



Parasitological, Serological and Molecular Prevalence of *Trypanosoma evansi* among Arabian Camels (*Camelus dromedaries*) with Evaluation of Antitrypanosomal Drugs

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Abstract

Purpose This study was carried out to assess the prevalence of *Trypanosoma evansi* infection in naturally diseased Dromedary camels in Dammam, Eastern region of Saudi Arabia. The detection of *Trypanosoma evansi* was performed using the parasitological, serological, and molecular diagnosis and a comparison between such methods were analyzed. In addition, evaluation of therapeutic efficacy of selected antitrypanosomal drugs, cymelarsan and quinapyrimine (aquin-1.5), was trialed for treatment of diagnosed infected cases.

Methods A total 350 randomly selected camels were evaluated using thin blood smear (TBS), RoTat1.2 PCR and CATT/*T. evansi* techniques.

Results The total prevalence was 6.9%, 7.7%, and 32.8% by TBS, RoTat1.2 PCR and CATT/*T. evansi* techniques, respectively. Although PCR detect *T. evansi* in more samples than TBS, the agreement was good ($K=0.9$). Among the CATT/*T. evansi* results, PCR detect *T. evansi* in 12 and 15 CATT positive and negative camels, respectively, with low agreement ($Kappa=0.1$). The use of cymelarsan and quinapyrimine sulfate in the treatment of naturally infected cases demonstrated a very efficient therapeutic response.

Conclusion It was found that

1. Comparing the CATT/*T. evansi* and PCR results, the positivity of CATT was higher than PCR detection, while the agreement was poor ($K=0.1$).
2. Cymelarsan and aquin-1.5 proved to be effective in the treatment of naturally infected camels, but cymelarsan presented with higher effectiveness (100%) than aquin-treated camels (83.3%).
3. The use of cymelarsan and CATT is recommended for disease treatment and control.

Keywords *Trypanosoma evansi* · RoTat 1.2VSG PCR · CATT/*T. evansi* · Cymelarsan

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Introduction

Trypanosoma evansi (*T. evansi*) is a subspecies of the Trypanozoon subgenus, which also includes *T. equiperdum* and *T. brucei* [1, 2]. It causes a disease called “Surra” that affects camels and domestic animals, as well as wild animals. The disease is prevalent in Africa and Asia, and to a lesser extent in Europe [3, 4], where *T. evansi* was recently reported in camels and horses in limited region in the Spanish mainland and France [5–7]. It is mechanically transmitted by biting flies and divided into types A and B. Type A has RoTat 1.2 VSG gene and is more common in Africa, Asia and Latin America in a wide host range and

type B lacks RoTat 1.2 gene and is restricted to dromedary camels in Kenya, Sudan, Chad, and Ethiopia [4, 8, 12].

Camel surra is commonly asymptomatic, however, can be fatal if not accurately diagnosed, and treated early [4]. The clinical picture ranges from the acute clinical form together with increased mortality to the chronic form, which is characterized by a significant effect on camels' health and their ability to work and productivity as well as financial losses in association to fertility impairment and trade limitations [13–15].

Over the past years, parasitological and serological diagnosis have been common, but their sensitivity and specificity have not been sufficient. However recently, the development and progress of molecular methods have overcome the limitation of parasitological and serological methods [13, 15] []. Molecular techniques provide better results when considering the diagnosis of *T. evansi* infection. This is due to its increased sensitivity and specificity that rely on its ability to detect acute and chronic infections, particularly low levels of parasitemia. Several primers were used targeting various regions such as ribosomal DNA, internal transcribed spacer region, and variable surface glycoprotein genes [19, 21]. However, the RoTat 1.2 VSG gene is a unique marker specific for the recognition of *T. evansi* by PCR [22, 23].

T. evansi spreads continuously towards the east, in the Arabian Peninsula, including Saudi Arabia and the neighbor countries [4, 14]. In Saudi Arabia, previous epidemiological, serological, and parasitological studies have been conducted in camels from Riyadh, Qassim, Eastern, Tabouk, Jazan, Al-Jouf and Northern Frontiers [20, 28]. However, there is little data on trypanosomiasis infections in Saudi, especially the Eastern region at the molecular level except recent studies reported the parasite by PCR targeting ITS-1 and RoTat 1.2 VSG genes in a dog and camels in Riyadh, Qassim and Taif [14, 26, 27]. Therefore, the present study was conducted to determine the prevalence of *T. evansi* in camels from Eastern region of Saudi Arabia, using parasitological, serological, and PCR targeting the RoTat 1.2 VSG gene and to study the effect of the most common drugs used in the field as cymelarsan and quinapyrmine (aquin-1.5) drugs for treatment of the natural infected cases. This study able to provide epidemiological information on the increase in the spread of trypanosomiasis in camels in eastern Saudi, which need more observation and interference to reduce the disease and increase the awareness of the farmers of the importance of periodic testing to early detection of the disease and treatment the infected cases. The study also aimed to contribute to the prevention and control of the disease among herds of camels, which are the main supply for the meat and milk for the Saudi peoples, and to reduce productive economic losses.

