

OIE NTTAT Network



1st International Conference on Non Tsetse Transmitted Animal Trypanosomosis

15th and 16th December 2016

Anses, 14 rue Pierre et Marie Curie
94700 Maisons-Alfort



WORLD ORGANISATION FOR ANIMAL HEALTH
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French agency for food, environmental
and occupational health & safety

1st International Conference on
Non Tsetse Transmitted Animal Trypanosomosis
OIE NTTAT Network

Thursday 15 December 2016

8 h 30 Registration of participants

9 h 00 **WELCOMING** by Philippe BÜSCHER

Session 1: General Session

9 h 20 **T1** - Activities of the World Organisation for Animal Health (OIE) in support of the surveillance and control of non-tsetse transmitted animal trypanosomosis
Morgane DOMINGUEZ - OIE, France

9 h 40 **T2** - Overview of research activities on NTTAT at OIE Reference Laboratory in Antwerp
Philippe BÜSCHER - Institute of Tropical Medicine, Belgium

10 h 00 **T3** - Overview and Research Activities on *Trypanosoma evansi* in Indonesia
April H WARDHANA - Indonesian Research Center for Veterinary Science, Indonesia

Session 2: Vaccination

10 h 20 **T4** - The application of irradiation technology as a tool for vaccine development in *Trypanosoma evansi*
Richard T. KANGETHE - FAO/IAEA Agriculture and & Biotechnology Laboratory, Austria

10 h 40 Coffee break

Session 3: Isolation and Genetic Characterisation

11 h 00 **T5** - Isolation, cultivation and molecular characterisation of a new *Trypanosoma equiperdum* strain in Mongolia
Ikko IGARASHI - Obihiro University of Agriculture and Veterinary Medicine, Japan

11 h 20 **T6** - Phylogenetic analysis of the *Trypanosoma equiperdum* strain responsible for the 2011 outbreak of dourine in Italy
Achim SCHNAUFER - University of Edinburgh, United Kingdom / Ilaria Pascucci - IZS, Italy

11 h 40 **T7** - *Trypanosoma equiperdum*: genome sequencing and failure of melarsomine hydrochloride (Cymelarsan®) treatment in experimentally infected ponies
Laurent HÉBERT - Anses, France

12 h 00 Lunch

Session 4: Treatment

14 h 00 **T8** - *In vitro* and *in vivo* efficacy of diamidines against *Trypanosoma equiperdum* strains
Kirsten GILLINGWATER - Swiss Tropical and Public Health Institute, Switzerland

14 h 20 **T9** - Isometamidium chloride and homidium chloride fail to cure mice infected with *Trypanosoma evansi* type A and B isolated in Northern Ethiopia
Birhanu HADUSH - College of Veterinary Medicine, Ethiopia

Session 5: Epidemiology

14 h 40 **T10** - A survey of trypanosomosis in sheep and goats from Cholistan Desert, Pakistan
Sonia TEHSEEN / Philippe BÜSCHER - Government College University, Pakistan

15 h 00 **T11** - Epidemiology of dourine in Mongolia and introducing diagnostics in the field
Sandagdorj NARANTSATSRAL - Institute of Veterinary Medicine, Mongolia

15 h 20 Coffee break

15 h 40 **T12** - History of a surra outbreak and treatment evaluations in horses in Thailand
Margot CAMOIN - CIRAD, France

16 h 00 **T13** - *Trypanosoma evansi* outbreaks in Spain: 2015-2016 follow-up
Carlos GUTIERREZ - University of Las Palmas de Gran Canaria, Spain

16 h 20 **T14** - Conclusions of an episode of surra in dromedary camels in Aveyron (France, 2006-2007/2013)
Sophie THÉVENON - CIRAD, France

16 h 40 **T15** - A review on camel trypanosomosis due to *Trypanosoma evansi* in Mauritania
Mamadou Lamine DIA / Philippe BÜSCHER - CNERV, Mauritanie

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Friday 16 December 2016

Session 6: Pathology

9 h 00

T16 - Clinical and pathological aspects of dourine in Mongolia and therapeutic interventions
Banzragch BATTUR - Mongolian University of Life Science, Mongolia

9 h 20

T17 - Comparative clinico-pathological observations in young Zebu (*Bos indicus*) cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse areas of Northwest Ethiopia
Shimelis DAGNACHEW - Faculty of Veterinary Medicine, Ethiopia

9 h 40

T18 - Cytokine responses during *Trypanosoma evansi* infections of high and low virulence isolates from Indonesia in DDY mice
Dyah Haryuningtyas SAWITRI - Research Centre for Veterinary Science, Indonesia

10 h 00

Coffee break

Session 7: Diagnosis

10 h 30

T19 - Recombinant antigens in serological testing for *Trypanosoma evansi* infection
Barrie ROONEY - University of Kent, United Kingdom

10 h 50

T20 - Multiplex detection of antibodies to *Trypanosoma evansi* and sequence analysis of a type B strain
Neil WATT - MV Diagnostics Ltd, United Kingdom

11 h 10

T21 - Development of a rapid antibody test for point-of-care diagnosis of animal African trypanosomosis
Théo BALTZ - CNRS, Université de Bordeaux, France

Session 8: Atypical human infections

11 h 30

T22 - Updated results on atypical human trypanosomoses caused by animal trypanosomes
Philippe TRUC - IRD, France

11 h 50

T23 - Atypical human infections by animal trypanosomes: evaluation of trypanocides against *T. lewisi* in laboratory rats
Marc DESQUESNES - CIRAD, Thailand

12 h 10

Lunch

14 h 00

General discussion on OIE NTTAT Network

16 h 00

Closing of the conference



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Talk Abstracts

Activities of the World Organisation for Animal Health (OIE) in support of the surveillance and control of non-tsetse transmitted animal trypanosomosis

M. Dominguez¹, F. Diaz¹, E. Erlacher-Vindel¹

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Abstract

Non-tsetse transmitted animal trypanosomosis (NTTAT) have represented an area of interest of the World Organisation for Animal Health (OIE) since the creation of the organisation in 1924.

Activities of the OIE in support of the surveillance and control of NTTAT worldwide promote: (i) transparency of the global epidemiological situation of NTTAT; (ii) disease prevention and control as well as sanitary safety of international trade in susceptible animal species and their products; and (iii) scientific expertise on NTTAT.

Disease caused by infection with *Trypanosoma evansi* (surra) or *Trypanosoma equiperdum* (dourine) is notifiable to the OIE. The 180 Member Countries of the OIE should report these diseases if they are detected on their territory. The OIE then publishes the information through the World Animal Disease Information (WAHIS) in order to ensure transparency in the global situation of these diseases.

The OIE has developed standards addressing the prevention and control of dourine as well as for the purpose of safe international trade in equids and their products (Chapter 12.3. of the OIE Terrestrial Animal Health Code). These standards are recognized by the World Trade Organization (WTO) as rules to safeguard world trade. In addition, standards relating to surra are being developed.

Internationally agreed diagnostic methods for surra and dourine are defined in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Furthermore, the OIE has approved four OIE Reference Laboratories to address all of the scientific and technical issues relating to the laboratory diagnosis of NTTAT:

1. the National Research Center for Protozoan Diseases in Japan (OIE reference Reference Laboratories for *T. evansi*);
2. the Antwerp Institute of Tropical Medicine in Belgium (OIE Reference Laboratory for *T. evansi*);
3. the All-Russian Research Institute for Experimental Veterinary Medicine (OIE Reference Laboratory for *T. equiperdum*);
4. the French agricultural research and international cooperation organization (CIRAD) (OIE Reference Laboratory for trypanosomosis of African origin).

To further promote scientific expertise on animal trypanosomes, the OIE set up an International expert Group on *T. evansi* in 1983. In 1991, the Group became an OIE *ad hoc* Group on Non Tsetse Transmitted Animal Trypanosomoses (NTTAT). In 2015, it was proposed to change the approach and create a broader network. The OIE NTTAT network was then created to strengthen multilateral cooperation, and to promote the exchange of knowledge, data and reference material. This network brings together the four relevant OIE Reference Laboratories, as well as other OIE Reference Laboratories, research institutes, non-profit organisations, ministries, international organisations, private companies, the OIE, and affiliated experts.

