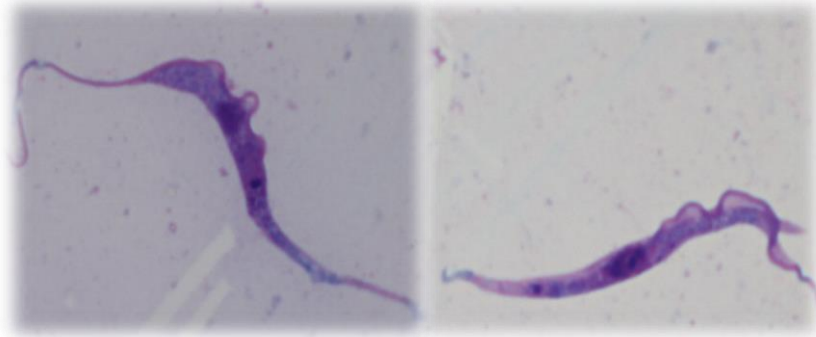


OIE NTTAT network



**3rd International Conference on
Non Tsetse Transmitted Animal Trypanosomosis**
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**North-West University
Potchefstroom
South Africa**



Oral presentation

Abstracts

Prevalence of *Trypanosoma evansi* in camels (*Camelus dromedarius*) in Ghardaïa district, south Algeria

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Abstract

Trypanosoma evansi is a protozoon parasite with worldwide distribution affecting many animal species. This parasite causes surra, characterised by anaemia, icterus, abortion and immunosuppression, which makes it a real threat to animal health. In Algeria, there is little information about the prevalence of *T. evansi*. The objective of this study was to estimate the prevalence of *T. evansi* in camels with different diagnostic methods.

The dromedary population in *Ghardaïa* district is about 11210 heads. One hundred and sixty camels were randomly sampled and tested for specific antibodies against *T. evansi* with serological tests (immune trypanolysis, CATT/*T. evansi*, ELISA/VSG RoTat 1.2, Sero-K-SeT) and for specific DNA with PCR (ITS1 PCR, 18S qPCR).

The prevalence of surra was 9.3% using immune trypanolysis, 8.1% using CATT/*T. evansi*, 8.7% using ELISA/VSG RoTat 1.2, 8.1% using Sero-K-SeT, 6.2% with ITS1 PCR and 13% with 18S qPCR.

The results indicate that *Ghardaïa* district is an endemic area and *T. evansi* has the potential to affect many other areas of the country. Therefore, authorities must pay attention to this parasite by increasing the epidemiological surveillance of animals in affected and unaffected areas.

The evaluation of GM6-based ELISA and ICT as diagnostic methods on a
Mongolian farm with an outbreak of non-tsetse transmitted horse
trypanosomosis

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Abstract

Trypanosoma equiperdum, which is the etiological agent of dourine, spreads through sexual intercourse in equines. The objective of this study was to evaluate the potential application of recombinant *T. evansi* GM6 (rTeGM6-4r)-based diagnostic methods on a farm with an outbreak of non-tsetse transmitted horse trypanosomosis. 97% homology was found between the amino acid sequences of *T. equiperdum* GM6 and the GM6 of another *Trypanozoon*, which also shared the same cellular localization. This finding suggests the utility of rTeGM6-4r-based sero-diagnostic methods for epidemiological studies and the diagnosis of both surra and dourine in Equidae. Fifty blood samples were examined from a herd of horses. The diagnostic value of an rTeGM6-4r-based ELISA and an rTeGM6-4r-based immunochromatographic test (ICT) were measured in comparison to a *T. evansi* crude antigen-based ELISA, which is a diagnostic method recommended by the OIE. However, this is not a perfect diagnostic method for trypanosomosis. Positive serum samples were detected in 46%, 42% and 28% of the tested horses using rTeGM6-4r-based ELISA, crude antigen-based ELISA and rTeGM6-4r-based ICT, respectively. The sensitivity of rTeGM6-based ELISA was 81%, the specificity was 79%, and the agreement was moderate. We conclude that rTeGM6-4r-based ELISA and ICT represent alternative options for baseline epidemiological studies and the on-site diagnosis of horse trypanosomoses in the field, respectively.

A preliminary study of *Trypanosoma evansi* infection in domestic animals in an endemic area of Southern Algeria

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Abstract

Trypanosoma evansi infection is endemic in dromedary camels of southern Algeria with an overall prevalence up to 32.4% with CATT and 21% with immune trypanolysis. In order to assess the presence of other potential reservoir species among domestic animals, a study was conducted in the Wilayate of Béchar, El Bayadh, Ouargla and Tamanrasset, between 2015 and 2016.