Materials and Methods

Animals and Sampling

The current study was carried out in Dammam which is in the Eastern region of Saudi Arabia. The number of camels in the Eastern province reached 20,578, according to General Authority for Statistics, Saudi Arabia (<https://www.stats.gov.sa/en>). Dammam's climate is classified as hot desert according to the Koppen and Geiger classification. The annual average temperature is 26.4 °C.

Cross-sectional research was conducted in 10 private farms between July 2020 and July 2021. Sampling size, was established at expected 95% confidence interval and 5% margin of error using Maple Tech International LLC (<https://www.calculator.net/sample-size-calculator.html>) formula:

$$n = \frac{1.96P(1 - P)}{\epsilon^2}$$

where n is the needed sample size, P is the population proportion and ϵ is the margin of error and at an expected prevalence of 22.2% [28] the sample size required was at least 263 camels. However, the size of the sample was increased to 350 camels to improve the accuracy of diagnostic estimates [29]. Five mL of blood were drawn from the jugular vein using vacutainer tubes without anticoagulant and then left at room temperature for clot formation and serum collection. Then serum sample, was stored at -20 °C until use in serological test (CAT/*T. evansi*). Additionally, five mL of whole blood were collected in vacutainer tubes with EDTA for PCR and blood smear.

Thin Blood Smear (TBS)

Blood samples collected in EDTA tubes from all camels were tested for *Trypanosoma evansi* using Giemsa-stain thin blood smear.

Card Agglutination Test for *T. evansi* (CAT/*T. evansi*)

Serum samples were collected from all 350 camels and tested with CATT/*T. evansi*. The test was performed as the instructions of the manufacturer (laboratory of serology, institute of tropical medicine, Antwerp, Belgium). Briefly, on a test area of the card put 25 µl of diluted serum sample, diluted up to 1:4 in CAT-buffer, then add one drop (about 45 µl) of the homogenized CAT antigen in each test area onto a plastic-coated test card, the reaction mixture was spread out using a clean stirring rod and allowed to react on a card with help of rotation for 5 min. Negative and positive

controls provided by the kit were included in each test. Appearance blue granular deposits reveal a positive reaction (visible to the naked eye).

DNA Extraction

DNA of each blood sample (200 µl) was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions and DNA extract was kept at -20°C until further use.

PCR Assays

Species-specific PCR assay was used to identify *T. evansi* type A using Rode *Trypanozoon* antigen type 1.2 variant variable surface glycoprotein (RoTat1.2 VSG) gene [10], using forward primer (5'-GCGGGGTGTTTAAAGCAATA-3') and reverse primer (5'-ATTAGTGCTGCGTGTGTTGCG-3'), amplifying a 205 bp PCR product. PCR was performed in a 25 µl reaction volume including 12.5 µl Emerald Amp Max PCR Master Mix 2X (Thermo Scientific™, UK), 1 µl of each primer (20 pmol), 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied BioSystem 2720 thermal cycler (Thermo Scientific™, UK) with initial cycle at 94°C for 5 min, and then 35 cycles of denaturation at 94°C for 30s, annealing at 52°C for 30s, and extension at 72°C for 30s and final extension for 7 min at 72°C . The 10 µl PCR product was separated by gel electrophoresis using a 1.5% agarose gel (Thermo Scientific™, UK) in 1X TBE buffer, stained with ethidium bromide (0.5 µg/ml). Gene-ruler 100 bp ladder (Thermo Scientific™, UK) was used and the gel was photographed by a gel documentation system (Thermo Scientific™, UK).

Trials of Treatment

The 24 infected camels by TBS were divided into two groups, one group treated intramuscularly with cymelarsan (Rone Merial, France) at two doses of 0.25 mg/kg body weight/72 h interval (12 camels), and the second group treated with quinapyramine sulfate (aquin-1.5) (Nicholas primal, India) at two doses of 4 mg/kg/72 h interval (12 camels). The selected two drugs in this study due to the most common commercial drugs used in the field to treatment and control the disease among infected camels. Camels were examined every 2 weeks by PCR for 3 months.