The main goal of the OIE NTTAT network is to establish a global strategy for the control of infections with NTTAT which continue to have a significant impact worldwide, especially in rural communities.

NTTAT-related research activities ongoing at the OIE Reference Laboratory for Surra, ITM Antwerp

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Abstract

The Unit of Parasite Diagnostics at the Institute of Tropical Medicine Antwerp is one of the two Reference Laboratory for Surra recognised by the World Animal Health Organisation (OIE). Apart from services to other laboratories in the form of reference diagnostic testing, ad hoc training and delivery of reference sera, trypanosome strains and derived products, we conduct research on the three non-tsetse transmitted pathogenic trypanosomes: *Trypanosoma evansi*, *Trypanosoma equiperdum* and *Trypanosoma vivax*. In this presentation, we give an overview of our research, that is mostly carried out in collaboration with research institutes all over the world. It focuses on improved differential diagnosis, including recombinant antigen expression, isolation and adaptation of trypanosome strains to *in vitro* culture, developing *in vitro* and *in vivo* models for drug screening and tissue tropism studies. To this end, we construct transgenic trypanosome strains expressing bioluminescent, fluorescent and enzyme reporter genes.

Overview and Research Activities on *Trypanosoma evansi* in Indonesia

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Abstract

Surra caused by *Trypanosoma evansi* was first reported in Indonesia, in 1897 attacking a group of horses in Semarang, Central Java and then it rapidly spread throughout Indonesian archipelago by livestock movement facilitated by the abundance of haematophagus flies and unavailability Surra drugs at that time. Only Sumba island was reported as a free region of *T. evansi*, however Surra outbreak occurred in 2010 when there was a traditional horse racing in Sumba. More than 4268 livestock was found to be infected and caused 1760 livestock died consisting of horses, buffaloes and cattle for 1159, 600 and 1 heads, respectively. The economic losses was increased from US \$ 4.22 million in 2010 to US \$16,72 million in 2012. Another Surra outbreak was occurred in Banten province (West Java) in 2013-2014 followed by some regions in Sumatra island. The current Surra case was found in 2016 attacking buffaloes and diary cattle in Bogor (West Java). Due to a major financial losses, the Indonesian goverment puts Surra into a list of strategic contagious animal disease. The Indonesian Research Center for Veterinary Science (IRCVS) located in Bogor, West Java is a National laboratory reference for animal diseases developing many research activities on Surra. A total 406 of *T. evansi* isolate was successfully collected throughout Indonesian archipelago and preserved into liquid nitrogen (Cryopreservation). Some isolates have been characterized using molecular technique and examined their virulence level including comparing genotype and fenotype among isolates from different geographical regions. Based on the pathogenic study in mice, *T. evansi* isolates from Indonesia were divided into three levels, i.e. high, moderate and low pathogen possessing 8 different pattern of parasitemia. The spleen size of mice infected by *T. evansi* isolates (Indonesia) differed from isolates of the Philippine indicating characteristic differentiation of those isolates. Further analyses were carried out on both high and low pathogen isolates. They also showed differentiation on PCV value, blood glucose, cytokine and polypeptides profiles. In addition, a multiplex PCR technique was developed and applied to identify Trypanosome species and vectors collected from the field. To control of Surra in Indonesia, the farmers uses diminazene aceturate and isometamedium chloride. Regarding to atypical human infection by animal Trypanosome issue, serological tests (ELISA and CATT *T. evansi*) were performed on farmers living in Sumba Island. The result demonstrated that 4 of 24 sera were found to be positive. It suggested that human population is frequently exposed to *T. evansi*. From those activities revealed that there is highly variable characteristics exhibited by *T. evansi* in Indonesia. Accordingly, for future work, the genetic diversity and molecular epidemiology studies are required involving larger isolates from various geographical regions in order to help a better understanding of the parasite's polymorphic features and its implacation for Surra control.

The application of irradiation technology as a tool for vaccine development in *Trypanosoma evansi*

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Abstract

Recent advances using irradiation in vaccine development against parasitic diseases such as malaria have reignited studies in related parasitic diseases such as trypanosomosis in livestock. The objective of this work is to utilise irradiation to produce living but non-infectious trypanosomes so as to understand mechanisms that are important for establishing an infection. Experiments with irradiated *T. evansi* parasites reveal *in vitro* cultures receiving irradiation doses above 200 Gy do not recover. However, inoculating Balb/c mice with parasites irradiated with doses below 200 Gy leads to an infection that is less virulent when compared to infections initiated with non-irradiated parasites. The RNA profiles of parasites irradiated using different irradiation doses at different time points was compared to non-irradiated parasites for using microarray. Parasites irradiated using 100Gy and analysed 20 hours post exposure show 68 genes with known function up-regulated and 18 genes down-regulated. This is in contrast to the up-regulation of 21 genes and the down-regulation of 267 genes when using a dose of 200Gy. Genes that are consistently down-regulated when using both doses include Metallo-peptidases, carboxypeptidases, Kinases and members of the Ubiquitin family. Further analysis of the data derived from these experiments will help elucidate how irradiation affects processes that are important for establishing disease in the mammalian host.

Dourine and *Trypanosoma equiperdum* in Mongolia
~Isolation, cultivation and molecular characterization of a new *Trypanosoma equiperdum* strain in Mongolia~

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Abstract

Classification of trypanosome species in subgenus *Trypanozoon* remains a controversial topic. It has been suspected that some of the reference strains of *T. equiperdum* were misclassified as *T. brucei* or *T. evansi*. Thus, there is a strong need for establishment of a new *T. equiperdum* strain directly isolated from the genital mucosa of a horse, which is clinically and parasitologically confirmed as dourine. Recently, we found that the epidemics of horse trypanosomoses in Mongolia by serological and PCR-based surveillances. In 2014, we obtained a stallion, which was definitively diagnosed as dourine by the detection of trypanosomes in the urethral tract mucosa. The parasites were directly isolated from the urethral tract mucosa and cultivated using 0.8% soft agarose HMI-9 medium at 37 °C in 5% CO₂. The phylogenetic analyses based on 18S rRNA and ITS sequences showed that the trypanosome was classified in the *Trypanozoon* clade. In a PCR characterization of the maxicircle kDNA genes, only NADH-dehydrogenase subunits 4 and 5 were amplified. Taken together, the trypanosome possesses typical clinical and molecular characteristics, such as edema in foreskin, very low blood parasitemia, many parasites in urethral tract mucosa, *Trypanozoon* clade, and defective maxicircle kDNA, we concluded that the trypanosome species is *T. equiperdum*. This is the first confirmed case of dourine in Mongolia. The trypanosome was cloned by limiting dilution and named as “*T. equiperdum* IVM-t1” strain.

Phylogenetic analysis of the *Trypanosoma equiperdum* strain responsible for the 2011 outbreak of dourine in Italy

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Abstract

Dourine is a sexually transmitted trypanosomosis that affects equids, historically attributed to a distinct etiologic agent, *Trypanosoma equiperdum*. The disease was first eradicated in Italy in the 1940s, but there was then a serious epidemic in the mid-70s. After sporadic reports at the end of the 1990s, an outbreak starting in May 2011 resulted in seven cases with confirmed serological and clinical signs (Pascucci *et al.*, 2013). The position of *T. equiperdum* as a distinct subspecies within the trypanozoon group is controversial (Claes *et al.*, 2005), and recent phylogenetic analysis of a number of isolates has proposed that both *T. equiperdum* and *T. evansi*, another non-tsetse transmitted trypanozoon closely related to *T. brucei*, should be regarded as subspecies of the latter (Carnes *et al.*, 2015). The same study also suggested that at least four groups of *T. equiperdum* and *T. evansi* have evolved from *T. brucei* independently and confirmed that some isolates historically classified as *T. equiperdum* cluster together with *T. evansi*. Molecular analysis of recent isolates from epidemiologically confirmed cases of dourine is critical to resolve the phylogenetic relationship of *T. (b.) equiperdum*, *T. (b.) evansi* and *T. brucei*. Here we present a phylogenetic analysis of the *T. equiperdum* strain responsible for the 2011 outbreak of dourine in Italy.

