OIE NTTAT Network

2nd International Conference on

Non Tsetse Transmitted Animal Trypanosomosis

18-19 December 2017

Institute of Tropical Medicine
Nationalestraat 155, 2000 Antwerp, Belgium

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2nd International Conference on Non Tsetse Transmitted Animal Trypanosomosis

Monday 18 December 2017

9 h 00 **Registration of participants and poster display**

9 h 45 Welcoming by Philippe BÜSCHER

10 h 00 The global distribution, host range and prevalence of *Trypanosoma evansi* causing surra: a systematic review and meta-analysis
Weldegebrial AREGAWI - Ethiopian Institute of Agricultural Research, Ethiopia

10 h 20 Bovine trypanosomosis in the Americas, with special emphasis on Ecuador
Armando REYNA BELLO - Universidad de las Fuerzas Armadas ESPE, Ecuador

10 h 40 Investigation of camel trypanosomosis (surra) in Egypt
Katerina DOLECKOVA - Keele University, United Kingdom

11 h 00 **Coffee break**

11 h 40 The diversity of trypanosomes and potential vectors outside the tsetse belt
Merid GETAHUN - International Centre of Insect Physiology and Ecology, Kenya

12 h 00 The prevalence of *Trypanosoma evansi* in camels (*Camelus dromedarius*) in South Darfur State, Sudan
Adel MAKI - University of Nyala, Sudan

12 h 20 Field investigation on *Trypanosoma evansi* in camels and horses from the Districts of Lahore, Kasur and Gujranwala in Punjab, Pakistan
Sonia TEHSEEN - Syed Chiragh Shah Degree College, Pakistan

12 h 40 Epidemiological investigations of *Trypanosoma evansi* infection in dromedary camels in the South of Algeria
Djamila BOUSHAKI - Ministère de l'Agriculture, du Développement Rural et de la Pêche, Algeria

13 h 00 **Lunch**

14 h 00 Evolution of *Trypanosoma evansi* and *T. equiperdum*: how, and how often?
Achim SCHNAUFER - The University of Glasgow, United Kingdom

14 h 30 Some lessons from the South: molecular and virulence studies on Venezuelan *T. evansi* and *T. equiperdum* strains
Maria Isabel GONZATTI - Universidad Simón Bolívar, Venezuela

15 h 00 **Coffee break**

15 h 40 Characterisation of newly isolated and culture adapted *Trypanosoma equiperdum*
Keisuke SUGANUMA - Obihiro University of Agriculture and Veterinary Medicine

16 h 00 Outbreaks of dourine in Mongolia and development of a therapeutic strategy
Banzragh BATTUR

16 h 20 Poster discussion

19 h 00 **Get-Together Dinner**

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Tuesday 19 December 2017

10 h 00 Resistance of recently isolated *Trypanosoma vivax* and *T. congolense* against diminazene diaceturate and isometamidium chloride hydrochloride using an infection model of trypanosomosis in cattle in Ethiopia
Fikru REGASSA - University of Addis Ababa, Ethiopia

10 h 20 Diversity of aquaglyceroporin genes (*TbAQP*) in *Trypanozoon* and their relation to melarsoprol and pentamidine cross-resistance
Nick VAN REET - Institute of Tropical Medicine Antwerp, Belgium

10 h 40 Comparing *in vitro* sensitivity and uptake of suramin, pentamidine and diminazene in *T. brucei*, *T. evansi* and *T. congolense*
Harry DE KONING - University of Glasgow, United Kingdom

11 h 00 **Coffee break**

11 h 40 Assessment of alternative diagnostic techniques for detection of *Trypanosoma equiperdum* infections in horses and donkeys in South Africa
Oriel THEKISOE - North-West University, South Africa

12 h 00 Recent developments in the diagnosis of NTTAT infections in domestic animals
Philippe BÜSCHER - Institute of Tropical Medicine Antwerp

12 h 20 Multiplex detection of antibodies to *Trypanosoma evansi*
Neil WATT - MV Diagnostics Ltd, United Kingdom

12 h 40 Dourine in Mongolia: isolation of *Trypanosoma equiperdum*
Badgar BATTSETSEG - Mongolian University of Life Sciences, Mongolia

13 h 00 **Lunch**

14 h 00 Purification of *Trypanosoma equiperdum* spiked equine semen by single layer centrifugation
Ahmed Yasine EBRAHIM - Wollo University, Ethiopia

14 h 20 Activities of the World Organisation for Animal Health (OIE) in support on the surveillance and control of non tsetse transmitted animal trypanosomosis
François Diaz - World Organisation of Animal Health (OIE), France

14 h 40 Problems to adapt coordinated methods for the control of NTTAT in the world
Louis TOURATIER, France

15 h 00 General discussion on OIE NTTAT Network and planning of the next conference

15 h 30 Closing of the conference

Talk Abstracts

The global distribution, host range and prevalence of *Trypanosoma evansi* causing surra: a systematic review and meta-analysis

Aregawi Weldegebrial[1,2], Büscher Philippe[2], Agga Getahun[3], Abdi Reta Duguma[4]

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Abstract

In contrast with the tsetse transmitted animal trypanosomoses, surra is severely neglected. A systematic review and meta-analyses (MA) methodologies were used to determine the geographic distribution, to identify the naturally susceptible domestic and wild animals and to critically estimate the prevalence of *Trypanosoma evansi* in the major mal host species. Four electronic databases, CAB Direct, EDS-ITM, PubMed and ScienceDirect were screened for relevant publications.
A total of 274 publicatons were included for qualitative analysis and 166 were used for meta-analysis. *T. evansi* was reported from 48 countries, seventeen 17 in Africa, seven in Southern America, twenty in Asia and four Europe. Rare outbreaks of surra in Europe were associated with import of infected dromedary camels while in the other continents the disease is endemic. Since 2009, 27 countries reported the presence of surra at least once to OIE. Seven of these countries were not represented in the publications retrieved for this review study. *T. evansi* was detected in almost all domestic mammals. It was mainly reported from camels in Africa and the Middle East. In East and South East Asia, buffaloes, cattle, dogs and horses were most affected. In South America, the acute form of the disease was reported in horses and dogs, while in bovine, surra is more chronic. A large range of susceptible wild animals was reported from South America and Asia. High prevalences of *T. evansi* were reported in capybara (*Hydrochoerus hydrochaeris*) and coatis (*Nasua nasua*). Outbreaks of surra were also reported in some endangered wild animals in Asia. Three confirmed human cases of *T. evansi* infection were reported recently in India and Vietnam.
The estimated pooled prevalence of *T. evansi* differed among the different diagnostic tests and host species. Generally, a higher prevalence was observed by serology and PCR. Host-wise, prevalence was relatively higher in camels followed by buffalo and cattle.
In conclusion, the systematic review in this study confirmed the wide geographic distribution and very large host range of *T. evansi* where it can naturally parasitise almost all domestic mammals and many wild hosts and even human. There is a need to develop, support and implement worldwide projects for the control and surveillance of *T. evansi*.

