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# Evaluation of various diagnostic techniques for *Trypanosoma evansi* infections in naturally infected camels

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**Abstract**

One hundred and eight camels (*Camelus dromedarius*) from *Trypanosoma evansi* endemic areas of the Thar Desert of Rajasthan State, India, were evaluated by various diagnostic tests including parasitological tests (wet blood film – WBF, stained thick blood film), chemical test (mercuric chloride), biological test (mouse subinoculation – MSI), and immunodiagnostic tests based on antibody detection (double immunodiffusion test – DID, card agglutination test – CATT), antigen detection (double antibody sandwich enzyme linked immunosorbent assay – Ag-ELISA). Of the tested camels 49 were found infected using the WBF of which nine gave false negative results with the mercuric chloride test. The efficacy of MSI was 87.03 percent, while the mercuric chloride test was 60.18 percent efficient. The diagnostic efficacy of CATT (72.22 percent) was found to be much better than DID (28.70 percent). Ag-ELISA was 86.11 percent efficient in detecting trypanosomal antigens. A good correlation was found between the positive results obtained by wet blood film, CATT and Ag-ELISA. It was inferred that CATT can be used to study the seroprevalenee of *T. evansi* with great ease, however, trypanosome antigen detection may give a more accurate idea of the prevalence of *T. evansi* in an endemic area.

*Keywords:* Dromedary camel; *Trypanosoma evansi*; Trypanosomosis; Diagnosis; Biological test; Mercuric chloride test; Card agglutination test; Double antibody sandwich enzyme linked immunosorbent assay

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## 1. Introduction

Trypanosomosis (surra), caused by *Trypanosoma evansi*, is one of the major parasitic diseases of camels causing 30 percent morbidity and 5 percent mortality (Higgins, 1983; Luckins, 1992; Pathak and Khanna, 1995). Surra runs a variable clinical course depending on the virulence of the parasite and the susceptibility of the host. It is difficult to diagnose surra because the clinical signs are varied and non-specific (Killick-Kendrick, 1968). Parasitaemia is intermittent, and it is not always possible to find trypanosomes in blood by wet smears examination (Nantulya, 1994). The objective of this investigation was to evaluate several diagnostic techniques for the diagnosis of surra in naturally infected camels and assess their suitability as epizootiological tools in cameline trypanosomosis survey.

## 2. Materials and methods

One hundred and eight camels (age 1–5 years), suspected of surra, from *Trypanosoma evansi* endemic areas of the Thar Desert namely Tanwarwala, Phattuwala, Barsalpur and Gajjewala villages of the Bikaner District of the Rajasthan State, India located near the India–Pakistan border, approximately 200 km away from Bikaner town, were selected for the study. Blood samples were collected from a jugular vein. Heparinized whole blood was used for detection of parasitaemia while serum from the blood of these animals was used for serological and chemical tests.

### *2.1. Direct detection of parasitaemia*

Approximately 0.2 ml blood from each sample was used for preparing wet blood films and Giemsa stained smears (MacLenan, 1957). The parasite was identified morphologically.

### *2.2. Mouse subinoculation (MSI) test*

The test was carried out as described by Godfrey and Killick-Kendrick (1962). Approximately 0.5 ml whole blood from each suspected camel was inoculated intraperitoneally in laboratory raised Swiss albino mice. The tail blood was drawn daily until 21 days post-inoculation and examined for the presence of *T. evansi* in the peripheral blood.

### *2.3. Mercuric chloride test*

The test was carried out as per the method of Gatt Rutter (1967).

### *2.4. Antigen preparation*

Trypanosome antigen was prepared from *T. evansi* originally isolated from a camel and maintained in dogs. Trypanosomes were separated from the blood of dogs (Purohit

and Jatkar, 1979) and whole cell soluble antigen was prepared (Pathak et al., 1993). The protein content of the antigen was determined by the method of Lowery et al. (1951). A drop of merthiolate was added to the antigens and stored at −20°C until further use.

### 2.5. Preparation of hyperimmune serum

Laboratory raised rabbits were immunized with the whole cell soluble antigen of *T. evansi* using 4 subcutaneous injections with doses gradually increasing from 1.0 to 3.0 ml, given 6 days apart. The rabbits were bled by cardiac puncture 7 days after the last injection; the serum was separated and stored at −20°C.

### 2.6. Preparation of conjugate

The immunoglobulin fractions of the hyperimmune rabbit serum were precipitated using 50 percent saturated ammonium sulphate. The precipitated globulins were washed twice with 50 percent saturated ammonium sulphate, dissolved in phosphate buffered saline (PBS) (pH 7.2) and then dialysed against PBS at 4°C. The protein content of the globulin preparations was determined photometrically and the antisera were then stored at −20°C. Each globulin preparation was conjugated with enzyme horseradish peroxidase (Sigma, St. Louis, MO) using the periodate method described by Wilson and Nakane (1978).

### 2.7. Double immunodiffusion (DID) test

The DID was conducted by the technique of Ouchterlony (1962) using somatic crude antigen and was first standardized with hyperimmune rabbit serum raised against the whole cell soluble *T. evansi* antigen.

### 2.8. Card agglutination test for trypanosomosis (CATT)

The serum samples were evaluated for the presence of antibodies against trypanosomes with CATT kit developed and supplied by the Institute of Tropical Medicine, Laboratory of Serology, Antwerp, Belgium. 50 μl of *T. evansi* RoTat 1.2 antigen mixed with 25 μl of camel serum diluted to 1 : 8 with PBS (pH 7.2) on the supplied cards. These were agitated in a circular motion for 5 min. The samples with blue granules were considered positive for antibodies against *T. evansi*.