References

Pascucci I *et al.* (2013). Diagnosis of dourine in outbreaks in Italy. *Vet Parasitol* 193(1-3):30-8. doi: 10.1016/j.vetpar.2012.12.006.

Claes F, Büscher P, Touratier L, and Goddeeris BM (2005). *Trypanosoma equiperdum*: master of disguise or historical mistake? *Trends Parasitol* 21(7):316-21.

Carnes J *et al.* (2015). Genome and phylogenetic analyses of *Trypanosoma evansi* reveal extensive similarity to *T. brucei* and multiple independent origins for dyskinetoplasty. *PLoS Negl Trop Dis* 9(1):e3404. doi: 10.1371/journal.pntd.0003404.

***Trypanosoma equiperdum*: genome sequencing and failure of melarsomine hydrochloride (Cymelarsan®) treatment in experimentally infected ponies**

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Abstract

Trypanosoma equiperdum is a flagellated protozoon that causes dourine in horses and other members of the Equidae family. This sexually-transmitted infection is a World Organisation for Animal Health (OIE) notifiable disease. *T. equiperdum* is closely related to the agents of surra (*Trypanosoma evansi*) and nagana in animals or sleeping sickness in humans (*Trypanosoma brucei*). Those three parasites constitute the Trypanozoon subgenus but recent phylogenetic analyses show that *T. equiperdum* and *T. evansi* are not monophyletic and should therefore be considered as subspecies of *T. brucei*.

In order to shed light on the classification of the Trypanozoon subgenus, we proceeded to the sequencing of the whole genome of *T. equiperdum* Onderstepoort Veterinary Institute (OVI), a strain isolated in 1976 from the blood of a horse in South Africa. The availability of this genome sequence constitutes an important insight for the development of new differential diagnosis tools and for the study of the genetic diversity between the causative agents of dourine, surra and nagana/sleeping sickness.

The question of the differentiation of trypanozoon is all the more problematic since the OIE terrestrial animal health code considers dourine as non-treatable and imposes a stamping-out policy for affected animals to recover a country free status unlike surra and nagana. To clarify the legitimacy of considering dourine as non-treatable we proceeded to the evaluation of the capacity of 4-melaminophenylarsine dihydrochloride (trade name, Cymelarsan®) to eliminate *T. equiperdum* OVI from the overall body of experimentally infected ponies. Our results show the inability of Cymelarsan® to cure animals presenting parasites in the cerebrospinal fluid and as a consequence, the OIE Terrestrial Code recommendation to practice a stamping-out policy for affected animals remains for us, the best way to control dourine until the validation of new effective treatments.

In vitro and *in vivo* efficacy of diamidines against *Trypanosoma equiperdum* strains

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Abstract

Trypanosoma equiperdum is a protozoan parasite, responsible for causing a debilitating neglected veterinary disease called dourine, which is found worldwide affecting only equids. It is the only pathogenic trypanosome species that does not require an invertebrate vector for transmission, thus being passed from animal to animal via coitus. At present, there is no officially recognised form of chemotherapeutic treatment as a potential control measure against dourine and therefore all confirmed (or suspected) cases of infected animals must be slaughtered immediately. For many global communities and farming populations, which rely heavily on their animals for their livelihood, such circumstances can greatly enhance the socio-economic problems attributing to poverty.

A selection of 37 diamidine molecules were chosen, based on their previous efficacy against *Trypanosoma evansi* infection and examined *in vitro* against two original *T. equiperdum* strains (OVI, South Africa and BoTat 1.2, Morocco). Drug sensitivity (IC₅₀) values of the 37 compounds ranged from 0.0002 - 0.212 µg/mL for both strains. *In vivo* drug profiles were then established, using diminazene aceturate and quinapyramine sulphate, against three original *T. equiperdum* strains (OVI, South Africa; Dodola, Ethiopia and BoTat 1.2, Morocco). The 17 most active compounds were then investigated further within *in vivo* mouse models of infection. Compounds were administered to mice as single bolus dose applications of 1 x 10 mg/kg, given intra-peritoneally. Parasitaemia was monitored for 60 days post-treatment to determine cures.

Since no reported studies have yet evaluated the potential for novel candidate compounds as effective chemotherapeutic agents against *Trypanosoma equiperdum*, these results are the first of its kind. The data acquired from this study greatly impacts our current understanding of *T. equiperdum* and could assist in the disease management for dourine in the future.

Isometamidium chloride and homidium chloride fail to cure mice infected with Northern Ethiopian *Trypanosoma evansi* type A and B

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Abstract

Trypanosoma evansi, mechanically transmitted by biting flies, affects camels, equines, other domestic and wild animals. Surra due to *T. evansi* type A that expresses the predominant RoTat 1.2 variant surface glycoprotein (VSG) is endemic in Africa, Latin America and Asia. *T. evansi* type B, that lacks this VSG gene, has so far been isolated only from dromedary camels in Kenya and Ethiopia. Unlike the many reports about drug resistance in tsetse transmitted animal trypanosomes, no information is available about drug resistance in Ethiopian *T. evansi*. Thus, this study was conducted with the objective of determining *in vivo* drug sensitivity of *T. evansi* type A and B from Tigray and Afar regions of Northern Ethiopia. Diminazene diaceturate (Veriben; Ceva Santé, Libourne, France), isometamidium hydrochloride (Veridium; Ceva Santé, Libourne, France), bis (aminoethylthio) 4 melaminophenylarsine dihydrochloride (Cymelarsan; Merial, Lyon, France) were purchased in Europe, while homidium chloride (Bovidium, Kela, Hoogstraten, Belgium) and diminazene diaceturate plus phenazone granules (Sequzene, Alivira Animal Health imited, India) were procured from veterinary drug shops in Tigray region. Cymelarsan at 2 mg/kg and Veriben at 20 mg/kg cured infected mice, while Cymelarsan at 0.125 mg/kg failed. Results were comparable to results obtained in earlier *in vitro* drug testing with the same strains. However, unlike *in vitro* observations, Veridium at 1 mg/kg and Bovidium were not effective, neither against kinetoplastic nor akinetoplastic *T. evansi* strains. Sequzen at 20 mg/kg was also not effective. In conclusion, use of isometamidium chloride, homidium chloride and diminazene diaceturate to treat surra in Northern Ethiopia may need to be adapted.

A survey of trypanosomosis in sheep and goats from Cholistan Desert, Pakistan

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Abstract

Trypanosomosis (surra) is a serious disease which affects both wild and domestic animals around the world. Sheep and goats are considered to be resistant to *T. evansi* infection, which is often reported to be sporadic in nature. A parasitological, molecular and serological-based investigation was carried out in randomly sampled sheep (n = 200) and goats (n = 200) belonging to different breeds, age groups, sexes, and localities from the district of Bahawalpur, Pakistan. Woo's test gave 0.5% prevalence with goats only. Card agglutination test (CATT/*T. evansi*) yielded a higher prevalence for goats (25.0 %, 95% CI: 19.0, 31.0) than sheep (10.0%, 95% CI: 9.53, 19.27). Immune trypanolysis (TL) gave a prevalence of 21.5% (95% CI: 15.81, 27.16) and 9.5% (9.5%, 95% CI: 5.44, 13.56) for goat and sheep respectively. Both PCRs (TBR 1/2 and Rotat 1.2) yielded similar results with a higher prevalence for goats (2%, CI: 0.06, 3.94) than sheep (1.5 %, CI: 0.18, 3.18). Based on CATT/*T. evansi* and TL, significantly ($P \leq 0.05$) higher prevalence estimates were recorded for females than males. For goats, a significantly higher ($P \leq 0.05$) prevalence was reported for the breed, Jattal than Beetal. Similarly, for sheep, higher prevalence estimates were recorded for the Cholistani (Khadali) breed in comparison to other breeds namely Sipli and Bucchi. The test agreement between CATT and TL is almost perfect with both sheep ($k=0.942$) and goats ($k=0.902$). This study suggests further investigations on a larger numbers of samples to assess the true status of *T. evansi* infection in the concerned animals, breeds and area.

