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The Pharma Innovation

**ISSN (E): 2277- 7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.23**
TPI 2021; SP-10(11): 1896-1900

www.thepharmajournal.com
Received: 28-09-2021
Accepted: 30-10-2021

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# A review of *Trypanosoma evansi* diagnosis

**Pradeep Kumar, Ravi Kumar, Ramkaran and Sukhdeep Vohra**

**Abstract**
Trypanosomiasis caused by *Trypanosoma evansi* is the most widely distributed pathogenic, mechanically transmitted vector borne haemoprotozoan disease of domestic livestock and wild animals in India. In tropical countries like India the disease is also called as *Surra* and is transmitted through a non-cyclical method by biting flies such as a Tabanidae. The disease can affect almost all species of mammals but from economic and zoonotic point of view, trypanosomiasis is an important disease of cattle, buffaloes, sheep, goats, horses and camels. It is a potential killer of livestock and causes economic losses to the farmers in terms of morbidity, mortality, abortion, infertility, reduced milk yield and fever. The diagnosis of trypanosomosis depends upon series of tests viz. direct microscopic examination (wet blood film, stained blood smears), concentration methods (hematocrit centrifugation technique), animal inoculation (using rats or mice), detection of trypanosome DNA (DNA probes and polymerase chain reaction), serological tests like IFAT, ELISA, card agglutination tests, latex agglutination tests and immune trypanolysis tests. The various available tests for leptospirosis diagnosis have been explained in this article.

**Keywords:** *Trypanosoma*, blood smear, haemoprotozoan, livestock, serological tests

## Introduction
*Trypanosomiasis* caused by *T. evansi*, commonly termed as *surra*, has wide host range and several wild or domestic animal including buffaloes carry latent infection and act as a source of infection for susceptible animals. Important symptoms observed in acute form are intermitted fever, redness and staring of eyes, lacrimation, drop in milk yield, deep and prolonged breathing, nervous symptoms such as circling movement or beating head to mangers, shivering of muscles, coma, collapse and death. There is dullness, lacrimation, intermitting rise in temperature, oedema of dependent parts of body and legs and abortion may occur. Besides causing economic losses, it also causes high mortality (20-90%) in valuable animals.

*T. evansi* is pathogenic to most of domestic animals but its effect on different hosts varies according to the virulence of the strain of parasite, the susceptibility of the host species and the local epidemiological conditions (Hoare, 1972; O.I.E. 2004) [17, 31]. Diseases may occur as an acute or chronic condition, and in the latter cases may persist for many months. Generally the disease is acute in camel, horse, donkey, mules and chronic in cattle, buffaloes and dogs. The clinical signs of *surra* are not sufficiently pathognomonic and diagnosis must be confirmed by laboratory methods. The disease in susceptible animals is manifested by pyrexia, progressive anaemia, loss of condition and lassitude. Recurrent episodes of fever and parasitaemia occur during the course of the disease. Oedema, particularly of the lower parts of the body, urticarial plaques and petechial haemorrhages of the serous membranes are often observed. Abortions have been reported in buffaloes in Asia (Lohr *et al.*, 1986) [24]. There are indications that the disease may cause immunodeficiency (Onah *et al.*, 1998) [32].

Trypanosomes consume large quantity of the blood sugar resulting in the breakdown of the liver function and the resultant hypoglycemia and fatal intoxication. There is an increase in the lactic acid production in the blood, which interferes with the normal absorption of oxygen by the haemoglobin resulting in asphyxiation. Mechanical blocking of the lung capillaries and pulmonary oedema by the obstruction of the capillaries by agglutinated trypanosomes leads to asphyxiation with fatal termination (Pathak and Singh, 2005) [38].

Economic losses caused by *surra* in Asia may be higher than those caused by the African trypanosomes, which are estimated to be US $ 1.3 billion in relation to the cost of meat and milk (Gupta, 2007) [15]. Most of the direct losses in the animals are due to mortality, cost of chemotherapeutic and chemoprophylactic interventions. The indirect losses due to infection have not been adequately defined, but remained an important factor in the field due to failure

of vaccination against bacterial and viral infections, due to severe immuno-suppression produced by the disease (Holmes, 1980). Further losses are occurring through abortions and reduced working capacity of the draught animals.

