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## Trypanosomiasis in equines: A brief discussion

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### Abstract

Trypanosomiasis causes by vector born haemoprotozoa in all mammals including human beings. In equines most common forms are Surra, Mal-de-caderus, Nagana, Dourine etc. It is a vector borne disease transmitted mechanically by various species of biting flies from the blood of infected animals. Surra is transmitted by tabanus and Stomoxys species. It is mostly prevalent during the monsoons and rainy season due to increase in the number of biting flies. The parasites are commonly observed in the swelling of oedematous lesion but less frequently seen in the blood. Diagnosis is based upon history of prevalence of infection, biting flies, clinical signs and symptoms. Laboratory examination of blood and body fluids by direct examination, chemical test, inoculation test and serological test helps in detection of disease. Treatment of the affected animals are done by preventive and curative measures. Chemotherapeutic agents like Diminazene aceturate (DA), Suramine, Quinapyramine, Isometanedium chloride and melarosomine dihydrochloride (Cymelarsan) are used. As vectors are the major transmitting agents thus control of vectors and changing in animal husbandry practice is best preventive measures for this zoonotic disease.

**Keywords:** Trypanosomiasis, equines, *Trypanosoma*

### Introduction

Trypanosomiasis is an infectious disease caused by several species of the genus *Trypanosoma*, a blood and tissue parasite found in mammals including human beings. The biting insects are the main transmitter of the disease in which biological transformation takes place (Desquesnes *et al.*, 2013) [1]. Here, we are going to mainly discuss about the diseases of horse caused by different forms of *Trypanosoma* species. In equines the most common forms causing disease are *Trypanosoma evansi*, causative agent of “Surra”; *T. equinum*, causative agent of “Mal-de-caderus”; *T. equiperdum* causative agent of “Dourine”. The other species affecting horses are *T. vivax*, *T. congolense*, *T. dimorphon*, *T. brucei* etc. (Mondal, 2012) [4] which cause “Nagana” in horses. The disease causes important economic losses in Africa, the Middle East, Asia and Latin America. In world *Trypanosoma evansi* was the first pathogenic mammalian Trypanosome to be discovered (Hoare, 1972) [9] in the blood of Indian equines and dromedaries in 1880 by Griffith Evans (Desquesnes *et al.*, 2013) [1]. Major obstacles to the local and global control of equine trypanosomiasis are the lack of vaccines, the inability of drugs to cure the neurological stage of the infections, the inconsistent case definitions and the limitations of current diagnostics.

### Taxonomy and Morphology

Taxonomical classification of parasite *Trypanosoma* is described in table 1

**Table 1:** (Mondal, 2012) [4]

Order	<b>Kinetoplastida</b>
Family	Trypanosomatidae
Genus	<i>Trypanosoma</i>
Species	<i>T. evansi</i> , <i>T. equinum</i> , <i>T. equiperdum</i> , <i>T. vivax</i> , <i>T. congolense</i> , <i>T. brucei</i> , <i>T. dimorphon</i>

It is a leaf-like, nucleated organism having flagella which is attached with the body by thin membrane known as undulating membrane. Blepheroplast is the dot like structure present posterior to the nucleus. Posterior to the blepheroplast, kinetoplast is present.

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From the blepheroplast, flagella originate. *Trypanosoma congolense* (subgenus *Nannomonas*) and *T. vivax* (subgenus *Duttonella*) are species that are clearly separated, both genetically and morphologically, from the other taxa within

the genus *Trypanosoma*. Table 2 summarises the major characteristics of *Trypanosoma* taxa that may cause trypanosomiasis in equines.