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Bovine trypanosomosis in the Americas, with special emphasis on Ecuador

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Abstract

Due to the appropriate climatic conditions, which allows the presence of the vectors, bovine trypanosomosis affects extensive regions of Africa, Asia and Latin America. The tsetse fly and the horsefly transmit the bovine trypanosomes in Africa. In Latin America however the hemoflagellates have been adapted almost exclusively to the horsefly (*Stomoxys calcitrans*), even losing their genes for survival in the genus *Glossina*. Cattle herds in Latin America are infected with *T. theileri*, *T. evansi* and *T. vivax*, with the last one the most pathogenic of three. *T. evansi* causes only mild disease and no clinical signs are associated with the infection of *T. theileri*. Countries with sound epidemiological information on bovine trypanosomosis in Latin America are Brazil, Colombia and Venezuela, demonstrating the presence of three species of trypanosomes in cattle with a prevalence ranging from 20 to 33%. In Peru and Bolivia the presence of *T. vivax* and *T. evansi* has been demonstrated in cattle with a reported prevalence of <10%. For Ecuador, studies on trypanosomosis are incipient. A preliminary study carried out in a slaughterhouse in Quito on 152 bovines showed 30% of the cattle positive for *T. vivax* by PCR. In another study with five blood samples from the same slaughterhouse and cloning and sequencing of the ITS region, for the first time the presence of *T. theileri* in Ecuador was shown. In a larger sample of 400 animals carried out in two cattle herds in a tropical region of the country (at the pacific coast), a seroprevalence of respectively 48 and 70% was found for *T. evansi*. In the central region of the country (lowlands), in a sample of 246 animals, 48% of the bovines were positive for an ITS-PCR; 1.9% having an amplicon compatible in size with *T. vivax*, 8.3% with *T. evansi* and 39.8% with *T. theileri*. These results show an extensive distribution of *Trypanosoma spp*. in cattle in Ecuador.

Investigation of camel trypanosomosis (surra) in Egypt

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Abstract

Surra, caused by infection with the parasite *Trypanosoma evansi*, has a huge negative impact on economic development in endemic regions. We have recently started a new project to investigate surra in camels and to initiate new work to identify novel lead compounds against *T. evansi*. Fifty five blood samples of camels have been collected from slaughter houses in Behera province, Egypt. Blood films were performed and examined by microscopy. Only two samples were suspected to be positive for *T. evansi*. We plan to use molecular and cellular techniques to determine disease prevalence in study regions and the extent of drug resistance in Egyptian isolates of *T. evansi*. We will also perform a drug screen to identify compounds and repurposed drugs with the ability to selectively kill *T. evansi* parasites. We will present findings to date and our future plans to improve our understanding of the impact of this disease in Egypt.
This study is funded by a Newton-Mosharafa Fund (British Council/Science and Technology Development Fund, STDF) Institutional Links grant.

The diversity of trypanosomes and potential vectors outside the tsetse belt

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Abstract

Animal trypanosomosis is an infectious disease that retards the productivity of domestic animals in many countries, where tsetse flies are present in Africa, and beyond the tsetse fly belt in other continents where trypanosomes are transmitted mechanically. *Trypanosoma vivax* and *T. evansi* have a worldwide distribution, including North Africa, Asia, Latin America and are reported in Southern Europe. Non-tsetse transmitted trypanosomosis has a significant impact outside the tsetse fly belt on the African continent, and is a major problem to livestock health and productivity. However, the flies responsible for transmission of trypanosomes and the diversity of clinically relevant trypanosomes both in biting flies and domestic animals outside the tsetse belt is not well studied. We investigated the potential vectors of *T. evansi* and other trypanosomes in areas outside tsetse belt, and found *Hippobosca camelina, Stomoxys calcitrans, Pangonia ruppellii, Tabanus sp.*, *Heamatopota sp*. as potential vectors of *T. evansi* and *T. vivax* based on molecular and microscopic analysis. The diversity and prevalence of trypanosome species varies between biting flies and domestic animals, showing variation in adaptation of pathogen-host interaction. Furthermore, there were complex mixed, dual to triple infections in a single vector (e.g. *H. camelina*) and camels. The blood meal analysis shows that these flies feed on diverse domestic animals. We observed a strong correlation of the prevalence and diversity of trypanosomes in domestic animals and biting flies. The diversity and complexity of trypanosomes in different biting flies and the strong correlation with trypanosomes prevalent in domestic animals demonstrate the potential of these biting flies in transmitting clinically important trypanosomes in the absence of tsetse flies, which are the known biological vectors of African trypanosomes.

The prevalence of *Trypanosoma evansi* in camels (*Camelus dromedarius*) in South Darfur State, Sudan

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Abstract

Trypanosoma evansi infection is considered as the most important disease of camels (*Camelus dromedarius*)in Sudan. The aim of this study was to investigate *T. evansi* infection in dromedary camels, using parasitological, serological and molecular tools. Jugular vein blood samples were randomly collected from 350 camels, during three successive seasons in Nyala area (South Darfur State) to study the prevalence of trypanosomosis. The samples were examined parasitologically for the presence of the trypanosomes by Giemsa stained blood smears (GSBS), serologically for detection of anti-trypanosomal antibodies by the card agglutination test (CATT/*T.evansi*) and molecularly for detection of the *T. evansi* DNA by PCR with *T. brucei* spp specific primers. Out of the 350 samples, 37 (10.6%) were positive in GSBS and 126 (36%) were positive in CATT/*T.evansi*, while 140 (40%) were positive in PCR. The obtained results showed that PCR has a higher sensitivity and specificity (90%), while CATT and GSBS were less sensitive, 69% and 31% respectively. The prevalence was significantly higher in the rainy season than in the dry season.