**Historical perspective**

*Trypanosoma evansi*, the causative agent of '*surra*', was the first pathogenic salivarian trypanosome discovered in India. *Surra* has been known in India since time immemorial, but its aetiology was first brought to the notice of the rest of the world by a British Veterinarian Griffith Evans in 1880. He discovered the parasite in the blood of equines and camels in Dera Ismail Khan District in erstwhile Punjab (now in Pakistan). The parasite was also reported by Steel as a spirochaete in 1885 in the blood of mules in Burma. Crookshank (1886) examined the smears of camel blood received from Evans and identified the parasite as a flagellate and first named it as *Haematomonas evansi* and then as *Trichomonas evansi.* This flagellate responsible for *surra* to the genus *Trypanosoma* (Hoare, 1972; Stephen, 1986; Gill, 1991) [17, 52, 11]. Hoare (1964) [16] divided the genus *Trypanosoma* into two sections as Stercoraria and Salivaria based on their development in insect vectors. Mechanically transmitted trypanosomes *viz*. *Trypanosoma evansi, T. equinum* and *T. equiperdum* are further classified under sub-genus Trypanozoon (Soulsby, 2005) [51].

Hoare (1972) [17] proposed that *T. evansi* might have originated from *T. brucei* infection. Camel was incriminated as the probable host which became infected with *T. brucei* in the marginal northern zones of tsetse fly infested area and spread the infection throughout northern Africa and then to Asia by camel caravans and through military campaigns (Loses, 1980). India is thought to be the major source from where, *surra* has spread within livestock throughout the continent of Asia and Islands of the Indian Oceans (Losos, 1980; Pathak, 1999) [25, 36]. Similarly, there was introduction of *T. evansi* from India to Australia when camels were imported from India to Port Hedland (Reid, 2002) [43]. *T. evansi* has been reported from warm and temperate countries that include North Africa, Egypt, Sudan, Somalia, Israel, Lebanon, Syria, Arabian Peninsula, Turkey, Iraq, China, Philippines, Indonesia, Mauritius, Central America and South America (Bhatia and Shah, 2001) [3].

*T. evansi* is transmitted mechanically by biting flies particularly of the genus *Tabanus*. The precise way in which *surra* is transmitted was described by Gill (1991) [11] who pointed out that the common disseminating agent of *surra* is *Tabanus striatus* and the mode of transmission is merely mechanical. He further described that this can occur only when the feeding is interrupted. There is no biological development of trypanosomes in the vector. The successful transmission can occur, if the transference of the parasite takes place within a few minutes after the bite. In addition to *Tabanus*, other haematophagus flies like *Lyperosia, Stomoxys* and *Haematopota* are also capable of transmitting *surra.*

In India, *T. evansi* infection is more prevalent, particularly in areas where the environmental conditions for the breeding of insect vectors like tabanid flies are congenial, thus being suitable for transmission. *Surra* is endemic in north India, particularly in Punjab, Haryana, Rajasthan and Uttar Pradesh. However, incidences are not common near the end of August and remain prevalent until midwinter, which coincides with the increase in number of blood sucking flies (Bhatia, 2000) [4].

**Prevalence of *T. evansi***

*Trypanosoma evansi* is present in India, China, Mongolia, Russia (from Kuibyshev, 53°N, to the Caucasus, 44°N), Bhutan, Nepal, Myanmar, Laos, Vietnam, Cambodia, Thailand, Malaysia, the Philippines, and Indonesia (Luckins, 1978) [26]. Muraleedharan *et al*. (1991) [29] reported a prevalence of trypanosomosis in buffaloes from Mandyai and Mysore districts of Karnataka and recorded the higher incidence in south-west monsoon (2.98%) followed by north-east monsoon (1.31%) and observed that the buffaloes of four to eight years age group were commonly affected. Krishnappa *et al.* (2002) [21] reported prevalence of trypanosomosis in domestic animals in Karnataka and analyzed that cattle were the commonly affected species followed by buffaloes and further reported that 38.46% occurrence of trypanosomosis was recorded in the age group of 9 to 10 years followed by 37.14% in one to two years and the lowest in the age group of 10 years and above.

The equine population in the endemic areas of *surra* in India had high prevalence in comparison to other species of domestic animals. In Mathura (U.P.), *surra* was responsible for 100% morbidity and 66.6% mortality rates (Kumar *et al.*, 1994) [22].

Season-wise highest incidence of *surra* was reported from July to December in the equines of Punjab (Soodan *et al.*, 1995) [50].