**Table 2:** (Büscher *et al.* Parasites Vectors, 2019) <sup>[8]</sup>

	Trypanozoon			Duttonella	Nannomonas
	<i>T. brucei</i>	<i>T. evansi</i> type A	<i>T. equipardum</i>	<i>T. vivax</i>	<i>T. congolense</i>
Distribution	Sub-saharan Africa	Africa, latin America, middle east Asia	Worldwide except Oceania USA and Canada	Africa, Latin-American	Sub-saharan Africa
Mammalian host range	Multi-species	Multi-species	Equines	Multi-species	Multi-species
Transmission	Tsetse	Biting flies, vampire bats, orally	Sexually (orally via milk)	Tsetse, biting flies	Tsetse
Morphology in mammalian host	Pleomorphic <sup>a</sup>	Monomorphic <sup>b</sup>	Monomorphic	Monomorphic	Monomorphic
Kinetoplast	Complete	Dyskinetoplastic <sup>c</sup> or akinetoplastic <sup>d</sup>	Dyskinetoplastic	Complete or dyskinetoplastic	Complete
Kinetoplast minicircle type		A	C or undefined		

a) Pleomorphic: present as long slender, short stumpy and intermediate trypomastigotes during an infection

b) Monomorphic: long slender trypomastigotes with anecdotal evidence for partial pleomorphism in *T. evansi*

c) Dyskinetoplastic: partial loss of kinetoplast DNA, in particular maxicircle DNA

d) Akinetoplastic: complete lack of kinetoplast DNA

### Epidemiology

Trypanosomiasis is distributed world-wide. *T. evansi* is reported in the USSR, North Africa, Sudan, Middle East, and Indian sub-continent, South East Asia, Phillipins and Indonesia. *T. evansi* occurs in Central and South America, South Argentina. Nagana is complexed disease caused by various *Trypanosoma* spp. mainly seen in Africa and then restricted to sub-saharan Africa. *T. equipardum* is found in the USSR, North Africa, South Africa, the Middle East, South East, Asia, Central and South America. Dourine has been reported from Italy recently. Surra in India is very old with records dating back from VIII centuries B.C. (Hoare, 1972) <sup>[9]</sup> with prevalence in almost all over the country, where environment for breeding of fly vectors is most suitable (Bhatia *et al.*, 2006) <sup>[14]</sup>. Occurrence of Trypanosomiasis depends upon the climatic and ecological condition which determine the distribution and abundance of the insect vectors of Trypanosomes involved. It is mostly prevalent during the monsoons and rainy season due to increase in the number of biting flies. Dourine transmitted during coitus; it is not influenced by environmental factors. Hence can occur anytime and anywhere. Dourine was present in North America and Canada until 1920s and in the Northern Europe and Balkans until 1940s. The recent outbreak of Dourine in Southern Europe underlined the need for constant vigilance and surveillance. The range of potential biting fly vectors of *T. evansi* is not known but where large number of biting fly population exist it might be possible that *T. evansi* gets introduced in imported livestock and get spread thereafter. Incidence of trypanosomiasis outbreak have been reported to be increased in camels after the advent of Indra Gandhi Canal and irrigation of vast tracts of arid land in Western Rajasthan (Pathak and Khanna, 1995) <sup>[10]</sup>. Again, the incidence of trypanosomiasis in bovines was found to be directly proportional to onset of monsoon to post monsoon in Panjab (Soodan *et al.*, 1995) <sup>[11]</sup>, Andhra Pradesh (Prasad *et al.*, 1997) <sup>[12]</sup>, West Bengal (Ray *et al.*, 1992) <sup>[13]</sup>, Bihar (Sinha *et al.*, 2006) <sup>[14]</sup>, Chhattisgarh (Agarwal *et al.*, 2003) <sup>[16]</sup> and Jammu (Raina *et al.*, 2000) <sup>[15]</sup>.