Epidemiology of dourine in Mongolia and introducing diagnostics in the field

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² National Research Center for Protozoan Diseases, OIE Reference Laboratory for Surra, OIE Collaborating Centre, OUAVM, Hokkaido 080-8555, Japan

Abstract

Prevalence of dourine in Mongolia is increasing steadily which previously had very limited distribution and affecting prosperous equestrian industry substantially due to increasing domestic and international horse trade. Epidemiological study of the disease was conducted by serological diagnostics using ELISA on 2621 sera collected from 20 provinces and state average was 9.4% and highest rate recorded in Tuv province as 38.4%.

Rapid diagnostics are essentially needed in practitioner veterinarians and confirmative diagnostic are also demanded in provincial level veterinary laboratories. Currently, within the frame of SATREPS project under the theme of 'Epidemiological Studies on Animal Protozoan Diseases in Mongolia and Development of Effective Diagnostic Measures' rapid diagnostic ICT will be developed and distributed to local practitioner veterinarians.

So far, we have successfully isolated 2 wildtype of *T. equiperdum* from an indigenous Mongolian breed horse indicating typical clinical symptoms of dourine and parasite is well adapted in *in vitro* culture system. Now we are initiating a research on morphology, motility and genomic studies of *T. equiperdum* by using *in vitro* culture adapted parasites which is very unique and valuable in the world.

More importantly, we are now experimenting to produce diagnostic crude antigens for IFAT, CFT and ELISA using *in vitro* culture of *T. equiperdum* successfully in order to search possibilities of utilizing it into diagnostics. Provincial veterinary laboratories are well equipped with devices necessary and diagnostic capacities of these laboratories will be improved through providing diagnostic antigens. This would allow us to initiate manufacturing diagnostic tools for the diagnosis of dourine in our country with cheaper expenses and supply provincial level veterinary laboratories demand.

Furthermore, we began to screen drug sensitivity and evaluate therapeutic methods against the disease in order to form controlling measurements and guidance against dourine.

Key words: Dourine, wildtype isolate, CFT, IFAT, ELISA

History of a surra outbreak and treatment evaluations in horses in Thailand

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Abstract

Trypanosoma evansi is the main pathogenic trypanosome of livestock in South-East Asia (SEA); it is the causative agent of surra, a disease mechanically transmitted by biting insects. Surra is of major importance as it can spread to many domestic and wild species. If cattle and buffaloes may develop acute form, they usually develop a chronic form with depression and reluctance to work, while horses undergo more acute forms often leading to death; on the contrary the disease rarely affects pigs and small ruminants in SEA. Surra's economic impact is consequently high as the disease can lower meat, milk and manure production, decrease work force and fertility and cause abortion and mortality. Surra also entails high treatment costs, hinders the efficacy of vaccination campaigns due to the induced immunosuppression and limits animal movements for sales as well as for touristic and sport events.

To illustrate the relative roles of those different domestic host species in the epidemiology of *T. evansi* in the SEA context, the history of a surra outbreak that occurred in 2011 in Surat Thani, Southern Thailand, in a mixed farm owning 41 Zebu cattle, 103 Angol goats and 12 local horses, is described. This outbreak also served as an opportunity to evaluate diminazen acetate and melarsomine hydrochloride treatments and to emphasize good practices for outbreak management. Additionally, treatment evaluations were carried out in horses naturally found infected in Nakhon Pathom Province (West Central Thailand).

In Surat Thani's farm, animals from the 3 species were examined for clinical signs and sampled for blood several times before and after treatment, especially horses; *T. evansi* infection was diagnosed through parasitological, serological and molecular methods. From this follow-up in a mixed farm with 3 host species grazing together, it appeared that mechanical transmission was very quick and effective in horses and bovines. Morbidity and mortality in horses was very high compared to bovines, which exhibited only mild clinical signs and very low mortality (only one cattle died); conversely, goats were almost not infected. Persistence or early relapses of clinical signs 1-2 weeks after treatments of horses demonstrated that diminazen acetate was no longer efficient, while melarsomine hydrochloride could be successfully used. However, not all animals were treated, and most of the animals were found infected again several weeks later. Finally, all infected horses died (11 out of 12).

Other evaluations made in Nakhon Pathom showed the inefficacy of diminazen acetate treatment while with quynapiramine or melarsomine hydrochloride treatment were successful in early stages. However, none of those drugs was efficient if used after the appearance of nervous signs.

Measures taken to eradicate *T. evansi* from an isolated farm are discussed and lessons learnt are highlighted.

Key words: *Trypanosoma evansi*, surra, outbreak, treatment, cattle, horses, goats, Thailand.

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Abstract

On mainland Spain, *T. evansi* was diagnosed in Alicante province in an equine and camel farm in 2008; the animals were treated twice (Cymelarsan®, Merial France, at 0.5 mg/kg b.w.) and periodically evaluated by serology (CATT/*T. evansi*), parasitology (Woo, blood smear examination) and molecular procedure (PCR). Six years later, after consistent negative results, the Regional Valencian Government declared the outbreak as eventually eradicated, although periodical blood examinations have been proposed for 5 more years in order to know the real status of the disease through time.

On the Canary Islands, surra was diagnosed by the first time in 1997 in a male dromedary camel imported from Mauritania. The disease was controlled and eradicated by culling or treating the animals after that first description. Currently, surra is absent in all the Islands except in a small camel farm, whose animals have shown antibodies, although seroconversion is expected to be occurred in the future. Official Vet Services of the Regional Canary Government are carrying out the evaluations nowadays.

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Conclusions of an episode of surra in dromedary camels in Aveyron (France, 2006-2007/2013)

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Abstract

A short outbreak of surra, due to *Trypanosoma evansi*, occurred in autumn 2006 shortly after the importation of dromedary camels from the Canary Islands into a farm of the Aveyron department (France), which already kept a small herd of camels for demonstrations and tourism. The Canary Islands were surra-infected some years ago by unduly imported dromedary camels originating from West-Africa. A first case of surra was suspected in a clinically sick imported animal after microscopic examination of Trypanozoon parasites on a blood smear. The whole herd was then investigated with parasitological (including mouse inoculation for parasite isolation), serological and molecular tests. Five animals were found positive by at least one test, among them some already living camels in the farm, which proved the local transmission of the parasite by biting insects acting as vectors (stomoxes were abundant on the farm). The control measures were based on the isolation of the farm, the treatment of all camels and the monthly monitoring with the set of internationally recommended specific diagnostic tests which all became progressively negative (in about 4 months). One animal presented a relapse 7 months after being negative to all tests. Following this event, all camels were treated with double doses of quinapyramine, and then melarsomine. The hypothesis of relapse was favored over a new infection from a reservoir, since no more case was noticed during the following monitoring which lasted four years, and a careful retrospective checking of the data indicated that this animal had received under dose of initial treatment. However, an animal, borderline for ELISA test and originating from the Canary Islands, was euthanized in 2013 following an administrative decision considering that the ELISA-VSG proceeded in an OIE surra reference laboratory (Anvers, Belgium) being positive, even slightly, the French regulations had to be put in force to obtain the disease free status of the infected farm and of the country.

This episode and associated control measures bring several lessons: (i) a lack of specific EU regulations regarding surra control; (ii) the difficulty to evaluate the evolution of *T. evansi* within an infected animal which can be a model to other trypanosomes of the subgenus *Trypanozoon*; (iii) The use of a specific drug the dose of which has to be rigorously adapted to the animal weight; (iv) The quality of the various available ELISA and other tests to evaluate the actual status of an animal.

A review on camel trypanosomosis due to *Trypanosoma evansi* in Mauritania

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Abstract

Camel trypanosomosis due to *Trypanosoma evansi* is transmitted mechanically by hematophagous diptera such as tabanids, stomoxes and hippoboscids. The acute form causes generalised weakness, abortions and mortality in 10 days to 4 months. The chronic form develops in 80% of the infections and is characterised by abortions, reduction in milk production, progressive weight loss, cachexia, etc.

T. evansi is widely distributed throughout the world as well in temperate and warm as in wet, arid and semi-arid climate. This is why the disease for which it is responsible, received various local names: *surra*, *tabourit*, *mal de caderas* etc.