Field investigation on *Trypanosoma evansi* in camels and horses from the Districts of Lahore, Kasur and Gujranwala in Punjab, Pakistan

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Abstract

Surra is a wasting disease which affects a wide range of hosts in South East Asia. A parasitological (Giemsa thick smear stain, GST) and molecular tests (TBR1/2 PCR and RoTat 1.2 PCR) based investigation was carried out in randomly sampled camels (n = 550) and horses (n = 215) reared by pastoralists in the three districts of Punjab Pakistan viz Lahore, Kasur and Gujranwala, from March 2017 to September 2017. GST positive camels (5%) were detected in Gujranwala only. Based on both molecular tests, significantly ($P \leq 0.05$) higher number of *T. evansi* positive samples were recorded in camels from Gujranwala (15%) followed by Lahore (8%) and Kasur (3%). Based on different tests, significantly ($P \leq 0.05$) higher numbers of *T. evansi* positive camels were recorded from the Province of Sindh followed by those based in Punjab and Baluchistan respectively. No significant differences were observed between different breeds, age groups and genders. *T. evansi* was only detected in one horse from Gujranwala using TBR1/2 PCR and RoTat 1.2 PCR.

Epidemiological investigations of *Trypanosoma evansi* infection in dromedary camels in the South of Algeria

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Abstract

An epidemiological study of *Trypanosoma evansi* infection in dromedaries was conducted in four localities (Wilayas) of Southern Algeria: Bechar, El Bayadh, Ouarglaf and Tamanrasset. In total, 1046 camels of different ages and both sexes, originating from 83 herds, were sampled between November 2014 and March 2017. The prevalence was determined by parasitological examination with the stained thin blood smear (STBS), serological tests (CATT/*T. evansi*, ELISA/VSG RoTat 1.2), and molecular tests (*T. evansi* type A-specific RoTat 1.2 PCR and *T. evansi* type B-specific EVAB PCR). The packed cell volume (PCV) was measured for 894 camels.

Among the 1046 dromedaries examined, the overall prevalence was 2.2% with STBS, 31.7% with CATT/*T. evansi*, 18.4% with ELISA/VSG RoTat 1.2 and 5.8% with RoTat 1.2 PCR.

The following variations were observed: the infection rate varied according to the origin of the dromedaries: El Bayadh was the most infected Wilaya regardless of the test in use, 11.3% with STBS, 73.3% with CATT/*T. evansi*, 41.4% with ELISA/VSG RoTat 1.2. However, Bechar showed the highest rate (11.8%) with RoTat 1.2 PCR versus 4.1% for El Bayadh.

Parasitological positive animals had an average PCV of 21.7 % (± 4.0) against 27.3% (± 4.3%) for negative animals. None of the 84 camels tested with *T. evansi* type B-specific EVAB PCR, was positive.

This first large-scale study on camel trypanosomosis in the South of Algeria,using parasitological, serological and molecular diagnostic tests, indicated that camel trypanosomosisis endemic and widespread in the South of Algeria.

Evolution of *Trypanosoma evansi* and *T. equiperdum*: how, and how often?

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Abstract

Dourine is a sexually transmitted trypanosomosis that affects equids and historically has been attributed to a distinct etiologic species, *Trypanosoma equiperdum*. In contrast, the trypanosomosis surra affects a wide range of mammalian animals, is typically transmitted mechanically by biting flies, and historically has been attributed to another distinct etiologic species, *Trypanosoma evansi*. In recent years, data from several labs have confirmed multiple independent origins for *T. evansi* and *T. equiperdum*[1-7]. In some of these studies, certain isolates of *T. evansi* were most closely related to isolates that had been classified as *T. equiperdum*. These findings challenge the taxonomic rank of a species for these parasites and have important implications for epidemiology, diagnostics and treatment. In this presentation we will review the current state of knowledge about the evolution of *T. evansi* and *T. equiperdum* and their phylogenetic relationship to *T. brucei*, and, in this context, present a phylogenetic analysis of an isolate from the 2011 outbreak of dourine in Italy[8]. Using microsatellite and kDNA markers we carried out a genetic comparison of the Italian isolate with 59 isolates classified as *T. b. brucei*, 13 isolates classified as *T. b. rhodesiense*, 49 isolates classified as *T. evansi* and 11 isolates classified as *T. equiperdum*. Our study strongly suggests that the Italian *T. equiperdum* is genetically distinct from other *T. equiperdum* and *T. evansi* isolates; it thus represents an important addition to the emerging panel of new isolates from cases of dourine with unequivocal etiology. Attempts to adapt this isolate to *in vitro* culture are underway.

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Some lessons from the South: molecular and virulence studies on Venezuelan *T. evansi* and *T. equiperdum* strains

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Abstract

Molecular studies have shown that *T. evansi* and *T. equiperdum* are closely related species or sub-species of *T. brucei*, that acquired significant genetic and biological differences throughout their evolution, including their mitochondrial DNA and mode of transmission. We characterised microsatellite markers and Procyclin PE repeats of nine Venezuelan *Trypanosoma spp.* isolates with various degrees of virulence and pathogenicity in a mouse model and compared them to a panel of *T. evansi* and *T. equiperdum* reference strains. Coinertia analysis revealed three distinct groups, one with seven isolates that grouped with *T. evansi* strains from around the world, a second group with the first confirmed *T. equiperdum* strains from Latin America (TeAp-N/D1 and TeGu-N/D1) and a third group, that included *T. brucei brucei* strains, that were originally classified as *T. evansi* and the BoTat 1.1 strain. The Venezuelan *T. equiperdum* strains clustered with a *T. equiperdum* strain from South Africa (OVI). Cysteine peptidases are responsible for the main proteolytic activity of trypanosomes and play a key role in their virulence. The *T. evansi* (TeAp-ElFrío01) and *T. equiperdum* (TeAp-N/D1) peptidases were characterised and compared. Using gelatin zymography and fluorimetry, higher cysteine peptidase and Cathepsin L activities were observed in the *T. evansi* extract, as compared to its *T. equiperdum* counterpart. The *T. evansi* CatL activity was fivefold higher than the corresponding *T. equiperdum*, while no differences were detected for the Cathepsin B activity of the two species. The BLASTP analysis of the translated sequences showed 99% identity between the *T. evansi* and *T. equiperdum* CATL sequences and the *T.b. gambiense* (DAL972) and *T.b. brucei* (TREU927) homologs. Interestingly, the two CatL orthologs differ in the Pro hinge region that separates the catalytic domain and the C-terminal end, with eight and nine residues for the *T. evansi* and *T. equiperdum*, respectively. These studies further support the importance of trypanopains in the host-parasite cross-talk with the potential as drug, diagnostic, vaccine and genotyping targets.