### Transmission

It is a vector borne disease transmitted mechanically by various species of biting flies from the blood of infected animals. Surra is transmitted by tabanus and *Stomoxys* species. Mal-de-caderus is transmitted by biting flies in a mechanical way. Dourine is transmitted through sexual intercourse where unrestricted and unsupervised mating is allowed. Nagana is influenced by degree of contact between horses and various types of Tsetse flies. *Trypanosoma equiperdum* is transmitted sexually, and *T. evansi* is transmitted mechanically by blood-sucking flies, vampire bats, and possibly sexually. Oral transmission of *T. evansi* via contaminated meat or carcasses.

### Forms of trypanosomiasis in Equines

**Surra:** It mainly occurs in horse and camel, and known "surra" in Asia and Africa means "rotten". It is also prevalent in cattle and dog. Incubation period is 1-4 weeks and may exceed up to 8 weeks. The disease shows symptoms like fever, anemia, emaciation, urticarial plaques and ulcerative lesion in the mucocutaneous junction. The animals on affection also shows dullness, weakness due to anorexia and subsequent weight loss, there are petechial haemorrhages and oedema in ventral parts, udder or scrotum and sheath, low milk and meat yield, poor traction power, increased abortion and death. In horses the disease is chronic in nature and lasts for 2-3 years commonly known as 'Tebersa'. Generally, it occurs in all age groups but mainly starts just after weaning.

**Mal-de-caderus:** The clinical signs shown in this case are conjunctivitis, eye lesions, keratitis, recumbency, fever etc. Nervous symptoms like periodic convulsions are also seen in some cases. Stumbling of fore- legs and dragging of hind legs is also seen. There may be signs of oedema in legs, brisket, abdomen, udder, testicles and sheath.

**Nagana:** Nagana is a Zulu word which means 'in low or depressed spirit'. The clinical signs of Nagana in horses is characterized by lethargic or depressed condition of animal, pyrexia (104°F rectal temperature), increase heart rate, tachypnea, severe anaemia with weakness and signs of ataxia.

Small oedematous nodule on the skin mainly on flank region. Mucous membrane become pallor due to haemolysis. Nagana mainly caused by *T. brucei brucei* in horses resulting in death within 2 weeks to 3 months. Among the inflected animals 20% of horses infected with *T. brucei* may develop keratitis and corneal opacity (Sandy Love, Tim S. Mair, 2012) [7].

**Dourine:** Meaning of Dourine is 'unclean'. Incubaion period is 8-12 weeks and this develops through three phages. First is 'stage of oedema' these shows visible symptoms like urethral or vaginal discharge, oedema of vagina and prepuce and the oedema extend until the belly. Second phage is 'dollar stage' circular urticarial plaques having 3 cm or more diameters are present beneath the skin mainly on flank region which is known as Dollar spots. Third phase is 'stage of paralysis' characterised by incoordination and unilateral paralysis of hind legs.

#### Postmortem lesion

Marked anaemia, emaciation, splenomegaly, enlargement of lymph nodes is seen. Petechiae may be observed on the serous surface and in the parenchyma of liver and kidneys. In *T. equiperdum* infection the carcass appears emaciated with muscular atrophy, oedematous infiltration of the perineal tissue and abdominal wall may be seen. Serous infiltration may be present along with large nerved trunk. The parasites are commonly observed in the swelling of oedematous lesion but less frequently seen in the blood.

#### Diagnosis

Diagnosis is based upon history of prevalence of infection, biting flies, clinical signs and symptoms. Laboratory examination of blood and body fluids by direct examination, chemical test, inoculation test and serological test helps in detection of disease.

#### Direct Examination: (Tewari *et al.*, 2013) [20]

It is done by demonstration of organism in fresh blood smear collected from infected animal. It is the confirmatory way of diagnosis. In acute infection organisms are readily detectable on freshly stained blood slides but in chronic cases thick and thin blood smear collected from lymphoid fluid should be studied because parasitaemia is low in chronic cases.

#### Chemical Test

Various types of chemical test are available for detecting the changes in chemical composition of blood by these organisms. Such disease induced alteration of serum proteins can occur in other infections as well. The tests, therefore are non-specific, less reliable but may be useful in the field for tentative diagnosis.