A total of 190 animals were sampled, including 48 equids (44 horses, 3 donkeys and 1 mule), 42 cattle, 49 goats, 40 sheep and 11 dogs. These animals were examined by parasitological (Giemsa stained thin smear, GST), serological (CATT/*T. evansi*, ELISA/VSG RoTat 1.2, immune trypanolysis) and molecular tests (*T. evansi* type A specific RoTat 1.2 PCR).

The CATT/*T. evansi* was positive in 10/42 cattle, 15/40 sheep, 27/49 goats, and 2/48 equids. On the other hand, 20/38 cattle, 17/44 goats, 31/39 sheep, 21/42 equids, and 1 dog were positive by ELISA/VSG RoTat 1.2. However, no positive results were observed with immune trypanolysis. In addition, *T. evansi* could not be demonstrated by either GST or PCR in any of the examined animals.

Based on seroprevalence only, this study suggests that *T. evansi* circulates in different domestic animal species that could act as reservoirs for the parasite that causes trypanosomosis in dromedary camels. This preliminary data suggested a need for a large scale epidemiological survey on a larger number of animals to confirm their putative role as reservoir of *T. evansi*.

In search of new small chemical leads against Surra

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Abstract

Surra (animal trypanosomosis) is a major debilitating disease in camels, equines, cattle and dogs, in which it can often be fatal in the absence of treatment. The disease causes major socio-economic losses especially among pastoralists and farming populations, who rely extensively on their animals as a main source of income. Current treatments for surra have limited efficacy and concerns of emergence of drug resistance to available treatments highlight the urgent need to identify new leads for development.

To address this need, we performed a drug screen of compounds from 3 different sources:

- (i) PhytoQuest proprietary library (PQL) consisting of pure, mainly non-polar compounds of known structure, natural products isolated from temperate zone plants
- (ii) inhibitors of N-myristoyl transferase (NMTi), an essential enzyme for many Kinetoplastids and well described drug candidate for human African trypanosomiasis
- (iii) a library of repurposed drugs (RDL), whose screening concentration is based on the maximal safe serum concentration of the given drug (1x human C_{max})

Using an established Alamar Blue Assay of bloodstream *T. evansi* parasites, we screened in total 671 compounds (544 PQL, 13 NMTi and 114 RDL). We identified 40 compound hits (12 PQL + 9 NMTi + 19 RDL) with the ability at 1 μ M (or at 1x in case of RDL) to reduce proliferation by 50%. Of these 40 hits, we have currently brought 6 PQL, 3 NMTi and 1 RDL hits forward for determination of EC50. All of these selected active compounds show a promising potency within the picomolar (NMTi) to nanomolar range (PQL, RDL).

Prospects for control and elimination of camel trypanosomosis due to *Trypanosoma evansi* (Surra) in Africa

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Abstract

More than 80 percent of the dromedary camels occur in Africa and are estimated to be 20-30 million heads. Camels can survive under harsh climatic conditions and provide numerous services and products contributing to food and nutrition security and to poverty reduction.

Camel trypanosomosis due to *T. evansi* (Surra) is causing direct economic losses through morbidity, 30% and mortality 3% as reported from Ethiopia. Biting flies, mainly Tabanids, are recognized as the mechanical vectors of Surra.

Controlling camel Surra and other major diseases should improve camel productivity and facilitate the access of their products to international markets

The elimination of camel surra in Africa looks more feasible than that of Animal African Trypanosomosis. Favourable factors for elimination of camel Surra are among others: unique trypanosome species (*T. evansi*), one main host (camel), seasonal character of the main vector (Tabanid spp.), and availability of 2 effective drugs, Melarsomine (Cymelarsan) and Anttrycide Pro-Salt and simple effective diagnostic tests (parasitological and CATT).

Main challenges are: little attention paid to camel diseases by national decision makers and donors; insufficient veterinary coverage of camel areas; some camel areas are dangerous to access due to the presence of armed groups; and insufficient understanding by the illiterate owners of the epidemiology and the benefits of preventing and controlling camel diseases

A three component strategy is suggested for the control/elimination of camel Surra in Africa: (1) Elimination of Surra (using trypanocidal drugs, mainly), (2) control of other camel priority diseases, and (3) reinforcement of capacities of national veterinary services, namely in camel rearing areas. It is recommended that the strategy should be implemented using the stepwise approach PCP, as suggested for other animal diseases including Animal African Trypanosomosis.