Characterisation of newly isolated and culture adapted *Trypanosoma equiperdum*

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Abstract

Classification of trypanosome species in the subgenus *Trypanozoon* remains a controversial topic. Thus, there is a strong need for establishment of *in vitro* culture systems of new *T. equiperdum* strains which are directly isolated from the genital mucosa of horses, which are clinically and parasitologically confirmed cases of dourine, for the further characterisation and analyses. We have successfully isolated *T. equiperdum* strains from the genital organ (IVM-t1, 2, 4 and 5 strains) or cerebrospinal fluid (t3) of dourine horses using soft agarose medium in Mongolia (Suganuma *et al.,* 2016). In this study, we have characterised these Mongolian *T. equiperdum* strains using molecular and morphological analyses.

Based on 18S rRNA and ITS sequences, they could not be genetically distinguished from *T. brucei* and *T. evansi*. The doubling times of t1 (mean ± S.D. hours: 10.0 ± 0.85) and t2 (9.1 ± 0.14) are significantly shorter than that of t4 (19.7 ± 1.86) and t5 (19.5 ± 1.86). At the beginning of isolation, the majority of them possessed kinetoplasts. However, except for the t2 strain, these strains lost their kinetoplast during long-term (6 months - > 2 years) cultivation, which was also confirmed by DNA staining level and transmission electron microscopic observation. In addition, the loss of the kinetoplast (maxicircles and minicircles) in t3, t4 and t5 strains was further confirmed by PCR. On the other hand, t2 lacks maxicircles but contains homogeneous sequences of minicircles even after long-term *in vitro* cultivation. The minicircle sequence of t2 strain was different from previously reported Type A and B minicircle sequences of *T. evansi* and *T. equiperdum*. At this moment, we cannot explain why these *T. equiperdum* isolates quickly lost their kinetoplast in the *in vitro* culture condition. In addition, the discovery of the new minicircle sequence (named Type C) suggests possible existence of various origins of *T. equiperdum* in each geographical area.

Outbreaks of dourine in Mongolia and development of a therapeutic strategy

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Abstract

Dourine is a venereal disease of Equidae, caused by the protozoan *Trypanosoma equiperdum,* and is an emerging disease in horses in Mongolia. The prevalence has increased and expanded in all 20 provinces of Mongolia, with prevalences of 1% to 45%. From 2015 to 2016, a total of 43 outbreaks of dourine were recorded in 12 provinces, such as Dundgovi, Govi-altai, Umnugovi, Khentii, Arkhangai, Orkhon, Selenge, Khuvsgul, Tuv, Sukhbaatar, Domod, and Ulaanbaatar city. Clinical signs, such as skin plaques, swollen abdomen, incoordination of hind legs, unilateral facial paralysis as nervous form, swelling of external genitalia, emaciation and abortions in mares were observed in 22, 25, 33, 33, 66, 90, and 100%, respectively. The clinical symptoms were significantly correlated with strong positive serological reactions measured by immunochromatographic test (ICT) and ELISA. Owners, breeders, and farmers have a deep interest for treating the horses due to their economic and sentimental values. We conducted the present study to evaluate a therapeutic strategy using diminazine aceturate and quinapyramine sulphate. In order to assess the treatment efficacy, all horses showing clinical signs were treated by the quinapyramine sulphate and diminazene diaceturate in combination. All the horses recovered from the clinical symptoms and their overall body conditions were improved, in which the clinical signs, such as unilateral facial paralysis, weakness, and ventral edema, disappeared within 20-30 days after treatment.

Resistance of recently isolated *Trypanosoma vivax* and *T. congolense* against diminazene diaceturate and isometamidium chloride hydrochloride using an infection model of trypanosomosis in cattle in Ethiopia

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Abstract

African animal trypanosomosis stands first in hindering the agricultural sector development by causing considerable morbidity and mortality in domestic animals, and causes huge economical losses. Diminazene diaceturate and isometamedium chloride are the most commonly used trypanocidal drugs since a long period of time. However, there have been increased reports of lack of response to these drugs under different experimental and field settings. Therefore, an exploratory study was conducted to determine the sensitivity of recent field isolates of *Trypanosoma vivax* and *T. congolense* to diminazene diaceturate and isometamidium chloride hydrochloride on experimentally infected local zebu calves in a fly-proof stable at the College of Veterinary Medicine and Agriculture, Addis Ababa, Ethiopia. Of the four filed isolates of *T. vivax*, two isolates successfully infected donor animals and all the four *T. congolense* field isolates established in the donor animals. Forty eight (48) calves were randomly allocated to 16 experimental groups each containing three animals and inoculated with blood from donor animals containing 100,000 trypanosmes/ml. At peak parasitaemia, these infected animals were treated with diamenazen diaceturate (Veriben®) at a dose of 7 mg/kg body weight and isometamidium chloride hydrochloride (Veridium®) at a dose of 1 mg/kg body weight. The study revealed that both *T. vivax* isolates are resistant against 7 mg/kg diminazene diaceturate as detected by both parasitology and PCR whereas the isolate are partially resistant against 1 mg/kg isometamidium chloride hydrochloride as detected by ITS-1 PCR in two animals. All the four *T. congolense* isolates showed resistance against both 7 mg/kg diminazene diaceturate and 1 mg/kg isometamidium chloride hydrochloride. Following rescue treatment of the 24 animals in the *T. congolense* group, all except two have shown relapses until the end of the study. This strongly suggests the presence of multiple drug resistance against the trypanocidal drugs even from genuine sources, at double of the recommended dose which calls for an immediate search for alternative trypanocidal drugs to practically limit the negative impact of animal trypanosomosis.