- A) **Mercuric chloride test:** 1ml of 1:25000 solution of mercuric chloride is taken in a test tube, then 1 drop of suspected serum is added to this test tube. In case the test is positive, there is appearance of white precipitate.
- B) **Stilbamidine test:** 0.5 to 2.5ml freshly prepared 10% solution of Stilbamidine is taken in a test tube, then 1 drop of serum is added. In positive cases coagulated mass is formed which begins to sink within half minute and gets dissolved within 5-10 minutes.
- C) **Formol Gel Test:** 2 drops of 40% formaldehyde is added with 1ml suspected serum in a test tube and shaken well. On standing, in positive cases gel is formed within an hour. If the content is kept for 24 hours at 4-degree Celsius white opalescence is formed.

- D) **Jone's nitric test:** 1ml of 1.8 percent w/v nitric acid is taken in a test tube add 1 drop of suspected serum is added. Positive cases show yellow colour turbidity in one hour.
- E) **Thymol Turbidity Test:** The test is similar to mercuric chloride test and it helps to detect excess serum globulin in infected animals. 3ml Alkaline Thymol buffer is mixed with 1 drop of suspected serum at 56 degree Celsius for 30 minutes. Positive cases give white turbidity within 1 hour.

#### Animal Inoculation Test

It is more fruitful than the direct microscopic examination. It can detect sub patent infection in blood of horses following chemotherapy. Albino mice and rats are suitable host for detecting sub patent infection. 0.5 ml of suspected blood with anti-coagulant is inoculated intra-peritoneally in white mice.

**Immuno-diagnostic test:** (Holland *et al.*, 2001; Desquesnes and Davila, 2002; Parashar *et al.*, 2015; Sudan *et al.*, 2014) [21, 22, 23]

Various type of immuno-diagnostic tests is there. Indirect haemagglutination test, complement fixation test, Enzyme linked Immuno Sorbent Assay, Immuno Fluroscient Antibody Test are recommended for diagnosis of Trypanosomiasis. Since long time serological test is useful for diagnosis of Trypanosomiasis. Compliment Fixation Test (CFT) is mainly done for *T. equiperdum*. Polymerase chain reaction (PCR) and DNA probes also helpful for detection of *Trypanosoma* infection in horses.

In allergic test, 0.1 ml antigen which consist of acetone dried trypanosomes with normal saline (1: 4000) also used for diagnosis of infection. 1ml of the antigen is injected intra-dermally on the side of neck in suspected animal. In positive cases there was no painful oedematous swelling on the side.

#### Treatment

There are four major groups of drugs which are used for treatment of *Trypanosomiasis* in horses. These drugs are divided into two categories i.e 'curative' drugs and 'preventive' drugs. Curative drugs are used to kill parasites but they cannot kill 100% of parasites. Preventive drugs are having chemoprophylaxis activity which helps to kill the parasites and also prevent the new parasites infection.

- **Diaminazine aceturate:** Diminazene aceturate (DA) is an aromatic diamidineIt is a curative drug mainly used for *T. evansi* @ 3.5mg/kg body weight deep intramuscularly given. Its use in horses and dogs is limited due to poor efficacy and tolerance in these species.
- **Quinapyramine:** Quinapyramine methyl-sulphate is used @ 8 mg/ kg body weight by subcutaneous injection. Quinapyramine sulphate and Quinapyramine chloride (Triquin) combination used as curative/preventive drug in horses given subcutaneously.
- **Suramin:** Is and uteric compound and 10% solution in sterile distilled water is given @ 4g/ 45kg body weight in 3 divided doses over a period of 3 weeks, intravenously. A combination of 8.9gm suramin and 10 gm Quinapyramine is also used.
- **Isometanidium chloride:** It acts as both curative as well as Preventive drugs and belongs to the phenanthridine family, as well as homidium chloride or bromide. As a curative it is given @ 0.5 mg/kg body weight and as a preventive @ 1 mg/kg body weight through deep intra-muscularly or subcutaneously. Diaminazine aceturate-



and Isometanidium chloride is used alternatively to induce 'senative effect' that means if one drug resistance develop another drug helps to prevent the infection.

