural and rural development dimensions, FAO Member Nations have recognized the need to establish focus and direction in the fight against T&T. This is pursued under the Programme Against African Trypanosomiasis (PAAT).

1.4. The Programme forms an international alliance and combines the forces of FAO, IAEA, AU (formerly OAU)/IBAR, WHO and other stakeholders. PAAT seeks to create a conducive environment to:

- Define viable strategies and technical guidelines for intervention; and
- Ensure direct involvement of technical staff, policy makers, planners of tsetse-affected countries.

1.5. In summary, the PAAT strategy is to link T&T intervention to overall policies and to Sustainable Agriculture and Rural Development (SARD), where the SARD goal is defined as:

"the enhancement of the productive capacity of the natural resources base as a whole, and of regenerative capacity of renewable resources, without disrupting the functioning of basic ecological cycles and natural balances or destroying the socio-cultural attributes of rural communities, but instead provide durable conditions for agricultural production".

1.6. In other words, T&T interventions are likely to yield maximal economic benefits if properly inserted into the broader policy of agricultural development.

1.7. In addition, PAAT mandated Organizations agreed to focus on those areas where the disease impact is most severe and where control provides the greatest benefits to human health, well-being and SARD. A way to achieve this objective is to base interventions on the area-wide integrated pest management (AW-IPM) concept.

1.8. Following the above principles, a set of criteria and guiding principles for prioritising intervention areas has been developed under the PAAT umbrella in collaboration with the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). These criteria led to the identification of two priority areas, one located on the boarder zone of Burkina Faso and Mali and the second one in the Southern Rift Valley of Ethiopia.

2. PAAT and PATTEC

2.1. In line with the Resolution 4/2001, FAO has been working to enhance synergy and harmonization between PAAT and PATTEC. The two programmes share the long-term objective of the removal of the trypanosomiasis constraint from sub-Saharan Africa.

2.2. In particular, the supportive role of FAO – through PAAT – to PATTEC, is to provide it with international expertise on various aspects of tsetse and disease management, and the associate issues of land use, environmental protection and sustainable livestock-agricultural and socio-economic development. PATTEC's action plan focuses on area-wide approaches to tsetse control.

3. FAO/PAAT support to PATTEC

3.1. FAO has been working together – through PAAT – in achieving international scientific and technical recognition for providing reliable science-based advice to Member Nations and PATTEC on tsetse and trypanosomiasis matters. The process is based on country-driven consultative advisory structure and advises on international technical policy, assist and promote regional and national programmes, as well as identify project guidelines, research priorities and strategies on T&T interventions.

3.2. FAO generates scientific/technical and normative principles on T&T and related development which are disseminated through the publication of bulletins, reports, positions and other media.

3.3. The main contribution provided to PATTEC by FAO – through PAAT – concerns technical and scientific support. FAO organizes annual meetings of the Panel of PAAT Advisory Group (PAG) Coordinators and of the Programme Committee, a decision-making body comprising senior technical advisers, representatives of affected countries, donors and international research institutions. In addition, FAO convenes international workshops, prepares internationally recognized guidelines and assists Member Nations and PATTEC in the process of priority setting in T&T interventions.

3.4. Also, FAO hosts and runs the PAAT Information System (PAAT IS) which allows PAAT and PATTEC partners to interact and communicate with the scientific and technical community, policy makers, donors and planners.

3.5. The close collaboration between FAO and PATTEC for jointly tackling T&T in sub-Saharan Africa is underpinned by the participation of PATTEC and members of the PATTEC Policy and Mobilization Committee in major international scientific meetings convened by FAO through PAAT, i.e. the 7th meeting of the PAAT Programme Committee, held in November 2002, in Geneva (Switzerland) and the 9th meeting of the Panel of the PAG Coordinators, held in September 2003, in Pretoria (South Africa).

3.6. FAO participated in relevant international discussion fora, such as:

- the 3rd PATTEC Policy and Mobilization Committee meeting, in Addis Ababa (Ethiopia), February 2003, with a view to foster understanding, harmonization and cohesion of efforts to address the T&T problem in the context of SARD;
- the 3rd AU/IBAR-FITCA (African Union/Interafrican Bureau for Animal Resources-Framing in Tsetse Controlled Areas) workshop on harmonization of national strategies for tsetse control/eradication, in Pretoria (South Africa), September 2003; and
- the 27th International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), in Pretoria (South Africa), October 2003.

4. Recent developments

4.1. At 32nd Session of the FAO Conference, November-December 2003, the FAO "Progress report on implementation of the plan of action for the Pan African Tsetse and Trypanosomiasis Eradication Campaign (Resolution 4/2001)" was unanimously endorsed by the Member Nations.

4.2. With regard of prioritization of normative activities, FAO convened two international workshops, respectively in July 2003, FAO HQ, and in February 2004, Ouagadougou (Burkina Faso) on planning T&T field programme proposals in priority areas of Burkina Faso-Mali and Ethiopia. The Concept Note of the Southern Rift Valley of Ethiopia is attached in Annex 1. A similar document for Burkina Faso-Mali is under development.