Treatment efficiency of combination therapy using diminazene aceturate and quinapyramine sulfate for a dourine horse

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Abstract

Dourine is a lethal protozoan disease for Equidae, and it is caused by *Trypanosoma equiperdum* infection via coitus. There are no authorized treatment strategies, therefore, the world organization for animal health (OIE) recommend stamping-out policy for dourine infected cases in dourine free countries. Our previous study has revealed a number of dourine suspected or confirmed cases in Mongolia. It is difficult to apply the stamping-out policy for dourine cases in Mongolia due to inadequate livestock guarantee system. In this study, an eight-year old stallion was diagnosed as dourine patient based on clinical signs, molecular analyses and microscopic observations of trypanosomes. Diminazene aceturate (DA, Demin[®]) and quinapyramine sulfate (QS, Triquin[™]-S) were administered as treatment. DA was injected three times at 3.5 mg/kg and QS was injected twice at 3 mg/kg one week after the treatment of DA treatment. Before the treatment, clinical signs, such as edema around the penis, accumulation of smegma and slight paralysis, were observed and trypanosomes were detected in urogenital tract mucosa specimen by microscopic observations and PCR. Moreover, the positive serodiagnosis was obtained by recombinant *T. evansi* GM6-based serological tests. After the treatment, these clinical signs and trypanosomes completely disappeared from genital organ. In addition, negative results were obtained by the serological tests. These results suggest that DA and QS combination treatment might be an effective cure for dourine.

In vitro evaluation of trypanocidal efficacy of benzyltriazole derivatives on
Trypanosoma evansi

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Abstract

Trypanosoma evansi causes surra affecting mainly camels and horses, even though water buffaloes and cattle can also succumb to the infection. Due to the adaptation to the mechanical transmission of *T. evansi* by biting flies, surra has spread outside the tsetse belt of Africa resulting in a wide distribution of the disease into North Africa, Asia, Central and South America. The infection is manifested by the clinical symptoms such as fever, progressive anaemia and paralysis of the limbs, untreated cases are fatal. Treatment is based solely on few available compounds

A total of 25 benzyltriazole derivative compounds synthesized at the pharmaceutical chemistry laboratory of North-West University, South Africa were subjected to throughput screening to assess the efficacy of the compounds on *T. evansi*, *in vitro*. Five effective compounds were selected to determine the IC₅₀ values which were recorded as; 7.55 ± 0.39 , 8.15 ± 0.79 , 5.61 ± 0.39 , 1.49 ± 0.49 , 5.10 ± 0.68 ($\mu\text{g/ml}$) for compounds NWU-FJS-103, NWU-FJS-203, NWU-FJS-401, NWU-FJS-402, NWU-FJS-403, respectively. Benzyltriazole derivatives in this study possessed moderate trypanocidal effects on *T. evansi*, *in vitro*. Various functional groups attached to the main benzyltriazole structure resulted in different trypanocidal efficacy of the compounds on *T. evansi*. The cytotoxicity effect of the compounds is yet to be conducted as well as the validation for the *in vivo* tests.

Dourine in Southern Africa: A neglected subject of research

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Abstract

Dourine is a disease of equines caused by the protozoan parasite, *Trypanosoma equiperdum*, which is transmitted by coitus from animal to animal. History of recognition of dourine in southern Africa dates as far back as 1907. There is contradictory stories of its introduction in the region, where some believe it was from horses imported to Namibia (formerly South West Africa) from Germany whilst others believe it already existed in the region before. Nevertheless, earliest record of recognition of the disease in South Africa is between 1907 – 1911. The presence of the disease was confirmed in 1917. Complement fixation test (CFT) is the OIE approved official test for confirmation of dourine in equines. In a nutshell, dourine has been reported as clinically diagnosed by veterinarians, confirmed by CFT in reference laboratories or reported by research in Botswana, Lesotho, Namibia, Swaziland and Zimbabwe. In 1935, *T. equiperdum* was demonstrated directly in the tissues of infected horses in South Africa. During that era there was relatively a good number of research conducted on dourine. In 1948, there is a report of the study on transmission of *T. equiperdum* to laboratory animals. Since then, there has been few research reports on prevalence, diagnostics, immunology and chemotherapy. Meanwhile, the veterinary sector continues to report clinical cases of dourine, particularly in South Africa which are as high as 905 dourine cases reported in the period 2000 – 2010 and 105 in the period 2011 – 2015. This paper, highlights there need for continued research aimed at improving dourine diagnostics, treatment and control in southern Africa.

