) Serological tests

Several methods have been used to detect specific antibodies to trypanosomal antigens, such as:

. **Indirect immunofluorescent antibody test (IFAT):** This test is time consuming and requires expert eye for the detection of actual and pseudo florescence. In this test, the blood from high parasitaemic animal is taken and slide is prepared for the source of antigen. Thereafter, such slides are treated with sera samples in different dilution from suspected animal followed by addition of fluorescent conjugate. In positive cases, there will be fluorescence and same will be absent in negative cases.

. **Enzyme linked immunosorbent assay (ELISA):** Different types of ELISA's namely indirect, competitive inhibition, antigen detection have been reported. Different antigens have been reported with varied efficacies. For example: Whole cell lysate (WCL) antigen, flagellar antigen, variable surface glycoprotein (VSG) and invariable surface glycoprotein (ISG) antigens etc. Through ELISA, both the presence of antigen and antibody in blood of the suspected can be detected. It is highly sensitive test. In this test, specific antigens or antibody are allowed to react on a solid phase polystyrene plates previously coated with reagents (Ag/Ab). Thereafter, enzyme conjugate is allowed to bind with antigen-antibody complex which later reacts with a suitable substrate to develop a colour. The intensity of colour is measured by colorimetric method. ELISA developed in our institute (NIVEDI) for the detection of surra antibody and antigen is competitive inhibition ELISA (CI- ELISA) (Fig. 5) and double antibody sandwich antigen detection ELISA (Ag-ELISA).

Fig 5: Competitive inhibition (CI) ELISA with variable surface glycoprotein and its corresponding monoclonal antibody developed in NIVEDI, Bengaluru.

. **Card agglutination test:** The card agglutination test for *T.evansi* is available. in this test, basically antigen and antibody are allowed to react. In positive cases, a colored agglutinate is visible under a white background. In this test, generally, IgM is detected, but the test may cross reacts with other species of trypanosomes.

. **Immune trypanolysis test:** In this test, live trypanosomes are incubated with test serum in the presence of guinea pig serum as the source of complement. In positive cases, 50% or more lysis of trypanosomes are observed. The disadvantage of the test is, for continuous supply of trypanosomes, it is required to rear the rodents.

) Biochemical tests

) **Mercuric chloride test:** In 1 ml solution of mercuric chloride (one in 30,000 dilution), one drop of suspected serum is added. In positive case, a white opalescence appears.

) **Stilbamide test :** One drop of suspected serum is added to 0.3% aqua solution of stilbamide. In positive case, precipitation will be observed.

As in biochemical test, the direct detection of parasite is not done, so it is not confirmatory. Such test can be used as a supportive.

Treatment

1) Aminoquinyl diamine

Quinapyramine salts: A mixture of quinopyramine sulphate and chloride salts are used. The sulphate salts reaches at therapeutic level very quickly. Thus, it is fast acting and is having quick therapeutic use. The chloride salt, on the other hand, slowly absorbs and is generally used as a prophylactic measure. Commonly, a mixture of sulphate and chloride salt are used for treatment.

2) Diamidine compound

Diminazene aceturate: This is moderately effective against *Trypanosoma evansi*. This compound is generally given by subcutaneously and intramuscular injection at the rate of 3.5 mg/Kg body weight.

3) Sulphonated naphthylamine

Suramin: This is also effective against *Trypanosoma evansi*. It can be given 2 to 4 grams/ 50 kg body weight intravenously.

4) Phenanthridine

Iso-metamidium chloride: 0.5 to 1 mg/body weight by deep intramascular route.

Duration of treatment:

The selection, route of administration, dose, and duration of treatment of anti- trypanosomal drug depends upon the species affected and health status of the animal. Therefore, it is always suggested that any anti trypanosomal drug should be administered under the proper supervision of a qualified veterinary doctor.

Control

1) In field condition, after the recovery of animals, there is an enzootic stability between host and parasite. In endemic areas, the animal get constant antigenic stimulation from the environment and the host immunity takes care the very low level of parasitaemia. If, due to any reason, the balance is disturbed, the parasite gets upper hand and causes the disease. So the asymptomatic carrier animals should be detected and thereafter should be treated with suitable drugs. These animals also serve as the source of the infection to the naïve population of the animals. Hence, the screening of such animals followed by a treatment is very essential for control.

