

## ABSTRACT

Surra is a wasting disease of multiple hosts and has a worldwide distribution. It is associated with great economic losses and adversely affects the health, working capacity, and productivity of animals. *Trypanosoma evansi*, the causative agent, is principally transmitted by hematophagous flies (*Tabanus*, *Stomoxys*, *Chrysops*, *Haematopota*, *Haematobia* and *Atylotus*).

In a cross sectional study, 1005 dromedary camels from the districts RahimYar Khan, Bahawalpur and Bahawalnagar in the Cholistan Desert were sampled to assess the prevalence of trypanosomosis due to *Trypanosoma (T.) evansi* (surra). Parasitological (Giemsa/Field stained thin smear), serological (formol gel test, CATT/*T. evansi*, ELISA/VSG RoTat 1.2, immune trypanolysis) and molecular tests (TBR1/2 PCR and RoTat 1.2 PCR) were employed to screen for *T. evansi* infection in different breeds, age groups and genders.

The overall prevalence of surra was 0.7% (95% CI: 0.08-1.30) with Giemsa stained thin smears (GST), 40.1% (95% CI: 37.07-43.13) with formol gel test, 47.7% (95% CI: 44.57-50.75) with CATT/ *T. evansi*, 44.2% (95% CI: 41.11-47.25) with ELISA/VSG RoTat 1.2, 40.0% (95% CI: 36.87-42.93) with immune trypanolysis, 31.9% (95% CI: 28.80-34.50) with TBR1/2 PCR and 30.5% (95% CI: 27.40-33.10) with RoTat1.2 PCR. The current study did not find molecular evidence for the presence of *T. evansi* type B in the studied population. Based on serological and molecular prevalences, this study declares the Cholistan Desert to be a high risk area for *T. evansi*. According to TBR1/2 PCR, the camels at Bahawalnagar are approximately two times more likely to be infected than those at Bahawalpur (OR = 1.9; 95% CI: 1.30-2.75). According to TL, the camels at Bahawalpur are approximately two times more likely to be infected than those at Rahimyaar Khan (OR = 2.1; 95% CI: 1.20-1.68). The test agreement of TL was moderate with CATT/*T. evansi* ( $k = 0.43$ ; 95% CI: 0.378-0.489) and ELISA/VSG RoTat 1.2 ( $k = 0.54$ ; 95% CI: 0.489-0.594) and was poor with the other tests. Test agreement between TBR1/2 and RoTat1.2 PCR was almost perfect ( $k = 0.96$ ; 95% CI: 0.950-0.984).

Combining all clinical symptoms, a moderate association between molecular prevalence ( $k = 0.4\%$ , 95% CI: 0.38-0.46) and a poor association between seroprevalence ( $k = 0.1\%$ , 95% CI: 0.071-0.182) was recorded. Using immune trypanolysis as a gold standard for antibodies, it was found that camels positive for *T. evansi* are 11.6 times more likely to be found in a poor body condition than those tested negative in immune trypanolysis. Using different tests, we also detected infection in goats, sheep, horses, cattle, deer and buffaloes from the same area.

Using different methods of detection, pathological aspects of surra in camels was manifested by the altered levels of hematological and serological parameters. Hematological parameters showed a significant decrease in red blood cells (RBCs), hemoglobin (Hb), lymphocytes % and mean cell hemoglobin concentration (MCHC) whereas significant increased levels of white blood cells (WBCs), mean cell volume (MCV), mean cell hemoglobin (MCH), neutrophils % eosinophils % were detected using different tests. A significant decrease was noted for levels of different biochemical parameters such as albumin, iron and alkaline phosphatase (ALP) whereas significant increased levels of aspartate transaminase (AST), alanine transaminase (ALT) and total serum proteins were recorded between *T. evansi* positive and negative camels

Reported vectors of surra, *Tabanus striatus*, *Tabanus rubidus*, *Tabanus megalops*, *Atylotus agrestis*, *Chrysops dispar* and *Stomoxys calcitrans* have been identified at Cholistan desert. *T. evansi* Type A was detected in *Tabanus striatus* and *Stomoxys calcitrans*. Organ preparations (mouthparts + gut) were found to be a better source of DNA than whole fly preparations for detecting trypanosomes in flies. The role of *Haematobia* as a potential vector of surra cannot be denied in Cholistan desert. Further, blood meal analysis of the gut preparations of flies and abundance of proposed vectors on camels, goats, sheep and cattle and hen suggests the possible role of these flies in transmission of the disease and is needed to be further investigated.

The current study is a first and novel work on the prevalence of surra in the Pakistan using a broad variety of diagnostic tests. The study supports the use of antibody detection tests (ELISA, CATT and TL), rather than parasitological and molecular examination, to assess surra prevalence in camels. It also calls for implementation of measures to control surra in the Cholistan. Because of unsuccessful

isolation of *T. evansi* during the studies, we recommend to isolate the *T. evansi* strains circulating in the Cholistan Desert for extended studies on their pathogenicity, genetic relationship with strains from other geographical origin and drug sensitivity. It is further recommend to carry out detailed studies on monitoring vector capacity of different hematophagous flies along with their routine activity patterns, relative abundance, and to carry out routine entomological surveys to establish control measures for the control of diseases.