Our extensive studies showed that *T. evansi* is generally present in Mauritania and that the disease is well known by stock breeders. Depending on the year and region, parasitological prevalence, assessed by examination of blood smears, varies from 1,1 to 17,6% compared to 13 to 58,8% seroprevalence in CATT/*T.evansi*, with Trarza being the most infested area. Potential vectors of *T. evansi* are Tabanidae (*Atylotus agrestis*, *Tabanus taeniola* and *T. sufis*), Stomoxynae (*Haematobia minuta* and *H. irritans*) and Hippoboscidae (*H. camelina* and *H. variegata*). Treatment of surra with Cymelarsan yielded spectacular results.

In view of climate changes and changing breeding practices in response to increasing demands for dromedary meat and milk, we conducted a study at the slaughter houses of Nouakchott using some newer diagnostic tools. This survey showed that 14,2% of the tested animals were positive in CATT but no animal was positive in buffy coat examination. None of the CATT seropositives was positive in RT-PCR, suggesting that they were intensively treated with trypanocides. Investigations in pharmacies and veterinary stores revealed an impressive number of trypanocides on the market in Mauritania. Some drug brand names are well known, others are not commonly known. Based on their aspect and sealing, some appear counterfeit.

All these results were discussed in relation to dromedary breeding practices in Mauritania, the agro-ecological environment, the camel breeders policy to choose which dromedaries to slaughter, the use of trypanocides, etc.

Key words: Mauritania, trypanosomosis, *T. evansi*, dromedary camel , mechanical vector, diagnosis, epidemiology, trypanocide.

Clinical symptoms in naturally infected dourine horses and treatment efficacy

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Abstract

Dourine is caused by *Trypanosoma equiperdium* which transmits through only reproductive pathway and the mortality rate in untreated cases is estimated to be 50-70%. In Mongolia, outbreaks of Dourine in horses have reported every year which caused by *T. equiperdium*.

During November 2013 to January 2014, the first outbreak of dourine occurred on an equestrian farm in Ulziit subdistrict, Ulaanbaatar city and second outbreak occurred in during middle of June of 2014 in Baga-khangai district of Tuv Province of Mongolia.

In 2015-2016, total of 43 outbreak of dourine documented in 12 provinces such as Dundgovi, Govi-altai, Umnugovi, Khentii, Arkhangai, Orkhon, Selenge, Khuvsgul, Tuv, Sukhbaatar, Dornod and Ulaanbaatar city.

Total of 20 horses suspected with dourine were examined by clinical, parasitological, serological analyses and DNA based techniques.

In-coordination of hind legs (6; 30%), unilateral facial paralysis as nervous form (7; 35 %), swelling of external genitalia (13; 65%) emaciation (18; 90%) and abortions in mares (7; 100 %) were observed. In the horses with swelling of external genitalia, trypanosomes were detected in swab from genital organs by Giemsa stain but no parasite was detected in blood smears and other body fluids. Clinical symptoms were significantly correlated with strong positive serological reaction by ICT and ELISA. Genital organ washes were examined by PCR using IST1 and KIN *Trypanosoma* specific primers, and results were positive. *T. equiperdium* infection is highly prevalent in horses of Mongolia by parasite detection, DNA-based PCR analysis and serological tests. In order to assess treatment efficacy, all horses showing clinical signs were treated by Cymelarsan and Diminazene diacetate (Demin). After the treatment, horses recovered from the clinical symptoms. Overall body condition was improved and clinical signs such as unilateral facial paralysis, weakness and ventral edema disappeared within 20-30 days after the treatment.

Key words: Outbreak, case, clinical symptom, diagnosis, therapeutic intervention.

Comparative clinico-pathological observations in young Zebu (*Bos indicus*) cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse areas of Northwest Ethiopia

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Abstract

The northwest region of Ethiopia is affected by both tsetse and non-tsetse transmitted trypanosomosis with a huge impact on livestock productivity. The objective of this experimental study was to determine clinical and pathological findings in young Zebu cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. A total of 18 animals divided into three groups of six animals were used. Animals in the first two groups (Group TT: tsetse infested and Group NT: non-tsetse infested) received 2 mL of infected blood from donor animals at 10^6 trypanosomes/mL, and the remaining group was non-infected control (NIC). Each group was observed for a period of eight consecutive weeks, daily for clinical signs and once per week for parasitaemia. Postmortem examinations were done on euthanized animals, and tissue samples were taken for histopathological analysis.

The prepatent period of the disease was earlier in the NT group 6 days post infection (dpi) than TT group 12 dpi. The infection was characterized by intermittent pyrexia and parasitaemia, enlarged lymph nodes, lacrimation, reduced feed intake and emaciation. Less frequently diarrhea, oedema and nervous signs were observed in both groups of infected animals. At necropsy, infected animals showed enlarged spleen, enlarged lymph nodes, pneumonic and emphysematous lung, enlarged liver, and haemorrhages on the brain and intestine. Histopathological analysis revealed lymphoid hyperplasia of the spleen, necrosis of the liver, encephalitis and hyperplasia of lymph nodes.

Trypanosoma vivax isolates from both tsetse infested and non-tsetse areas showed a variety of virulence factors leading to the development of acute clinical signs, gross and histopathological lesions. However, the parasitaemia and clinical signs appeared earlier in the NT compared to TT groups.

Keywords: Cattle; Clinical findings; Gross lesions; Histopathological lesions; *Trypanosoma vivax*; Northwest Ethiopia

Cytokines responses during *Trypanosoma evansi* infections of high and low virulence isolate from Indonesia in DDY mice

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Abstract

Trypanosoma evansi is a pathogenic extracellular blood protozoan that cause Surra disease which affects several animal spesies. Disease progression and the clinical, hematological and pathological aspects in the host can vary according to the strain's virulence, host susceptibility and epizootic conditions. It is known that different virulence of *T. evansi* display differential susceptibilities, parasitemia pattern and prepatency periodes to the same mouse strain. Unlike other trypanosomosis, information about the immunological mechanisms playing a role in *T. evansi* infection is limited. It is still not known the pattern of cytokine level of different virulence *T. evansi* isolate in mice. There is no data regarding the serum levels of proinflammatory cytokines (IFN- γ , TNF- α) and antiinflammatory cytokines (IL-10) in infected animals. The aim of this study was to measure the levels of these cytokines in the serum of mice experimentally infected with high (Bang87 isolate) and low virulence (Pml287 isolate) *Trypanosoma evansi* from Indonesia. Initially, 20 DDY mice divided into two group (10 animals/group): Group A-high virulence (represented by Bang87 isolate) and Group B-low virulence(represented by Pml287 isolate). Each groups were intraperitoneally inoculated with cryopreserved blood containing 10^4 trypomastigotes/0,3 mL blood per animal with Bang87 and Pml 287 *T. evansi* isolate respectively. Ten animals (group C) were used as negative controls and received 0.3 mL of saline by the same route. Observations were carried out every two days (level of parasitaemia) and four days on the hematokrit and cytokine levels. The blood samples were collected from tail peripheral blood to perform hematokrit, degree of parasitaemia and the determination of IFN- γ , TNF- α , and IL-10 levels using an ELISA quantitative sandwich. Infected mice with *T. evansi* showed anemia during the experimental period marked by reduced hematokrit value (27,1%) at 4 day post infection Bang87 isolate and 31,2-50,7% at 8-24 day post infection with Pml287 isolate. These value significantly different compared to the controls ($P < 0,05$). IFN- γ levels increased significantly in mice infected with Bang 87 isolate on 4th day post infection (dpi) having a significant negative correlation ($p < 0,05$) with increased IL-10 levels, whereas in mice infected by PML 287 isolate, IFN- γ levels were positively correlated with IL- 10 levels. Early death in mice infected with Bang87 isolates was caused by systemic inflammatory response syndrome (SIRS).

Key words: *Trypanosoma evansi*, IFN- γ , TNF- α , IL-10, mice.

Recombinant antigens in serological testing for *Trypanosoma evansi* infection.