Diversity of aquaglyceroporin genes (*TbAQP)* in *Trypanozoon* and their relation to melarsoprol and pentamidine cross-resistance

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Abstract

Melarsoprol and pentamidine cross-resistance (MPXR) is linked to mutations within the *TbAQP2* gene, both in laboratory-induced resistant *Trypanosoma brucei* (*T.b*.) strains and in clinical isolates of *T.b. gambiense*. While *TbAQP2*deletion is sufficient to explain this phenotype, trypanosomes often resort to chimerisation of *TbAQP2* by incorporation of different proportions of the highly similar gene *TbAQP3* into the *TbAQP2* coding sequence. Such *TbAQP2/3* chimeric genes are thought to be responsible for the inhibition of both melarsoprol and pentamidine drug uptake. Interestingly, genotyping the *TbAQP2-TbAQP3* locus of different *Trypanozoon* strains revealed the existence of highly diverse forms of these *TbAQP2/3* chimeras between and within strains. In this study, we performed *in vitro* drug sensitivity tests on a panel of *Trypanozoon* strains that carry either one or multiple *TbAQP2/3* chimeras. Indeed, *T. b. gambiense* strains that have only *TbAQP2/3* chimeras, and no wild-type *TbAQP2*, display various levels of MPXR. However, *T. b. gambiense* strains that contain a *TbAQP2/3* chimera, but also a wild-type *TbAQP2* gene, remain melarsoprol and pentamidine sensitive, similar to *Trypanozoon* strains that harbor only wild-type *TbAQP2.*Intriguingly, some *T. evansi* and *T. equiperdum* strains*,*organisms that never came in contact with pentamidine or melarsoprol, also carry*TeAQP2/3* chimeras, in addition to wild-type *TeAQP2, TeAQP3* and even *TeAQP3/2* chimeras, and remain fully sensitive to pentamidine, melarsoprol or even diminazene and isometamidium. Therefore, the presence of *TbAQP2/3* and *TeAQP2/3* chimera(s) in certain *Trypanozoon* strains does not necessarily indicate a MPXR phenotype.

Comparing *in vitro* sensitivity and uptake of suramin, pentamidine and diminazene in *T. brucei*, *T. evansi* and *T. congolense*

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Abstract

It is known that different species of African trypanosome are differentially sensitive to the few available treatment options used against animal trypanosomiasis. However, little is known about the biochemical causes of these differences. We have extensively studied drug resistance mechanisms in *Trypanosoma bruce*i and found that sensitivity and resistance usually correlate with the rate by which the drug is internalised by the parasite. This is certainly the case for the diamidine drugs pentamidine and diminazene, and recent results indicate this is also the case for suramin. Here, we evaluate whether three trypanosome species involved in livestock infection, *T. b. brucei*, T. *congolense* and *T. evansi*, display differential sensitivity to these drugs, and whether this correlates with the rate and efficiency of uptake.

Assessment of alternative diagnostic techniques for detection of *Trypanosoma equiperdum* infections in horses and donkeys in South Africa

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Abstract

Dourine is a disease of equids caused by a protozoan parasite called *Trypanosoma equiperdum* which is transmitted sexually from animal to animal. Complement fixation test (CFT) a serological assay, is the only diagnostic method used for confirmation of *T. equiperdum* infections in equines in South Africa. In this study serological assays and PCR were assessed for their efficiency to detect *T. equiperdum* infections from samples collected from equids in South Africa. A total of 256 blood and serum samples were collected from donkeys (n=32) and horses (n=224) which were from Free State (FS), Mpumalanga (MP), Northern Cape (NC) and North West (NW) provinces. The overall prevalence of dourine by PCR in horses was 17%, 16%, 12% and 13% in MP, FS, NC and NW respectively, whilst 8.3% and 10% positive for donkeys from NC and NW respectively. The sero-prevalence in horses was 14.3% in FS, 12.8% MP and 17.9% NC and 29.9% NW by TeGM6-4rELISA whilst TeCA-ELISA detected 8.6% FS, 20.2% MP, 7.7% NC and 22.9% NW. In donkeys sero-prevalence was 0%, 58.3%, and 5% for FS, NC and NW respectively by TeGM6-4rELISA whilst TeCA-ELISA detected 0% in FS, 25% NC and 0% NW. We conclude that PCR and ELISA with recombinant and crude antigens are capable of detecting *T. equiperdum* infections in donkeys and horses in South Africa.

Recent developments in the diagnosis of NTTAT infections in domestic animals

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Abstract

Diagnosis of NTTAT infections is challenged by usually low parasite load in body fluids and tissues, by the variety of trypanosome taxa and strains, and the diversity of host species. In addition, market failure prohibits investments of commercial companies in NTTAT diagnosis and the tests recommended by the OIE are not readily available. As a result, no single properly validated serological or molecular diagnostic test exists for NTTAT infections. Yet, several research groups and commercial companies are developing and evaluating improved diagnostic for *Trypanosoma vivax*, *T. evansi* and *T. equiperdum*. This presentation gives an overview of the recent literature on this topic.

Multiplex detection of camel antibodies to *Trypanosoma evansi*

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Abstract

We have developed an ELISA-type multiplex test for simultaneous detection of camel antibodies to four recombinant proteins expressed by *T. evansi* type A, Variant Surface Glycoprotein RoTat 1.2 (VSG), Invariant Surface Glycoprotein 75 (ISG75), calpain tandem repeat domain (GM6) and oligopeptidase B (OligoB). OIE reference sera, 'true positive' (TP, n=44) and 'true negative' (TN, n=44), as determined by CATT and/or immune trypanolysis and/or blood parasite detection, were used to establish multiplex conditions. Of 44 TP sera 43 recognised one or more of the antigens, whereas one serum did not recognise any. Of 44 TN sera one recognised two of the antigens. Kappa statistics showed 'very good' agreement between the multiplex and the OIE panel of tests (κ=0.995, SE±0.032, 95%CI 0.892-1.00). Sera (n = 100) were analysed from camels in Gran Canaria, Spain which is regarded as free of *T. evansi*. Only one animal showed a positive reaction in the multiplex. The discrepant samples will be analysed further to determine which test is most likely correct. We have applied the test to field samples from Saudi Arabia, including 51 sera from an outbreak of abortion in camels and shown significant antibody titres in aborting and in-contact animals.
The *T. evansi* multiplex ELISA offers an efficient, cost effective means to detect infection at herd and individual level, with the potential 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. Further diseases could be added to the multiplex to enhance its value for surveillance.

Dourine in Mongolia: isolation of *Trypanosoma equiperdum*

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Abstract

Prevalence of dourine in Mongolia is steadily increasing and affecting prosperous equestrian industry substantially, although it had a limited distribution previously. Recently, an epidemiological study of the disease was conducted with ELISA on 2,621 equine serum samples that had been collected from 20 provinces, and the state average was determined as 9.4%, in which the highest rate (41.5%) was recorded in Tuv province. Currently, only one out of 21 provinces is free from the disease. In this study, we successfully isolated five *T. equiperdum* strains in three different provinces, from infected horses that showed typical clinical symptoms of dourine. The parasites were adapted in an *in vitro* culture system using a HMI-9 medium. Regarding the isolates, four of them had been isolated from urethral swabs from stallions that showed clinical signs of reproductive organs and neurological symptom, while another isolate was obtained from the cerebrospinal fluid from a mare that showed lip and ear nerve paralysis. Today, we are producing diagnostic crude antigens for IFAT, CFT, and ELISA using the *in vitro* cultured *T. equiperdum*. It would allow us to initiate manufacturing diagnostic tools for the serological diagnosis of dourine in our country. Furthermore, we will screen the drug sensitivity of *T. equiperdum* using the *in vitro* culture system to establish therapeutic methods against the disease in horses.