Statistical Analysis

Chi-square test was calculated to evaluate significant differences ($p < 0.05$) between the results of TBS and PCR in *T. evansi* detection in camels using online statistical tools (socsistatistics.com), whereas the kappa agreement test was

calculated using online statistical tools (<http://vassarstats.net/kappa.html>).

Results

Among 350 camels tested, 24 (6.9%) were positive by blood smears examination (Fig. 1) and 27 (7.7%) were positive by RoTat 1.2 VSG PCR (Supplementary data) and the differences are not statistically significant ($p = 0.7$). Based on CATT/*T. evansi* findings, seroprevalence was 32.8% (115/350).

Comparing the Positivity of Blood Smear, CAT/*T. evansi* and PCR

All the 24 blood samples positive by blood smear were also positive by PCR (100%). Although 326 samples were negative in the blood smear examination, 3 were positive by PCR, giving the total PCR positivity to be 27 samples, and the agreement was a very good ($K = 0.9$) in the case of these samples. Of the 115 seropositive sera, 12 (10.4%) were PCR-positive and 15 (6.4%) out 235 CAT-negative samples were PCR positive, and the agreement was poor ($K = 0.1$). Of the 220 seronegative samples, none was positive by RoTat 1.2 VSG PCR, which was consistent with the putative absence of infection in these camels and confirmed the test specificity. Additionally, 15 seronegative camels were PCR-positive, which confirming that those camels are in an early stage of infection (Fig. 2).

Post Treatment Results

PCR test after three weeks of treatment showed no detectable *T. evansi* in all (100%) camels in the cymelarsan-treated group and the other group treated with aquin-1.5 showed

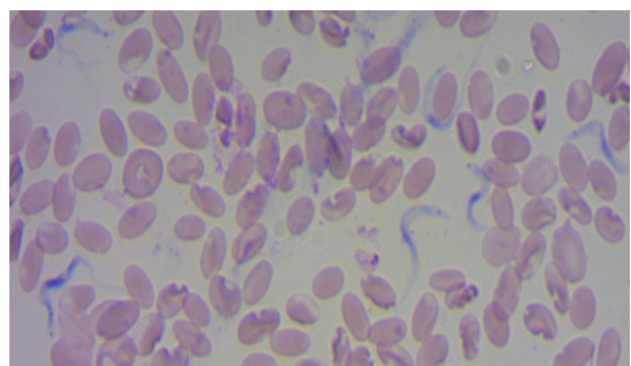


Fig. 1 Blood smear stained by Giemsa stain showing *Trypanosome evansi* X1000

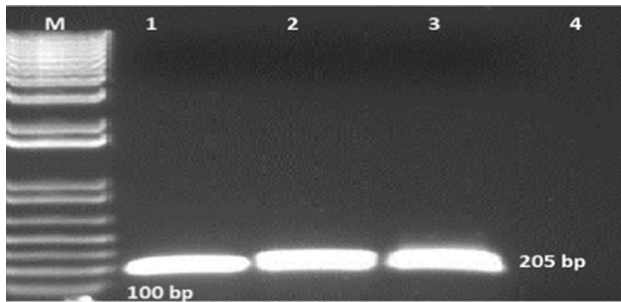


Fig. 2 PCR detection of *T. evansi* using RoTat 1.2 VSG PCR (205 bp) product, Lanes (1 and 2) indicate positive results, Lane 3 (positive control), Lane 4 (Negative control), Lane M, DNA ladder (100 bp)

no *T. evansi* in 10 (83.3%) camels and two camels stayed positive.

Discussion

In the current study, we investigated the prevalence of *T. evansi* in camels in the Dammam, Saudi Arabia using various diagnostic tests including detection of the parasite, CAT/*T. evansi* and RoTat 1.2 VSG PCR.

We observed that the prevalence of *T. evansi* varies from low (6.9%) using TBS to high (32.8%) using CAT/*T. evansi*. Previous studies from Saudi reported TBS prevalence ranged between 0 and 40% [14,24,25,27,30]. Further, a lower TBS prevalence has been reported compared to our results from different regions of the world, 0.7% in Pakistan [13] and 5.3% in Nigeria [15]. On the other hand, a higher TBS-based prevalence was reported in Egypt (12%) using parasitological methods [17]. The same authors reported a higher PCR and serology prevalence of *T. evansi*.