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Abstract

The need for inexpensive, user friendly tests are necessary for the detection of all forms of Surra. The production of antigens by a recombinant system such as *Leishmania tarentolae* can provide a reproducible source of materials. Recombinant Invariant glycoprotein (ISG) from *T.evansi* was purified and tested against a panel of sera from Moroccan camels. Results on sensitivity and specificity will be presented.

Multiplex detection of camel antibodies to *Trypanosoma evansi* and transcriptome analysis of two type B strains

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Abstract

Current diagnostic tests for *Trypanosoma evansi* suffer from a variety of imperfections. A robust serological assay is needed for surveillance and disease studies and for management of infection. We have developed an ELISA-type multiplex test for simultaneous detection of camel antibodies to four recombinant proteins expressed by *T. evansi* type A. We have compared results with CATT, parasite detection and immune trypanolysis assays on samples from the OIE Reference Lab for Surra, ITM, Antwerp. We have applied the test to approximately 400 field samples from Saudi Arabia. RNA from two type B strains of *T. evansi* was sequenced.

Serum samples were screened by indirect chemiluminescence against non-glycosylated recombinant proteins arrayed as discrete spots in microtitre plates. Antigens used were Variable Surface Glycoprotein RoTat1.2 (VSG), Invariant Surface Glycoprotein 75 (ISG75), Calpain tandem repeat domain (GM6) and Oligopeptidase B (Oligo). OIE reference sera, 'true positive' (TP, n=55) or 'true negative' (TN, n=49), as determined by CATT and/or immune trypanolysis and/or blood parasite detection, were used to establish initial multiplex conditions. RNA from two *T. evansi* type B strains (KETRI 2479 from Kenya, ref 1; MU10 from Ethiopia, ref 2) was sequenced to identify conserved and expressed genes.

VSG, ISG75 and GM6 were used for the analysis. Oligopeptidase B did not 'spot' properly making it impossible to discriminate TP and TN sera. Of 55 TP sera 50 recognised one or more of the antigens, whereas 5 sera did not recognise any. Of 49 TN sera 2 recognised at least one of the antigens, with one recognising three antigens. Kappa statistics showed 'very good' agreement between the multiplex and the OIE panel of tests ($\kappa=0.865$, SE 0.049, 95%CI 0.769-0.962). Further samples (serum and milk) will be screened from Gran Canaria which is free to *T. evansi* (n=100). An optimised panel of antigens will be used to screen 400 camel sera from farms in the Riyadh area of Saudi Arabia. These further results will be reported at the meeting. We expect to have type B sequence data to report too. Initial studies have shown that GM6 and Oligo are highly conserved between type A and B strains, but the situation with ISG75 is more complicated.

The *T. evansi* multiplex showed very good agreement with OIE results on TP and TN samples. The discrepant samples will be analysed further to try and determine which test is most likely correct. Sequencing of type B strain RNA will be reported to determine if the genes for the diagnostic targets are expressed and therefore are possible diagnostic targets. Further work is needed to optimise the Oligopeptidase B antigen to this test.

The *T. evansi* multiplex ELISA offers an efficient, cost effective means to detect infection at herd and individual level, to categorise herds according to likely infection risk, and to aid the targeting of treatment and control measures. The multiplex test format is suitable for serum and milk and could provide an effective tool for disease surveillance and management schemes.

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Development of a rapid antibody test for point-of-care diagnosis of animal African trypanosomosis

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Abstract

Trypanosoma congolense and *T. vivax* are the main causative agents of animal African trypanosomosis (AAT), a disease which hinders livestock production throughout sub-Saharan Africa and in some parts of South America. Although two trypanocidal drugs are currently available, the level of treatment is low due to the difficulty in diagnosing the disease in the field. The major clinical signs of AAT such as anaemia, weight loss, and infertility, are common to several other endemic livestock diseases. Current diagnostic methods, based on the visualization of the parasite in the blood, or on the detection of its DNA or the antibodies it triggers in the host, are not suitable for direct use in the field as they require specialized equipment and personnel. Thus, we developed a quick-format diagnostic test (15 min) based on the recombinant TcoCB and TvGM6 antigens for detection of *T. congolense* and *T. vivax*, respectively, aimed at providing farmers and veterinarians in the field with the means to conduct a quick diagnosis. The prototype was evaluated using both sera from experimentally infected cattle and fresh blood from animal under current experimentation. The test, which includes both antigens, shows a specificity of 95.9 % and a sensitivity of 92.0 % for *T. congolense* and 98.2 % for *T. vivax*. The high levels of sensitivity and specificity of this rapid test, the possibility of using directly whole blood, and the ease of interpreting the result, all contribute to make of this test a valuable candidate to contribute to the control of AAT in the field.

Updated results on atypical human trypanosomoses caused by animal trypanosomes

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Abstract

There are only two classical human forms of trypanosomoses, they are sleeping sickness in Africa (*Trypanosoma brucei* spp.) and Chagas' disease (*T. cruzi*) mainly in South America respectively. Other trypanosomes can infect a wide range of wild and domestic animals (fish, reptile, amphibians, mammals including cattle), but they are not supposed to be infective to human beings. However, several human cases infected by animal trypanosomes have been recently reported, in particular *Trypanosoma lewisi* (a *Rattus* trypanosome usually transmitted by fleas), and *T. evansi* (found for instance in cattle, camels, and mechanically transmitted by blood sucking insects such as tabanids or stomoxes).

High density lipoprotein (HDL) in normal human serum (NHS) contains several compounds (e.g. ApoL-1) which protect us against African trypanosomes. The Indian patient infected with *T. evansi* reported in 2005 because of a genetic deletion was confirmed in the ApoL-1 gene in this patient, while another naturally *T. evansi* infected patient reported in Viet Nam in 2015 had a normal ApoL-1. The mode of transmission suspected in both cases was direct contamination via a wound while butchering raw beef. Both patients were cured successfully by using suramine, a drug for the acute form of sleeping sickness.

Human infected with *T. lewisi* was mainly reported in babies. Although most of cases were transient infections, other required treatment or died. A recent case died from *T. lewisi* infection in India in 2015. It has been demonstrated that this parasite is resistant to NHS. Thus, *T. lewisi* is potentially a human pathogen or zoonotic pathogen. We present the new cases either described or suspected since the 2012, and previous cases as well (including infection by *T. b. brucei*, *T. congolense*). The problem of diagnosis and treatment will be considered, and the potential risk of emergence of a new zoonotic disease will be discussed.

Atypical human infections by animal trypanosomes: evaluation of human and animal trypanocidal drugs against *Trypanosoma lewisi* in Wistar rats

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Abstract

Trypanosomosis is a disease of medical and veterinary importance, mainly distributed in tropical areas of Africa, Latin America and Asia. Some *Trypanosoma* species are typically pathogenic for animals, such as *Trypanosoma vivax*, *T. congolense*, *T. evansi* etc, and others are zoonotic, such as the agents of sleeping sickness in Africa (*Trypanosoma brucei* ssp.), or Chagas disease in Latin America (*T. cruzi*). Beside these 2 “typical” human trypanosomes, there is a growing number of reported “atypical” human infections due to *Trypanosoma evansi*, a livestock parasite, or *Trypanosoma lewisi*, a rat commensal, especially in Asia. Drugs available for the treatment of *T. brucei* ssp in humans are obviously of choice for the control of *T. evansi* because it is derived from *T. brucei* lineage; indeed, in 2 recent cases of human infection by *T. evansi*, successful treatments were obtained using suramine. However, concerning *T. lewisi*, there is a need to determine the efficacy of trypanocidal drugs for the treatment in humans. In a recent study, pentamidine and fexinidazole were shown to have the best efficacy against one stock of *T. lewisi* in rats, they have thus been explored amongst others.