*T. evansi* was also recorded in the wild animals including circus tigers, cubs and elephant from Andhra Pradesh (Rao *et al.*, 1995) [50] and Orissa (Parija and Bhattacharya, 2001) [34] by stained blood smear examination.

**Diagnosis of *T. evansi* in domestic animals:**

Trypanosomosis is responsible for fluctuating nature of parasitaemia, which is often difficult to be detected by the commonly used parasitological methods. The situation becomes even worse in chronic infections, which are of common recurrence in cattle, buffaloes and camels. The limitations in terms of low sensitivity of parasitological diagnostic techniques had been a driving force for researchers to work out an alternative technique such as serological and DNA based methods, which have got a great potential for unequivocal identification of the causative agent with higher sensitivity. Hence, none of the tests, presently used is 100% sensitive and so for the foreseeable future, a combination of parasitological and serological tests will continue to be need for the diagnostic trypanosomiosis.

**Diagnosis of *T. evansi* by parasitological methods**

Wet blood film, stained blood smears and microhaematocrit technique (MHCT) are widely used parasitological methods for diagnosis of *T. evansi* infection.

Identification of *T. evansi* from the blood of suspected animals gives a definitive diagnosis but fluctuating level of parasitaemia in host makes this test insensitive as parasites cannot always be demonstrated (Nantulya, 1994) [30]. Even sero-positive animal may appear negative during routine parasitological tests (Gracia *et al.*, 2000) [14]. Microscopic examination of wet and dry preparations is most commonly practiced. Wet blood preparations donot require use of stains and parasites are readily seen because of their jerky movements under field microscopy at 400x. Detection of trypanosomes in wet blood preparation is difficult when the parasitaemia is less than 1 $x10^4$ per ml of blood (Turner, 1999) [54]. Dry preparations include Giemsa and Leishman

stained thin blood smears, thick blood films and buffy coat smears. Lymph node aspirates and cerebrospinal fluid smears can also be examined microscopically.

The advantage of blood smear test is that apart from light microscope no other special equipment is required, thus, making it a cheap and technically simple mean of detecting parasites particularly in *T. evansi* endemic countries (Killick-Kendrick, 1968) [20].

The MHCT involves centrifugation of blood in a capillary tube and microscopic examination of buffy coat-plasma interface for detecting the presence of motile *T. evansi* (Woo, 1969; Paris *et. al.*, 1982; Davison *et al.*, 2000; Reid *et al.*, 2001) [55, 35, 9, 44].

According to Reid *et al.*, (2001) [44], the limit of detection of the HCT is approximately 83 parasites per ml of blood. Mini anion exchange chromatography test (MAECT) with DEAE cellulose uses either whole blood or the buffy coat, which increase the sensitivity. Up to 1.25 trypanosomes in 2 ml of blood were detected when using buffy coat MAECT. All of these above mentioned tests are suggested for diagnostic use in animals during the first few months after infection when possibility of parasitaemia is the highest.

Mouse inoculation test is more sensitive than other parasitological tests (Reid *et al.*, 2001; Tuntasuvan *et al.*, 2003) [53, 44] and is generally considered the gold standard test, if buffy coat is used for inoculation rather than the whole heparinized blood (Reid *et al.*, 2001) [44]. In 95% of cases the sensitivity of mouse sub inoculation test was 3-25 trypanosomes per ml of blood and that was increased 10 fold by substituting the whole blood with buffy coat.

Direct microscopic tests must be performed as soon as possible after collection because trypanosomes get rapidly metabolized and lysed. Therefore, keeping them in cool, dark place or collection in Alsever's solution helps them to survive for a few hours (Chappuis *et al.*, 2005) [5]. The samples must be kept away from direct sunlight as it can kill the parasites. Likewise, MIT should also be performed as soon as possible; nevertheless, Reid *et al.*, (2001) [44], found that sensitivity of MIT was not measurably reduced with the infected blood having relatively high parasitaemia (25 *T. evansi* per ml of blood), which was refrigerated for up to 21 hours. This indicates that refrigerated transport of samples to a laboratory could be undertaken as a substitute of maintaining laboratory mice in the field.

Monoclonal antibody-based simple and field-oriented latex agglutination test (Suratex) was developed by Nantulya (1994) [30] for the detection of circulating invariant trypanosomal antigens in *T. evansi* infections. In an initial evaluation, Suratex detected the antigens in 53 (88.3%) of 60 blood samples collected from experimentally infected rabbits in comparison to 22 (36.7%) and 2 (2.3%) by buffy coat and WBF, respectively. Analysis of field sera from 2 camel herds, which were experiencing *T. evansi* outbreak, confirmed the superior sensitivity of Suratex.