Improvement of molecular diagnostics for *Trypanosoma equiperdum* infection in horses and donkeys from South Africa

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Abstract

Dourine is a disease of equines caused by *Trypanosoma equiperdum* which is sexually transmitted from one animal to another. The OIE recommended diagnostic technique is a serological assay called complement fixation test which confirms exposure to infection. The lack of simple and reliable diagnostic methods is an obstruction in the effective control of diseases such as dourine. The aim of this study was to develop DNA based diagnostic assays including conventional polymerase chain reaction (conPCR), real-time PCR (qPCR) and loop-mediated isothermal amplification (LAMP) for the detection of *T. equiperdum* infections in South African equids. Primer sets and probes were designed from the repetitive insertion mobile element (RIME) gene. The 3 assays namely conPCR, qPCR and LAMP specifically amplified *T. equiperdum* DNA when tested against other parasites which co-infect equines. However, the specificity of qPCR was not stable and requires analyses using melting curves. The detection limit of conPCR and LAMP for serially diluted DNA was 1×10^{-5} and 1×10^{-7} for conPCR and LAMP respectively which is equivalent to 1 and 0.001 trypanosome cells/ml respectively. Whilst the SYBR green and probe based qPCR had 1×10^{-5} detection limit which is equivalent to 1 trypanosome/ml. The conPCR, qPCR and LAMP assays were used to screen DNA extracted from blood collected from horses and donkeys in South Africa. The detection performance of LAMP was higher than that of real-time qPCR, conPCR with 70.8%, 52.1% and 62.5% respectively. These assays will now be evaluated in large scale epidemiological survey of dourine in equines in South Africa.

Poster presentation

Abstracts

Diagnosics and countermeasures of dourine in Mongolia

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Abstract

Epidemiological study of dourine has been conducted in Mongolia past few years using randomly collected horse samples including all ages of horses and castrated males. The disease was diagnosed in all 21 provinces of Mongolia and prevalence was 4.5% on average. On the other hand, horse owners and equestrian farmers requested 401 racing and stud horses consisting from mares and stallions to be examined in first half of 2018 and prevalence of dourine was 19.7%. From requested farms, 48.5% of them had dourine exposure and their initial propose of dourine examination were before or after purchase of racing or stud horse, determining unclarified illness or as general preventive measures. We have conducted necropsy on one of infected mares with facial paralysis and atrophy and mononuclear cells were diffused slatternly throughout muscle fibre of left levator labii maxillaris.

Under the frame of SATREPS project, 'Epidemiological Studies on Animal Protozoan Diseases in Mongolia and Development of Effective Diagnostic Measures' on site, rapid diagnostic immunochromatographic test (ICT) was developed and officially registered in veterinary pharmacopeia of Mongolia and approved by Ministry of Food, Agriculture and Light Industry of Mongolia. Total of 1420 "Dourine rapid test" produced at Institute of Veterinary Medicine of Mongolia, distributed to 15 provincial veterinary laboratories for screening test in August 2018. Also, rTeGM6-4r antigen based ELISA kits were produced and distributed to the provincial veterinary laboratories as for confirmative diagnosis.

Dourine countermeasure guideline was officially formulated by our research team this year and recognised by Ministry of Food, Agriculture and Light industry, although the disease was listed as notifiable disease of equidae in Mongolia since 2011.

Symptomatology and diagnosis of Dourine in horses infected by insemination or transfusion

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Abstract

Diagnosis of dourine (*Trypanosoma equiperdum*) with a short-term parasitaemia and aspecific serological tests is difficult. Diagnosis depends on symptomatology and serology.

This study describes the symptomatology and diagnostic expectations after *T. equiperdum* infection. Clinical signs in stallions (N=4), infected by blood transfusion ($\approx 100,000$ *T. equiperdum* Dodola 943) and mares (N=4), by insemination of spiked semen ($\approx 36,000$), were recorded and parasitological (Woo and wet smear), serological (CATT) and molecular (PCR using ITS1 primer) tests were performed.

In the preclinical stage when parasitaemia was positive, semen was collected on day 7 (N=3) and day 13 (N=1) and epididymal semen at 120d post infection when stallions were again aparasitaemic. In mares when parasitaemia was negative (chronic stage), a uterine biopsy (N=3) was taken for PCR.

In stallions, clinical symptoms were obvious especially preputial oedema (from 10 days on) and nervous signs (55-102 days). Cachexia and corneal opacity were observed later. Five days post-inoculation *T. equiperdum* were observed. Stallions became CATT positive on day 17. Semen on day 7 was negative and PCR-positive on day 13. Epididymal semen from aparasitaemic stallions was PCR positive.

In mares, oedema of the vulva (11 days), depigmentation (38 days) and nervous signs (44 days) were recorded and cachexia and corneal opacity developed later. The *T. equiperdum* DNA was found in the bloodstream (day 5), trypanosomes from day 6 and seroconversion from day 15. Later, in aparasitaemic stages, uterine biopsies were PCR positive.

Parasitological, serological and molecular tests were positive after infection along with clinical signs regardless of the route of infection.

In the aparasitaemic, chronic stages, epididymal semen and uterine biopsies were PCR-positive. *T. equiperdum* DNA detection in semen and uterine biopsies might be an alternative approach to overcome false negative results in these aparasitaemic stages of the disease.

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