2) Haematophagus tabanid fly transmit the disease. So the control of fly by insecticidal spray in the vicinity of the animal sheds or shelter also helps in controlling the disease.

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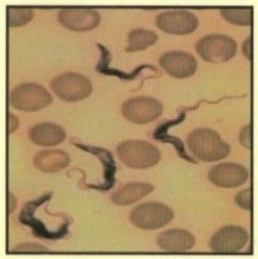
Trypanosomosis (Surra) in animals



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Trypanosomosis (Surra) in animals

Trypanosomosis in animals or human is caused by the sub-genus species Trypanozoon in different parts of the world. In India, mainly *Trypanosoma evansi* causes trypanosomosis in animals. This disease is basically chronic in nature and is characterized by fluctuating fever, chronic weakness, emaciation, inappetance, hypoglycemia, reduced animal production (in terms of body weight gain, milk yield and draughtability), abortion in pregnancy and even death, if untreated (Fig.1). The disease is also called as 'surra' locally. The meaning of surra in hindi is 'rotten' as it is a wasting type of disease.

BUFFALO CATTLE CAMEL HORSE

Fig 1: *T. evansi* affected animals

Transmission

In India, the disease is mechanically transmitted by the bites of the tabanid fly (*Tabanus, Stomoxys*, and *Lyperosia sp.*) (Fig.2). In case of carnivores such as dogs, tigers, leopards, lions etc., infection can be transmitted through the ingestion of tissues from infected animals.

Fig 2: Tabanid fly (Source: Internet)

T.evansi affects a wide range of domestic and wild carnivores and herbivores including bovine, bubaline, equine, dromederine, canine and feline. The disease has been reported most severe among camels, horses and dogs. The buffaloes are known as the carrier of the disease.

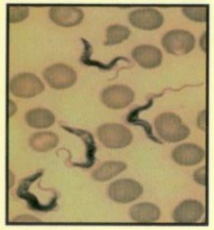

Fig 3: *Trypanosoma evansi* in blood smear

Morphology

T.evansi was first identified by Griffiths Evans, a British veterinarian from Indian sub continent. The organism is 15-30µm in length and kinetoplast is sub terminal; the undulating membrane is well developed with a free flagellum (Fig.3). The organisms multiples by longitudinal binary fission.

Epidemiology

Surra has been reported from different parts of the world normally Asia including Indian subcontinent and the far East, Africa, central and south America. In India, the disease has been reported among cattle, buffalo, camels, horses, dogs and other domestic and wild animals. Due to high diversity and geographical demography in its territory, the presence and/or spread of both the disease (surra) and the responsible vectors (tabanid flies) are significant. It's been reported that overall mortality rate due to *Trypanosoma evansi* in India is found to be 12.7% in horses and 40% in bovines in eastern India (Laha and Sasmal , 2009). The available mortality report in cattle due to trypanosomosis from 1990 – 2011 has been shown in Fig. 4. The untreated recovered animals can harbor low parasitaemia with latent infection which is apparently symptomless, but if there is any stress to the immunity of the animal the infection may flare up even up to the clinical infection with high parasitaemia. Moreover such carrier animals may act as a source of infection to other healthy animals through fly bites. Also it has been reported that there is a significant reduction in animal production in carrier animals (Pholpark *et al*., 1999). Therefore it is very essential to detect and diagnose the carrier animals for subsequent treatment to stamp out the disease.

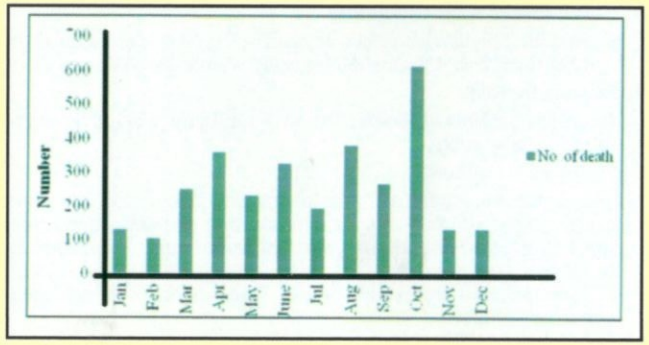

Fig 4: Monthwise mortality reported due to trypanosomosis in cattle during 1990-2011 (Source: Annual report, PD_ADMAS, 2011-2012).