Purification of *Trypanosoma equiperdum* spiked equine semen by single layer centrifugation

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Abstract

Trypanosoma equiperdum (T. equiperdum) is a venereal transmitted infection in horses. Purification of semen by single layer centrifugation (SLC) is proven to be successful in reducing venereal transmitted diseases in other pathogens (Morrell et al., 2013). The objective of this study is to evaluate the purification of *T. equiperdum* spiked semen by SLC.
Semen was spiked using cryopreserved *T. equiperdum* stabilates (Dodola strain isolate 943). Viability of the parasite was checked beforehand using microscopic examination. In total, 6 spike concentrations, varying from 1+ to 6+ (Paris et al. 1982), where added to semen samples. Subsequently, SLC was conducted following standard procedures (Morrell et al. 2013) to obtain spiked-SLC-purified semen samples to inoculate 35 (i.e. 5 per concentration) mice. Spiked semen of the 6 different concentrations with no centrifugation as positive control and non-spiked semen as negative control were included in the experiment.
All the spiked-SLC-purified sperm pellets were found to be negative on microscopic examinations. All mice in the positive controls exhibited parasitaemia (5/5). Mice inoculated with low grade parasitic (1+ to 3+)-spiked-SLC-purified semen remained free of parasitaemia, similar to the negative controls. However samples spiked with parasite concentrations of 4+ - 5+ to 6+ were able to infect some mice, respectively 2/5 and 3/5.
This experiment indicates that a low level of parasite infection in stallion semen can be cleared off using SLC.

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

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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. Diseases caused by infection with *Trypanosoma evansi* (surra) or *Trypanosoma equiperdum* (dourine) are notifiable to the OIE. The 181 Member Countries of the OIE should report these diseases when 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 recognised 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.

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.

The missions of the OIE rely heavily on the Veterinary Services of each Member Country. It is the reason why in parallel and in synergy with the development of Standards, the OIE provides a continuing support to Veterinary Services and laboratories to enable OIE Member Countries to implement them. Example of OIE support is the OIE Laboratory Twinning Programme which is an exchange of expertise between an OIE Reference Centre and another laboratory.

The OIE has also published publications on NTTAT: Livestock Trypanosomoses and their vectors in Latin America (2004), and Trypanosomosis in Camelid Infections Disorders (2014).

Problems to adapt coordinated methods for the control of NTTAT in the world

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Abstract

At the 1st International Conference on Non Tsetse Transmitted Animal Trypanosomosis, the opinion paper "Developing a Progressive Control Pathway for African Animal Trypanosomosis" by Diall et al (2017) in Trends in Parasitology was mentioned. The need to extend this Progressive Control Pathway to NTTAT was briefly discussed. Consecutively a detailed survey was carried out with the efficient help of the World Animal Health Information System (WAHIS) to estimate the number of OIE member countries that report on NTTAT. Surprisingly, only a few countries (15) notify surra while many others notify “trypanosomosis“ which means “trypanosomosis, tsetse transmitted" in the OIE listed diseases. After correction of these discrepancies, a coordinated control method might be considered to be progressively extended, first to Africa, then to other regions. The need of such a difficult task appears in the recommendations of the 34th General Conference of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) and 16th PATTEC National Coordinators Meeting, 11-15 September 2017, Livingstone, Zambia, as follows:

"8. It was noted also with concern that PATTEC Country reports lacked information and detail on non-tsetse transmitted trypanosomiasis, and recommended that efforts be made to give adequate prominence to this very important area."

"11.f. Non-tsetse transmitted trypanosomiasis continues to be an important area and it is recommended that efforts should be enhanced in the development of new olfaction and visual baits for the control of the vectors to maximise control of Trypanosoma evansi infections."

"12. The Progressive Control Pathway including road maps for AAT was presented. However it was noted that non-tsetse transmitted trypanosomiasis was not included. The meeting recommended that this be further looked into, working closely with OIE."

This information deserves to be communicated to interested OIE Regional Commissions.

Review of the situation of camel trypanosomosis (surra) and control methods in CILSS countries and recommendations for the way forward

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Abstract

CILSS member countries are: Burkina Faso, Cabo Verde, Chad, Guinea-Bissau, Mali, Mauritania, Niger, Senegal and The Gambia. Out of those nine countries, four do own around one million heads of camels, each; those are Mauritania, Mali, Niger and Chad. Two other countries, Burkina Faso and Senegal have very limited numbers of camels, while the last three, Cabo Verde, Guinea-Bissau and The Gambia do not raise camels at all.
In the camel rearing CILSS countries, trypanosomosis due to Trypanosoma evansi (surra) is known as a major animal health burden and biting flies like *Tabanus*, *Stomoxys* and *Hippobosca* are recognised as the mechanical vectors of surra.
Recent epidemiological data on the situation of surra in these Sahel countries are rare or inexistent. Old data have indicated relatively high prevalences in the southern parts of the Sahel where rainfalls ranging from 200-250 mm allow the presence of above mentioned vectors and hence the transmission of surra.
The control of surra relies mainly on the use of tryanocidal drugs, the most notable of those are anthrycid and for a brief period, cymelarsan. This last drug was dropped out due to its high price, while the first one has come back on the market after a first withdraw due to chemoresistance. Antrycid is mainly used in Eastern Africa and rarely found in Sahel countries. Presently, camel owners use more available but less effective trypanocidal drugs which are diminazene and isomethamidium.
Due to the mechanical nature of the transmission, mass trypanocidal treatment targeting the season of high risk, may be a good solution for exhausting the source of infection. The challenge in this respect is poor effectiveness of the most current trypanocidal drugs. Research should be encouraged to get new drugs or to design better mass treatment protocols with available drugs and taking into account the high risk period. A surra research network should also be established with its coordination based preferably at CIRDES, Burkina Faso.