### 2.9. Enzyme linked immunosorbent assay (ELISA)

The presence of antigen was detected by a sandwich enzyme linked immunosorbent assay (Ag-ELISA) technique described by Bidwell and Voller (1981) and modified by Pathak et al. (1993). Controls were included with each plate consisting of wells reacted with substrate only, negative control wells had serum from uninfected animals shown to be parasitologically negative by the mouse subinoculation test, to which antigen of *T.*

Table 1
Efficacy of various diagnostic techniques in 108 camels clinically suspected of *Trypanosoma evansi* infection

| Parameters | Clinically suspected cases | Number of positive cases | Percent positive cases |
|---|---|---|---|
| Wet blood film exam. | 108 | 49 | 45.37 |
| Thick blood film exam. | 108 | 42 | 38.88 |
| Mercuric chloride test | 108 | 45 | 60.18 |
| MSI test | 108 | 94 | 87.03 |
| DID test | 108 | 31 | 28.70 |
| CATT | 108 | 78 | 72.72 |
| Ag-ELISA | 108 | 93 | 86.11 |

*evansi* had been added. An optical density (OD) value of more than 0.209 measured photometrically at 450 nm was considered as the cut off value for the positive sera.

## 3. Results

Confirmed morphologically, *T. evansi* was found to be the causal organism in all the camels positive for trypanosome infection, out of the 108 camels clinically suspected of surra.

Table 1 summarizes the number and percentage of camels found positive for trypanosome infection employing different tests. 38 camels which were not positive by wet blood film examination and thick blood film examination were positive by the mercuric chloride test while out of 49 parasitologically confirmed cases, nine gave negative results with the test. In three camels the mercuric chloride test gave negative results while these camels were positive by MSI, CATT and Ag-ELISA. On the other hand two camels which were negative by all other tests were positive by the mercuric chloride test. MSI gave positive results in six camels in spite of the fact that these camels were tested negative with the other tests.

In Ag-ELISA the OD varied from 0.077 to 0.300 (mean $0.266 \pm 0.021$) for negative control sera, while for *T. evansi* positive sera, the OD varied from 0.310 to 0.461 (mean $0.445 \pm 0.002$). There was a highly significant difference between the mean Ag-ELISA optical density values obtained for parasite positive and healthy camels. Out of the 49 parasitologically confirmed camels, four did not show detectable circulating antigen. Six camels found negative by MSI gave a positive reaction for antigenaemia.

## 4. Discussion

The present investigation documents cameline trypanosomosis in the western part of Rajasthan. The percent efficacy of wet and thick blood film in the present study was 45.37 and 38.88 percent respectively, which is similar to earlier reports (Killick-Kendrick, 1968; Raisinghani and Lodha, 1989).

The mercuric chloride test has an excellent record of detecting camel surra for the last 60–70 years (Bennett, 1929; Raisinghani and Lodha, 1989). Though the mercuric chloride test, in the present study, appeared to be a reliable test as 30 camels which were

not positive by wet blood film examination were found to be positive by the mercuric chloride test it also gave negative and doubtful results. Pegram and Scott (1976) also found similar results using the mercuric chloride test in cameline surra and got a poor correlation between this test and the patent parasitaemia. Goel and Singh (1971) and Boid et al. (1985) found the mercuric chloride test unsuitable as it gave non-specific reactions. In the present study in a few animals which were positive by wet blood film examination and the thick blood smear test, the mercuric chloride test was negative.

In the case of many camels, trypanosomes, though invisible in the wet blood film and the thick blood film, were detected by bioassay in mice. Though the efficacy of MSI was found to be 87.03 percent, it can not be recommended for field diagnosis due to the cost of the laboratory animals, lack of such facilities in the field and the time taken (at least 3 days) to reveal the infectivity of the samples. However, in the laboratory it can be used as a confirmatory test.

In the present study the low efficacy of DID may be due to the fact that in some young camels, inadequately mobilized defence mechanisms are unable to increase the globulin contents, and the use of crude somatic antigen showed also a lower efficacy. CATT is a new commercial assay in which antigen consists of Trypanosome RoTat 1.2, a variable surface antigen type common to all *T. evansi* stocks. The antigens are agglutinated by antibodies. A better correlation was found between the percentage of positive results obtained with CATT and the presence of trypanosomes in the blood. The CATT has advantage over the DID test by being a test quick, easy to perform and to read. Therefore, the test can be used to study the seroprevalence of *T. evansi* with great ease and is sensitive under field conditions (Diall et al., 1994).

In the present study detection of circulating antigen of *T. evansi* was found to be a more sensitive means (86.11 percent) of practical diagnosis and a more reliable method of detection of current and latent infections. Rae and Luckins (1984) reported that in infected camels trypanosomal antigen detection is a better method because antibody levels decline slowly following trypanosomal drug therapy and remain high for many weeks after the parasites have been eliminated (Luckins et al., 1979). The detection of antigenaemia indicates the current status of the infection as well as detects the latent infection (Nantulya et al., 1989; Waitumbi and Nantulya, 1993). As an epidemiological tool for use in the survey of trypanosomosis, the test could prove to be a useful immunodiagnostic test for active trypanosome infection (Pathak et al., 1993). The test could be extremely effective since it does not require access to freshly collected blood. There were five cases in which sera from parasitologically proven infection did not show detectable circulating antigen. Similar findings have been reported by Waitumbi and Nantulya (1993). According to them the possible explanation could be that during the trypanosome multiplication phase, there may be parasite destruction insufficient to produce detectable levels of antigen in circulation and therefore the test would produce false negative results.

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