Rayulu *et al.*, (2007) [42] developed monoclonal antibody-based immunoassays (LAT and Ag-ELISA) for the diagnosis of *T. evansi* antigens in domestic animals. The diagnostic sensitivity and diagnostic specificity were recorded as 95.38% and 59.74% for LAT and 83.08% and 67.14% for Ag-ELISA, respectively using MHCT as reference test, and 90.33% and 88.30% for LAT using Ag-ELISA as reference test. The mAb based LAT developed had several advantage over other tests for diagnosis of *T. evansi.*

**In laboratory animals**

High susceptibility of laboratory animals particularly mice and rats makes them suitable hosts for detecting sub-patent trypanosome infection and also for maintenance of infection through serial passages.

*T. evansi* isolates from cattle (Gobel and Denning, 1981) [13], buffaloes (Bansal *et al*., 1986) [1], ponies (Sarmah, 1998) [46] and camels (Pathak *et al.*, 1997; Shahardar *et al.*, 2002) [39, 48] have been maintained in laboratory animals by serial passages. Seven different stocks of *T. evansi* collected from buffaloes, cattle and horses were maintained and propagated in albino rats through serial passages of infected blood at 48-72 hour intervals (Singh *et al.*, 1995b) [49]. Mongrel dogs have also been used to maintain and propagate camel and cattle isolates of *T. evansi* (Pathak *et al.*, 1993 and Pareek *et al.*, 1999) [37, 33].

The mild strains of *T. evansi* were observed to be virulent after being passaged in mice and such strains could kill them within a week (Lingard, 1907) [23]. The prepatent period of *T. evansi* has been reported to be of 7 to 12 days in mice inoculated with a single parasite (Luckins *et al.*, 1978) [26].

**Serological tests**

Several serological tests have been developed to detect circulating antibodies in the serum samples of *T. evansi* infected/suspected animals for diagnosis. Complement fixation (CFT) test was used to detect *T. evansi* antibodies in camels (Schoening, 1924) [47], cattle and buffaloes (Manresa and Gonzalez, 1935) [27] and horses and mules (Randal and Schwartz, 1936) [40]. In India, CFT for diagnosis of *surra* was evaluated by Minnet (1946) [28] and Ray (1948) [41]. Complement Fixation Tests, however, are prone to interference by anti-complementary activity in sera from several animal species and there can be difficulties in the preparation of satisfactory complement fixing antigens (Gill, 1970; Sabanshiev, 1973) [12, 45].

Gill (1964) [10] observed that indirect haemagglutination test was more sensitive in the detection of anti-*T. evansi* antibodies but at the same time it failed to differentiate between homologous and heterologous antigens. Bansal and Pathak (1971) [2] employed gel diffusion test for demonstrating *T. evansi* antigen and antibody in sera of infected camels, horses and buffaloes. Jatkar and Purohit (1971) [18] used fluorescent antibody techniques to determine *T. evansi* antibodies in the infected sera.

Clausen (1986) [8] found that the micro-ELISA test was suitable for detecting trypanosome specific antibodies of both Ig classes (IgG and IgM) in camels experimentally infected with *T. evansi.* The antibodies were found in detectable levels in the sera of goats experimentally infected with *T. evansi* from 9 days to b17 weeks post infection by ELISA. These assays distinguished effectively between infected and non-infected camels.

**Conclusion**

Numerous tests are available to diagnose Trypanosomiasis. Depending on the situation appropriate test can be selected. Diagnosis of *T. evansi* usually starts with clinical symptoms. However, conclusive evidence of *T. evansi* infection relies on detection of the parasite directly in blood or by other indirect methods. The successful control of *surra* requires an integrated approach which includes immunodiagnostics methods, chemotherapy and vector control (Chaudhri, 2007) [6]. The choice of drug, dosage and route of administration

vary with the species affected, drug availability and presence or absence of drug resistance. Currently available drugs for the treatment of *surra* in domestic animals in India, include diminazine aceturate (Berenil), quinapyramine sulphate and quinapyramine chloride (Triquin) and Antrycide Prosalt (Juyal *et al*., 2007) [19]. Early case detection and prompt treatment and creating awareness about the disease among the people and public health professionals are the steps that could be taken to reduce the magnitude of the problem.

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