Pathogenesis

Basically, surra is a chronic wasting type of disease. Emaciation and edema are very commonly seen at the legs and lower parts of the body. Sometimes articarial flex are also visible which is necrosed at the centre and hemorrhages may occur at the junction of skin and mucous membrane. Enlargement on the lymph nodes and spleen are very common characteristics in this disease. Intermittent fever may be present also. Petechial hemorrhages on the seroous surface and parenchyma on the liver and kidneys.

Diagnosis

Clinical symptoms: Fluctuating fever, loss of appetite, enlargement of lymph nodes, progressive weakness are the common signs of this disease. The animal showing such symptoms should be subjected to further laboratory investigation for confirmation.

Identification of agent:

1) **Wet blood smear examination:** On 2 to 3 µl of blood with anticoagulant, a clean glass slide is placed and observed under microscope (10X). Moving trypanosomes will be visible in positive case.
2) **Stained thick/thin blood smear examination:** For thin smear, approximately 3-5 µl of blood is placed at one end of the slide and a thin film with the help of another slide is made. The blood film is allowed to air dry completely and is fixed with methanol for 1 min. After fixing, Giemsa's stain is added for about 45 mins and washed slide is observed under oil immersion objective.
3) **Lymph node biopsy:** A suitable lymph node is punctured with a sterile needle and lymph node material is drawn into a syringe attached to the needle. Lymph is later smeared on to the slide and observed under microscope after staining.

Concentration methods

1) **Haematocrit centrifugation technique (Woo's technique or HCT):** Approximately 70 µl of blood are collected into heparinised capillary tubes and centrifuged at 12,000 rpm for 5 mins. The capillary tube is examined and the value is expressed as a percentage of packed red blood cells (RBCs) to total blood volume. The capillary tube is then placed in a groove on the slide. Trypanosomes are clearly visible when observed under microscope, as they are large cells that concentrate between the buffy coat and the plasma. This technique can detect around 50-200 trypanosomes/ml of blood (Desquesnes & Tresse, 1996).
2) **Phase-contrast buffy coat method/ Dark- ground (also known as Murray's technique, or BCM):** This procedure is similar to hematocrit centrifugation technique, where after centrifugation, capillary tube is broken below the buffy coat layer. Thus the upper part contains a small top layer of RBCs, the buffy coat (white blood cells and platelets) and some plasma. Approximately 5-8 µl of plasma is partially expelled on to the slide and observed under microscope. Trypanosomes are mostly present at the periphery of the thick buffy coat material.
3) **Anion exchange centrifugation technique:** This technique is mostly used for the purification of parasites from the blood and later concentrated.

Animal inoculation: This test is most reliable and the standard test for the diagnosis of trypanosomosis. The mice are generally used for this purpose. Approximately 1 ml of suspected blood is injected intraperitonially in the mice and observed.

Detection of trypanosomal DNA: Detection of trypanosomosis can be done by using,

1. **DNA probes:** As specific DNA probes have to be used to detect trypanosome DNA in infected blood or tissue, this is not routinely applied as further evaluation needs to be made (Basagoudanavar *et al*., 2001). PCR techniques are generally preferred.
2. **Polymerase chain reaction (PCR):** PCR is considered to be the most sensitive technique for the detection of the infection. The sensitivity of the PCR being dependent on the amount of DNA available, it is proportional to the parasitaemia. DNA preparation is an important step and it can be done from blood, tissue, buffy coat etc. PCR is found to be more sensitive in highly susceptible hosts (camels, horses, dogs, etc) than in hosts of mild susceptibility (cattle, buffalo, pig, etc). Applying the PCR method, it is possible to detect as low as 0.15 trypanosome/ml and considering the number of parasite to DNA concentration, the PCR method has a sensitivity of 0.015 pg /ml. Thus it has been found that, the application of PCR has favored over the parasitological methods for the detection of the early and /or chronic stage of surra in domestic and wild animals (Sengupta *et al*., 2010).