Prevalence of the parasite estimated by PCR was not significantly much higher (7.7%, $p=0.7$) than Giemsa-stained blood smear (6.9%), which is expected to present a high sensitivity of PCR, a finding agrees with previous reports by [13, 15, 27]. The slight increase in positivity for the PCR assay than the stained blood smear could be attributed to it detects low levels of parasitaemia and/or chronic stage of the infection [15, 17, 31]. A previous study concluded that the PCR testing provides accurate results with greater sensitivity and specificity than traditional parasitological methods and serology and that it overcomes non-specific reactions for serological diagnosis [15]. The PCR estimates a prevalence of (7.7%) in Dammam which was lower than that observed in camels in other regions of Saudi Arabia, where it was 9.8% in Taif, 46% in Qassim, 39% in Riyadh and 25% in Al-Jouf [20, 26, 27]. Also, the detected prevalence in present study was lower than that reported in dogs in Riyadh [14], these controversial results are probably due to variability in

vector activity, season of sample collection, and ecology of the animals under study [32].

Although the CAT/*T. evansi* assay identified more cases (32.8%) among the examined camels studied than using the PCR and TBS, this serological test was unable to distinguish between active cases and recovered or treated cases, as the antibodies remain in detectable levels in the blood circulation up to 4 weeks after infection and treatment constituting a diagnostic problem [13, 15]. Necessitating the collecting of full history of previous infection and treatment with antitrypanosome drugs as well as combined with appropriate diagnostic test. In this study, although there was poor agreement between CAT and PCR, 12 seropositive and 15 seronegative samples were positive in the PCR test, except for 220 samples were negative in CATT and PCR, which confirm the absence of the infection in these camels and confirmed the tests specificity targeting RoTat 1.2 VSG gene. Furthermore, differences in the positivity rate of each test may be due to false negative PCR results in the case of very low parasitemia and/or the presence of non-RoTat 1.2VSG *T. evansi*, as well as false positivity of the CATT due to treatment [33–35].

In present study, cymelarsan at 0.25 mg/kg body weight IM showed a 100% therapeutic effect by eliminating all trypanosomes confirmed by negative PCR results in all tested camels within 3 weeks after treatment compared to aquin-1.5, which showed an efficacy of 83.3%. The finding of cymelarsan at 0.25 mg/kg body weight IM is endorsed by a previous study from Sudan. Despite high efficacy of both drugs against pathogenic trypanosomes, both drugs appear to be toxic and the toxicity of these drugs remains a major problem with their use in the chemotherapy [36] and should be addressed in future studies. Treating sick camels with quinapyramine showed the existence of two infected cases post treatment using the PCR, and this may be attributed to drug resistant. The finding confirms the results of who found in Sudan, a resistant cases of *T. evansi* against quinapyramine under field conditions, he also recorded a side effect of treated animals. The use of cymelarsan is recommended in treatment while using of quinapyramine required repeated examination to detect the resistant cases not responded to treatment.

Conclusions

This study emphasizes that *T. evansi* infection is a possible threat to the health of camels in Dammam, Saudi Arabia, and that the disease is prevalent either by clinical course or by laboratory diagnosis. There is a difference in the rate of CAT/*T. evansi* positivity when compared with the results of blood smear and RoTat 1.2VSG PCR used in such a study, indicating the need for additional studies to provide

high-quality molecular and epidemiological data, and the use of cymelarsan in treatment of clinical cases (acute or chronic cases) of surra without side effects is recommended.

The data revealed a high prevalence of *T. evansi* and are relevant for *T. evansi* surveys in different regions and hosts for disease prevention and control.

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Author Contributions All authors contributed to the study conception, design, material preparation, data collection and analysis. All authors read and approved the final manuscript.

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Data Availability The data used in this manuscript are publicly available.

Code Availability The code will be made available under request to the corresponding authors.

Declarations

Conflict of Interests The authors have declared no competing of interest.

Ethical Approval This research did not include any experimentation on animals and all collection procedures were performed for the camels benefit and standard diagnostic purposes. All methods used in this study were carried out in accordance with relevant guidelines and regulations. The study protocols were approved by the ethical committee on the animal experiments of the ministry of environment, water, and agriculture, Saudi Arabia. The study was carried out in compliance with the ARRIVE guidelines.

Informed consent Not applicable.

Consent for publication Not applicable.

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