Poster Abstracts

Development and validation of new serological tests for diagnosis and confirmation of dourine

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Abstract

Dourine is a parasitic venereal disease of equines caused by the flagellate protozoan *Trypanosoma equiperdum*. Specific antibodies are found in infected animals, whether they display clinical signs or not, but diagnosis of dourine must include history, clinical, and pathological findings in addition to serology. The complement fixation (CFT) is the recommended test for international trade and it is applied to confirm clinical suspicions and to detect latent infection; however, some uninfected equines may give inconsistent or nonspecific reactions to CFT due to the anticomplementary effects of their sera.

In this study we developed an indirect ELISA (iELISA) and an immunoblotting assay (IB) that could be used to confirm positive serological cases or to solve inconclusive results obtained by CFT. A total of 606 CFT negative sera, collected in 2017 during the national surveillance plan for Equine Infectious Anaemia in the Abruzzo and Molise regions, and 140 sera positive to CFT and indirect immunofluorescent assay (IFA) (collected at different stages of infection from 20 clinical cases of dourine occurred in Italy in 2011) were tested by iELISA. A *Trypanosoma equiperdum* derived from Onderstepoort Veterinary Institute (*T.e.*OVI) was used as antigen. Results were expressed as percentage of positivity PP and the optimum cut-off value, determining a sensitivity and specificity of 100% (CI_{Se} = 97.9-100; CI_{Sp} = 99.5-100), was determined using ROC curves. All positive sera, tested by IB with *T.e.*OVI, were confirmed as positive. Specifically, all infected animals recognised bands ranging between 6 and 37 kDa. Twenty seven sera low-positive at CFT and negative for IFA were additionally tested with iELISA and IB. All samples resulted negative to IB and one turned positive to iELISA.

Our results suggest that iELISA and IB should be used as alternative or supplementary confirmatory tests whenever other recommended serological methods are inconclusive or doubtful.

Identification of *Trypanosoma equiperdum* potential biomarkers useful in the serodiagnosis of dourine

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Abstract

Dourine is a contagious disease of equids caused by *Trypanosoma equiperdum* and transmitted directly from animal to animal during coitus. Diagnosis of dourine is mainly based on serological tests (CFT, IFAT, ELISA), and recognition of clinical signs, since isolation and identification of the parasite are tricky. *T. equiperdum* is closely related to other Old World *Trypanosoma* species of the Trypanozoon subgenus, in particular *T. evansi* and *T. brucei*, and serological cross-reactions among them are currently observed. Suspected dourine cases have been confirmed by analysing history, clinical and pathological findings. Actually, serodiagnosis of dourine is made using the *T. equiperdum* whole antigen; no specific recombinant proteins are available. To improve diagnosis, *T. equiperdum* specific subunit antigens and monoclonal antibodies to them are necessary.
In this work, *T. equiperdum* strain OVI (Onderstepoort Veterinary Institute) proteins were separated by SDS-PAGE; six electrophoretic bands with molecular weight ranging between 37 and 10 kDa, recognised only by antibodies from infected horses, were subjected to standard in-gel destaining, reduction, alkylation and trypsin digestion. Peptides were then analysed using an UPLC EASY-nLC 1000 system coupled to a Q Exactive-HF mass spectrometer, in order to identify immunogenic proteins that could be used as biomarkers in the diagnosis of dourine.
A total of 167 proteins were identified. Their amino acid sequences were compared, by similarity searching, to all sequenced proteins of other *Trypanosoma* species (*T. evansi*, *T. brucei brucei*, *T. b. gambiense*, *T. b. rhodesiense, T. congolense*, *T. cruzi*, *T. rangeli* and *T.vivax*) to establish their degree of conservation. Thirty-seven proteins out of 167 resulted as specific for *T. equiperdum* and could be potential candidates for development of diagnostic assays for dourine.

Experimental model for assessing drug efficacy against *Trypanosoma equiperdum* infection in horses

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Abstract

The causative agent of dourine, *Trypanosoma equiperdum*, may cross the brain-blood-barrier and lead to the development of nervous clinical signs in infected horses. This location participates to the protection of the parasite from most (if not all) existing chemotherapies. In this context, the OIE terrestrial code considers dourine as a non-treatable disease and imposes to practice a stamping-out policy for affected animals to recover a country free status. The use of this practice remains controversial but the lack of suitable tools for studying treatment efficacy against dourine hampers the development of an alternative strategy. The present study reports on the development of an experimental infection model for assessing drug efficacy against the nervous form of dourine that combines the infection of horses by *T. equiperdum* and the detection of trypanosomes in cerebrospinal fluid (CSF) thanks to an ultrasound-guided cervical centesis protocol.

A development phase, involving 4 horses, allowed us to select an infection process consisting in the intravenous inoculation of 5.10^4 *T. equiperdum* OVI parasites to adult horses. The efficiency of this procedure was confirmed on eight horses for which, parasites were observed in blood (2 days post infection), in CSF (12.5 ± 1.6 days post infection) and that developed an immune response detected 8.25 ± 0.5 days post infection. The 8 animals developed fever (> 39 °C), anemia (hematocrit < 27 %), and ventral edema (7.9 ± 2.0 days post infection) and other inconsistent clinical signs were observed including edema of the vulva (6 over 8) or cutaneous plaques (3 over 8).

This protocol of dourine infection allows the detection of parasites in CSF of infected horses within a period of time compatible with animal experimentation constraint. This suitable model can be used to evaluate *in vivo* efficacy of anti-*Trypanosoma* treatments.

Development of phage display library for the selection of peptides binding to targets against *T. evansi*

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Abstract

Trypanosoma evansi is a hemoparasite, which causes the disease known as "Surra" and has been described in several species. It is responsible for direct losses in the livestock. *T. evansi* is transmitted by insects (Tabanidae and Stomoxydae) and hematophagous bats and iatrogenically. With the objective of identifying new compounds for the control and/or diagnosis of *T. evansi*, phage display technology associated with Kunkel's mutagenesis technique was used. The library retained a structural protein of an attenuated toxin with Kunitz Domain present in the venom of *Mesobuthus tamulus* scorpion. The mutations were directed, with the support of the crystallographic structure of the toxin, to specific residues in the N-terminal portion of the α-helix in the smaller loop of the protein. The mutant DNA was purified and amplified by the Selective Rolling Circle technique. Clones from the library were sequenced, where a close to 100% rate of insertions of specific residue mutations was evidenced. The library was used in affinity selections against *T. evansi*. After one round in vivo and two in vitro, six clones were selected for individual studies of potency, binding specificity and toxicity. Among all clones, in particular, clones 5 and 1 showed a more significant potential for binding to T. evansi and lack of binding against other trypanosomatids (*T. cruzi* and *T. rangeli*). Clones 1, 2 and 5 proved to be toxic to *T. evansi* causing a mortality of 13%, 31.5% and 19.7%, respectively. DNA from clones 1, 2 and 5 were sequenced, demonstrating that mutations were inserted. The toxin-derived library, as well as clones 1 and 5 identified, have the potential for the development of new tests for the direct diagnosis of *T. evansi*. Clones 1, 2 and 5 that demonstrated toxic activity might serve as the basis for other studies aimed at the development of new drugs with trypanocidal activity.