In order to explore efficient trypanocidal drugs, attempts were made to treat groups of 3 rats experimentally infected by *T. lewisi*, using low and high doses of the available human and veterinary trypanocidal drugs: diminazen acetate (DA; 14 and 28 mg/kg), isometamidium chloride (IMC; 2 and 4 mg/kg), quinapyramine sulfate and chloride (QSC; 8.3 and 16.6 mg/kg), cymelarsan (Cym; 0.5 and 1 mg/kg), suramine (20 and 40 mg/kg), pentamidine diisetonate (Pt; 8 and 16 mg/kg), eflornitine hydrochloride (Efl; 800 and 16000 mg/kg), nifurtimox (Nt; 30 and 60mg/kg), benznidazole (Bz; 20 and 40 mg/kg) and fexinidazole (Fex; 200 mg/kg). At the exception of Nt, Bz and Fex which were administered peroral route, all drugs were intramuscularly injected. All treatments at all doses failed to clear parasites from rat’s blood.

To confirm the potential efficacy of fexinidazole, a mixed infection protocol was set up in cyclophosphamide immunosuppressed rats. Animals were infected successively by *T. lewisi* and *T. evansi*, and received 10 daily peroral administrations of 200 mg/kg fexinidazole or 0.5 mg/kg Cym. *T. evansi* was cleared from the rat’s blood within 24 to 48 hours; however, the treatment did not affect *T. lewisi* which remained in high number in the blood until the end of the experiment. Results are discussed and further studies suggested. Because of its potential as an emerging parasite in humans, identifying efficient trypanocides against *T. lewisi* is required.

Key words: *Trypanosoma lewisi*, trypanocidal drugs, fexinidazole, melarsomine hydrochloride, rats, *Trypanosoma evansi*.

Poster Abstracts

Adaptation of an antibody-ELISA for *Trypanosoma evansi* infection (surra) in buffaloes and its application to a serological survey in Thailand

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Abstract

Surra, caused by *Trypanosoma evansi*, is a neglected disease due to frequent subclinical evolution, especially in bovines in Asia. However, acute and chronic signs are regularly observed, with significant sanitary and economic impacts. In this study, we standardized and applied an antibody-ELISA test for the detection of anti-*T. evansi* immunoglobulin G in buffaloes using anti-bovine conjugate. Based on buffalo reference sera from the Philippines, a TG-ROC analysis was conducted to define an optimal cut-off value; sensitivity and specificity were estimated at 92.5% and 94.2%, respectively. A cross-sectional serological survey was carried out in the major buffalo breeding areas of Thailand; 892 buffaloes from 8 provinces were sampled in North, North-Eastern and Southern Thailand. Seropositive buffaloes were found in all 8 provinces, on 20.3% of farms for an overall prevalence of 12.2% (95%CI: 10.2-14.5%). Nearly one third of the sampled population was exposed to infection. Broader sampling would be necessary but is not possible in the southern half-wild breeding systems. The impact on livestock systems and husbandry practices is discussed. According to our results, buffaloes may constitute a large and robust reservoir for *T. evansi*, which is a permanent threat to other livestock such as cattle, and horses as well as wild animals such as elephants in South-East Asia.

Keywords: *Trypanosoma evansi*, buffalo, ELISA *T. evansi*, Thailand.

Potential of the oriental house rat (*Rattus tanezumi*) to act as reservoir of *Trypanosoma evansi* in Thailand?

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Abstract

Trypanosoma evansi (Steel, 1885) Balbiani, 1888, is a protozoan blood parasite and etiologic agent of “surra,” a disease affecting a wide range of domestic and wild mammals, some identified as potential reservoirs. Although *T. evansi* has been detected in several small wild rodent species, their role in the epidemiology of surra is unclear. There is molecular evidence of *T. evansi* in wild rodents in Asia, but it is not known whether they can carry the parasite for sufficient time to significantly contribute to the epidemiology of surra. We assessed the receptivity and susceptibility of the Oriental house rat (OHR; *Rattus tanezumi*) to *T. evansi* infection. Five adult male OHRs trapped in Bangkok district, Bangkok, Thailand, and five laboratory Wistar rats (*Rattus norvegicus*), as positive controls, were experimentally infected with a local strain of *T. evansi*. The five controls and three of the five OHRs were highly susceptible and rapidly exhibited high levels of parasitemia as usually observed in Wistar rats. They died or were euthanized just prior to expected death. Two OHRs presented fluctuating levels of parasitemia, without obvious clinical signs, throughout 40 days of monitoring. These results highlight the moderate susceptibility of some OHRs and their ability to carry the infection for a significant period of time. Along with the molecular evidence of *T. evansi* in captured OHRs (demonstrated elsewhere), our results bring new information on the potential role of OHRs in the complex epidemiology of surra.

Keywords: Experimental infection, *Rattus tanezumi*, reservoir, surra.

Tabanids: morphology, biology, direct effects and pathogen transmission

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Abstract

Tabanids are nuisance pests for humans and livestock because of their painful bite and persistent biting behavior. About 4,400 tabanid species have been described; they are seasonally present in all kinds of landscapes, latitudes, and altitudes. Thus, high populations of tabanids can have serious economic impact on outdoor activities, tourism and agriculture. Tabanids have so far received little attention and can then be considered as neglected subject of research. This poster tends to show that they are important vectors of disease agents and provide a brief summary of tabanid morphology, biology, and life cycle. Tabanids are also vectors of animal disease agents, including viruses, bacteria and parasites such as equine infectious anemia virus, *Bacillus anthracis* or *Trypanosoma evansi* responsible of surra. Direct annoyance and stress generated by tabanids are also responsible of immunosuppressive effects which increase the effect of inter-current diseases in their hosts.

Keywords: Tabanid, horse fly, deer fly, cleg, pathogens, livestock, direct effects, mechanical transmission.

Wild rodents as potential reservoirs of *Trypanosoma* spp. in Southeast Asia: a link towards human infections ?

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Abstract

The present study investigated the molecular prevalence of *Trypanosoma lewisi* and *Trypanosoma evansi* in wild rodents from Cambodia, Lao PDR and Thailand. Between 2008 and 2011, rodents were trapped in 10 localities and 584 of them were tested using three sets of primers: TRYP1 (amplifying ITS1 of ribosomal DNA of all trypanosomes), TBR (amplifying satellite genomic DNA of *Trypanozoon* parasites) and LEW (amplifying ITS1 of ribosomal DNA of *Trypanosoma lewisi*). Based on the size of the PCR products using TRYP1, 10% were positive for *Trypanosoma lewisi* and 2.6% positive for *Trypanozoon*. Results were confirmed by sequencing PCR products and by using more specific primers (LEW and TBR). The specificity of TRYP1 primers however failed as rodent DNA was amplified in some instances. Using LEW, the positive samples for *Trypanosoma lewisi* were confirmed both by PCR and sequencing. In Thailand, *T. lewisi* was found in *Rattus tanezumi*, *R. exulans* and *Berylmys*; in Lao PDR, in *R. tanezumi* and *R. exulans* and in Cambodia in *R. tanezumi*, *R. exulans* and *R. norvegicus*. Using TBR, the positive samples for *Trypanozoon* were confirmed by sequencing; as *T. evansi* is the only species of the *Trypanozoon* sub-genus possibly present in Asian rodents. These results confirmed its presence in rodents from Thailand (*R. tanezumi*), Lao PDR (*R. tanezumi* and *R. nitidus*) and Cambodia (*R. tanezumi*, *Niviventer fulvescens* and *Maxomys surifer*). We tested how habitat structure affects the infection of common murine rodents, inhabiting human-dominated landscapes in South East Asia, by *Trypanosoma* species. For this, we used geo-reference data of rodents investigated for *Trypanosoma* infection and land covers developed for seven sites in Thailand, Cambodia and Lao PDR. Infection by *T. lewisi* was found in rodents living near human settlement and in areas with high cover of built-up habitat. Increased patchiness and high cover of rain-fed agriculture lands were the likely habitat explaining the infection of rodents by *T. evansi*. These results suggest a likely role of wild rodents as reservoir and possible source of atypical human infection by animal trypanosomes.