- **Melarsomine dihydrochloride:** The latest trypanocidal drug is melarsomine dihydrochloride (Cymelarsan), first available in 1992 for commercial use (Desquesnes, M. *et al*, 2013) <sup>[1]</sup>. In case of horse it is given @ 0.25-0.5 mg/kg body weight via deep intramuscular injection.

**Alternative approaches in treatment:** Currently, *in vivo* and *in vitro* trypanocidal activity of free and nano encapsulated curcumin against *Trypanosoma evansi* is evaluated and found significant positive result to reduce parasitemia on adult male wistar rats. Treatment with essential oil of *Achyrocline satureioides* in rats infected with *Trypanosoma evansi* revealed that essential oil did not eliminate the parasites from the bloodstream, but it reduced the number of trypanosomes, mainly by its nano encapsulated form so, association of this natural product with a trypanocidal drug may enhance its curative effect. Study on pre-clinical mouse model indicated that Heat shock protein 90 (Hsp90) from protozoan parasites may as a potential drug target of trypanosomiasis. Alchorneidine a new guanidine alkaloid from the leaves of *Alchornea glandulosa* was found effective against trypanosomiasis. *In vitro* trypanocidal activity of macela (*Achyrocline satureioides*) extracts against *Trypanosoma evansi* was also evaluated (Baldissera *et al.*, 2014b) <sup>[19]</sup>.

#### Zoonotic aspect

Trypanosome has a wide range of zoonotic importance. *Trypanosoma brucei* spp., the causative agents of human sleeping sickness. In 2005, a human case of trypanosomiasis caused by *T. evansi* was reported in a farmer from the Chandrapur district in the Maharashtra State, India. In this case, the man had fluctuating trypanosome parasitaemia associated with febrile episodes for several months and treated successfully with suramin. Immunosuppressed individuals living in the regions where *T. evansi* is endemic are under risk.

#### Prevention and Control

*Trypanosomiasis* is one of the important disease of horses which causes high mortality. As it is a vector-borne disease so control of vector is most important. Vector is controlled by different types of insecticides and fly repellents. Various type of trap is used to catch the flies i.e. Nzi trap, vavoua trap etc. Identification and isolation of infected animal from healthy animals. Protection of animals by using mosquito net in the stable and smoke released to protect the horses from biting of flies. As vampire bat is also transmit the infection 'Japanese net' is used to catch the vampire. *T. equiperdum* is sexually transmitted so mating should be allowed with infection free animal or semen should be collected from healthy animal for artificial insemination. For *Trypanosomiasis* no vaccine is available as the organisms change their surface glycoprotein rapidly to prevent immune response. Another reason is that the parasite undermining the host's capacity to mount an efficient immune response and to maintain its immunological memory termed as immunodeficiency to the host (Pays *et al.*, 2004) <sup>[18]</sup>. Although a solitary report, wherein formalin inactivated *T. evansi* (2×10<sup>6</sup> count) were administered in mice and found protective against homologous challenge (Tewari *et al.*, 2009) <sup>[17]</sup>. Immunization with *T. evansi* recombinant beta-tubulin, induced some protection against *T. evansi*, *T. equiperdum* and *T. brucei* infection in mice.

#### Conclusion

*Trypanosoma* is an important blood protozoa causes worldwide losses in equine population and also has economic impact. Vectors are main transmitting agent of this zoonotic parasite. Thus control of vectors is the best method to prevent this parasitic hazards. Although there is the lack of specific diagnostic methods, but early treatment with most effective drugs can save the animals.

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