4.3. FAO, in collaboration with PATTEC, is preparing a regional Technical Cooperation Programme (TCP) for the mobilisation of resources for "Capacity building and programme development in support of PATTEC". One of the objectives of the TCP project is to build the necessary regional and national capacity for integrated AW-T&T intervention schemes in agreed priority areas. Pending the delay in TCP approval, FAO AGA and AGE opted to jointly accelerate the implemenation of activities – to the extent possible – under RP funding, The two international workshops mentioned above should be seen in this context and constitute further FAO efforts to in support of PATTEC and in the advancement of strategic planning of field programme proposals.

4.4. In order to respond to the severe problem concerning the presence on the African markets of poor quality or counterfeit trypanocidal drugs, FAO initiated a collaboration with the International Federation for Animal Health (IFAH) – private sector (IFAH represents

- Pursue internationally and scientifically agreed standards, protocols and guidelines for Quality Control of trypanocides;
- Make accessible, on equal basis, to any company and stakeholders the generated scientific information; and
- Transfer the methodology and technology to African laboratories.

4.5. The dialogue between FAO/PAAT stakeholders, including GFAR partners, like EMVT and ILRI is active and concretizes in the participation of EMVT and ILRI staff in official meetings (e.g. PAG Coordinators and PAAT Programme Committee meetings, etc.) organized by FAO. In this regard, it should be mentioned that the GFAR Secretariat is hosted in FAO.

4.6. FAO investment in normative actions – through PAAT and its partners, including IAEA – concretized in the publication of position papers, in the PAAT Technical and Scientific Series. In addition to the paper entitled "Integrating the sterile insect technique as a key component of area-wide tsetse and trypanosomiasis intervention", two additional papers were published:

- "Socio-economic and cultural factors in the research and control of trypanosomiasis"; and
- Economic guidelines for strategic planning of tsetse and trypanosomiasis control in West Africa.

4.7. The first paper reviews a broad spectrum of socio-economic and cultural information on T&T intervention, mainly in PAAT-PATTEC agreed priority areas, and analyses it from "macroplanning" (large-scale, government-managed schemes) and "microplanning" (small-scale, community-based programmes) perspectives. The analysis helps to evaluate when and how it might be appropriate to involve communities and individual livestock owners in T&T interventions.

4.8. The second document covers the main methodological issues involved in the economic assessment of AW-T&T control programmes. The dynamics of benefits over time are examined. The economic analysis developed is used to characterize types of situations in West Africa where tsetse control is likely to be economically profitable and those where long-term AW-projects are not likely to show good returns.

4.9. Two additional publications are in the pipeline:

- "Role of trypanotolerant livestock in the context of the methodological approach for tsetse intervention strategies"; and
- "Long-term tsetse and trypanosomiasis management options in West Africa".

4.10. In collaboration with DFID, FAO has initiated a study linking quantitative economic variables to a Geographic Information System to produce new insights and refine and reinforce the decision-making process for T&T intervention, through estimations of the potential benefits (mapping the benefits) resulting from intervention within a series of livestock management systems in dry and moist ecosystems.

4.11. In 2003, the PAAT Website was reviewed and harmonized with all other AGA

version is in progress and should be ready in 2004. The PAAT Website can be found at the following internet address:
http://www.fao.org/ag/againfo/programmes/en/paat/home.html.
The PAAT Website comprises six main sections: About PAAT, The Disease, Maps (of tsetse flies), PAAT Information System (PAAT-IS), Information Resources and Calendar. Each section is further divided in sub-sections.

4.11. It has to be mentioned that all the PAAT activities are conducted in consultation and with inputs of the members of the PAAT Secretariat (AU/IBAR, FAO, IAEA and WHO).

4.12. With a view to enhance national and regional capabilities in data management, analysis and decision making for the formulation of T&T management strategies, FAO has provided 13 tsetse-affected countries with more than 50 copies of the GIS software ArcView and Spatial Analyst.

4.13. On 29 September 2003, at the Opening Cerimony of the 27th meeting of the International Scientific Council for Trypanosomiasis Research and Control, Pretoria, South Africa, chaired by H.E. the Vice-President of the Republic of South Africa, FAO and PAAT were awarded of a Certificate of Appreciation by the Chairman of the Commission of African Union (AU) and the Governments and People of all AU Member States. The Certificates of Appreciation were awarded [to FAO and PAAT] "in recognition to the support, cooperation, active participation and most valuable contribution toward the control and suppression of Human and Animal Trypanosomiasis and their vector Tsetse Flies from the 37 affected countries in Africa".

5. Conclusions

5.1. Polic issuey

FAO, through the inter-agency Programme Against African Trypanosomiasis (PAAT), has continued to provide support to PATTEC, collaborate with PAAT partners, and assist the concerned African Union's Member Nations in setting and refining, criteria, guidelines and principles to alleviate the obstacles which tsetse and trypanosomiasis pose to agricultural development, poverty alleviation and food security.

5.2. Highlights on important aspects on normative function

The normative function of FAO and PAAT has also served the policy and technical dialogue on norms and standards for T&T intervention programmes. Substantial progress has been made in achieving concertation and consensus on a common approach to tsetse and trypanosomiasis intervention duly placed in the context of SARD. International consensus involving all relevant stakeholders now concerns focal areas for action in Burkina Faso, Mali and Ethiopia.