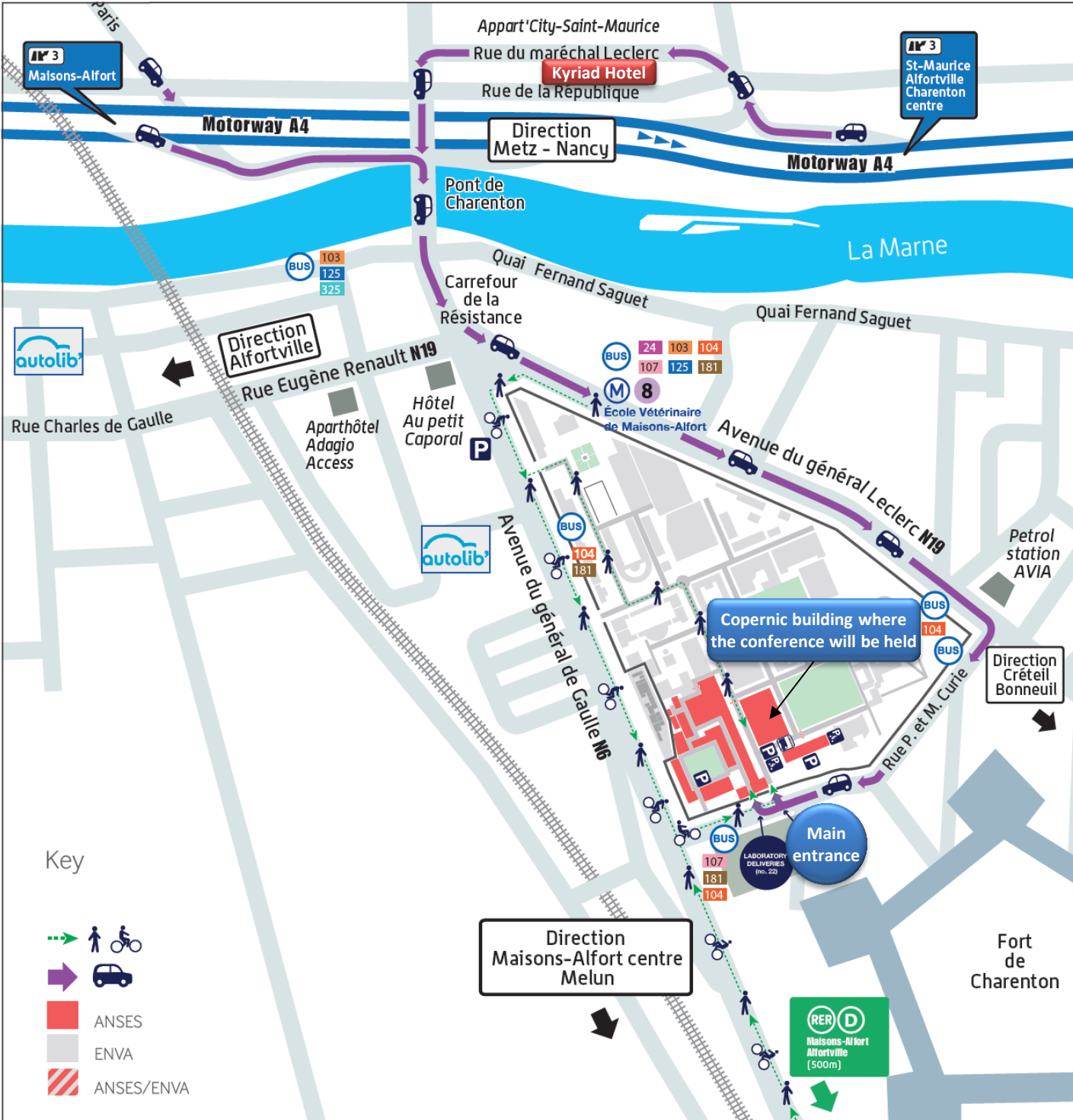
Keywords: *Trypanosoma evansi*, *Trypanosoma lewisi*, PCR, wild rodents, habitat, land covers, Southeast Asia, human settlement.

General information

New address:
 14 rue Pierre et Marie Curie
 94700 Maisons-Alfort

- > Visitor access (pedestrians, 2-wheelers and cars):
 main entrance, 14 rue Pierre et Marie Curie
- > Deliveries: main entrance,
 14 rue Pierre et Marie Curie
- > Deliveries to the laboratories: 22 rue Pierre et Marie Curie
- > Autolib stations:
 - 16 Rue Charles de Gaulle, Alfortville
 - 42 avenue du Général de Gaulle, Maisons-Alfort

Visitors' map



Dinner Thursday 15 December 2016

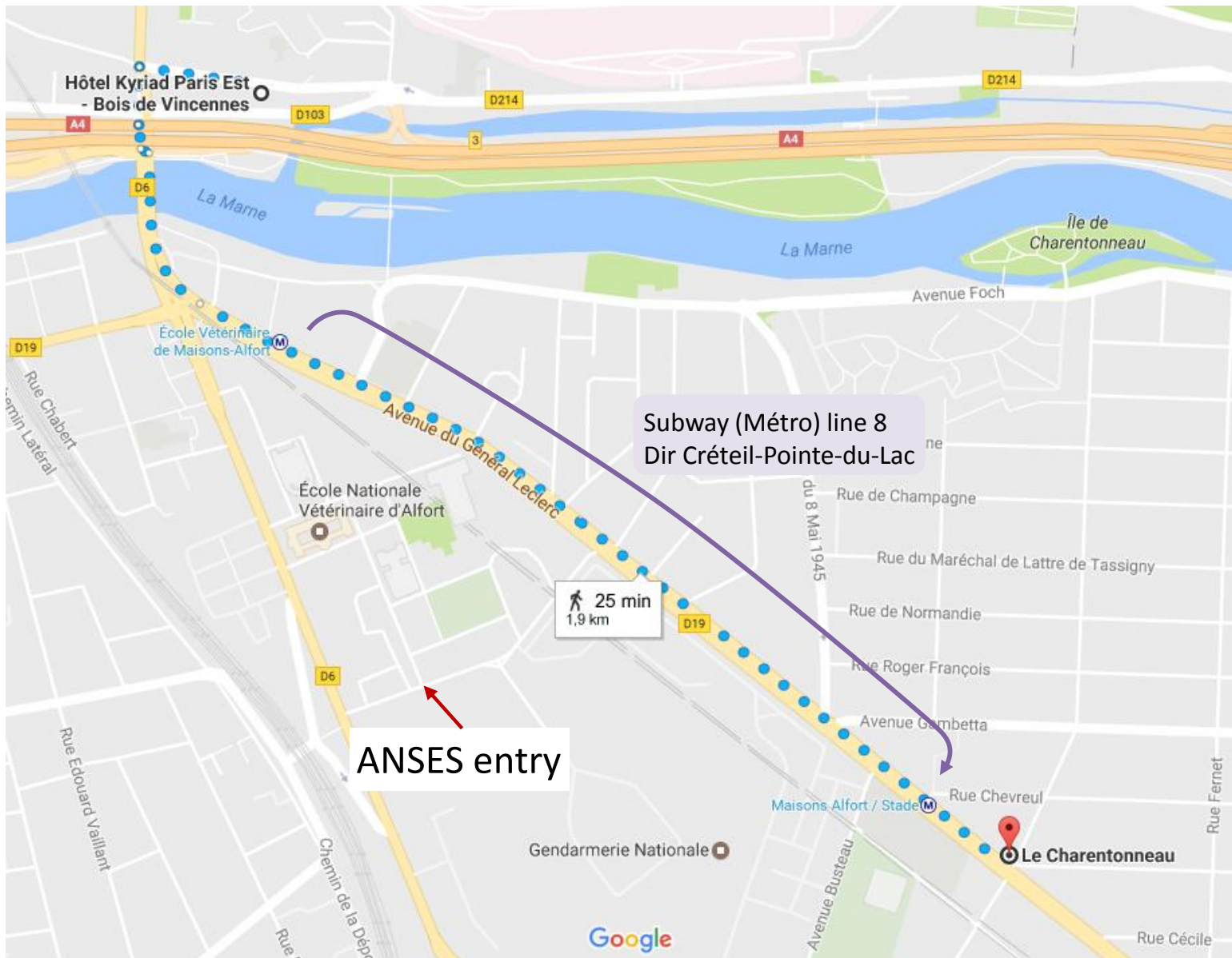
Restaurant *Le Charentonneau*

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Starter, main course, dessert and coffee + mineral water or wine for 40 €

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