A PCR-based survey of animal trypanosomosis among domestic animals herded together in Mongolia

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Abstract

Trypanosoma evansi affects a wide range of domestic animal hosts and can be cross-transmitted mechanically by biting flies when various animals graze together. Such conditions can be observed in nomadic systems of livestock husbandry. The Mongolian livestock industry depends on nomadic systems of husbandry in which various animals are herded together. It is characterised by low input and large herds grazing across pastures in the open country.

We screened cattle, yaks, camels, horses, sheep and goats that were herded together from the Mongolian provinces of Bayan-Ölgii and Hovd for animal trypanosomes, using a KIN (ITS1) PCR; 21.3% of the samples tested positive. The highest prevalence was observed among the goats (35.7%) followed by sheep (26.4%). The prevalence in small ruminants was significantly higher than that in other domestic animals, (p<0.0001). At 7% (cattle) and 5% (yaks), bovids had the lowest prevalence. The prevalence in camels and horses was 17.4% and 15.8% respectively. The prevalence in males (25.6%) was significantly higher than that in females (19.8%; p<0.05). The prevalence in adult animals (22.1%) was significantly higher than that in young animals (9%; p<0.01). Generally, there were no significant differences in the prevalence in the 2 provinces; however, the prevalence in the sheep in Hovd province (34.5%) was significantly higher than that in the sheep in Bayan-Ölgii (16.8%; p<0.01). In contrast, the prevalence in horses in Bayan-Ölgii (29.5%) was significantly higher than that in the horses in Hovd (2.7%; p<0.0001).

This is the first study to highlight the prevalence of animal trypanosomosis in various domestic animals grazing together in the Mongolian grasslands. The study also highlights the significance of small ruminants as possible reservoirs of trypanosomosis. It also shows the possible relationship of the herd structure, age and sex to the prevalence of trypanosomosis in Mongolia.

General information

http://www.itg.be/E/contact

From Brussels

From Brussels National Airport (https://www.brusselsairport.be/en) (Zaventem)
SN Brussels Airlines Expressbus (https://www.brusselsairport.be/en/passngr/to-from-brussels-airport/bus) to Antwerp Central Station

- Brussels-Antwerp : every hour
  first bus at 5 am (sundays at 7 a.m)
  last bus at 12 pm
- Antwerp-Brussels: every hour
  First bus at 4 am (Central Station) and 4:10 am (hotel Crowne Plaza) (sundays 6 and 6:10).
  Last bus at 11 pm and 11:10 pm
  Price : € 10,00 (driver sells the tickets)

By train from the station Brussels-Airport.
http://www.belgianrail.be/en

Public transport in Antwerp

- From Antwerp Central Station
  http://www.belgianrail.be/en
- Subway n° 9 or 15 - stop Groenplaats - take exit Nationalestraat - follow Nationalestraat for 1 km
- From Berchem Station
  Tram n° 4 - stop 'Tropisch Instituut'

Additional information

- More information on trams and buses: De Lijn
  https://www.delijn.be/EN

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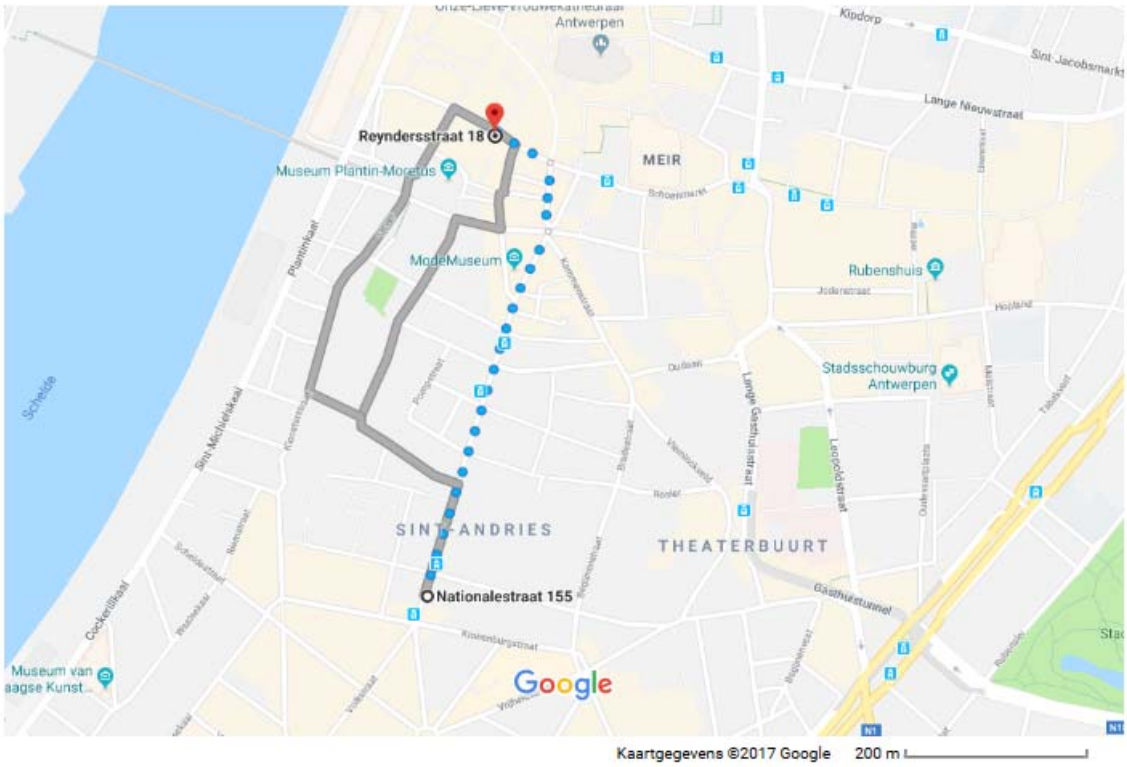

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