

PREVALENCE OF CLINICAL AND SUBCLINICAL FORMS OF *TRYPANOSOMA EVANSI*
INFECTION IN BUFFALOES OF MUMBAI REGION (MS) OF INDIA

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ABSTRACT

This study reports the present status of clinical and subclinical of *Trypanosoma evansi* infection in buffaloes of Mumbai region of India. Seventy five out of 253 buffaloes screened by different methods (Blood Smear Examination and PCR) revealed *T. evansi* with the overall prevalence rate of 29.64% which is alarmingly high as compared to previous studies conducted in the region in the recent past. The prevalence of *Trypanosoma evansi* was found to be highest at Karjat (67.06%) followed by Wawarley (51.72%) and it was lowest at Govandi (14.28%). The level of parasitaemia was mild in 82.2%, moderate in 11.29 and high in 6.45% amongst the infected buffaloes. Out of 75 positive animals only 10 (13.33%) buffaloes were exhibiting one or the other typical clinical signs suggestive of trypanosomiasis. The parasitaemia did not coincide precisely with pyrexia as three of the 10 confirmed clinical and 65 subclinical cases did not reveal pyrexia. These findings confirm the status of buffaloes as a reservoir host for *T. evansi* and increased chances of buffalo population at risk of its infection in the region.

Keywords: *Trypanosoma evansi*, bubaline surra, buffaloes, Trypanosomiasis

INTRODUCTION

Trypanosomiasis (surra) caused by *Trypanosoma evansi* is a major haemoprotozoan disease of equines, camels, dogs, cattle and buffaloes. The disease, mechanically transmitted by haematophagous flies viz., *Tabanus* spp. and *Stomoxys* spp., occurs ubiquitously in India affecting domestic and wild mammals. It is associated with tremendous economic losses due to high mortality, loss of production and expenditure of management of the disease. The organisms have also been reported in humans in India (Banerjee and Garg, 2011) alarming the workers about future possible threats of zoonosis.

The clinical disease is chronic in cattle and buffaloes which act as reservoir hosts and play a vital role in the epidemiology of the disease. To fulfill the huge demand of milk in Mumbai city for human consumption, a large population of buffaloes is reared locally. The hot and humid climate of Mumbai region is very favorable for propagation of tabanid flies, the vectors of surra which raise the possibilities of the disease in the area. The adverse effects of trypanosomiasis on the animal health, production, reproduction; its possible zoonotic threat and potential of buffalo as reservoir hosts of the disease points the need of current surveillance. The present study was therefore, conducted with aim to investigate the prevalence of *Trypanosoma*

evansi and to ascertain the subclinical status of bubaline surra.

MATERIALS AND METHODS

During eight months from July, 2010 to February, 2011, 253 buffaloes from 7 localities Viz., Karjat (west), Kadaw, Govandi, Palghar, Panvel, Wawarley and Goregaon were screened for *Trypanosoma evansi* infection. The peripheral blood smears from tip of ear pinna and whole blood samples from jugular vein were collected from apparently healthy as well as pyrexia cases, were brought to the laboratory and processed immediately.

The blood smears stained (BSE) with field's staining technique (Fleck and Moody, 1993) were sequentially observed under low (10x), high (40x) and oil immersion (100x) objectives of research microscope (LaboMed India Ltd.) for detection of the intercellular flagellate organisms. The level of parasitaemia in the peripheral blood smear was also noted and accordingly the cases were categorized into three groups viz., mild (<5), moderate (6 to 10) and heavy (>10 organisms per high power field) prasaemia.

The samples which were detected negative by BSE were subjected to molecular technique (PCR). Genomic DNA was isolated from the whole blood by using Genie Pure blood Genomic DNA purification Kit (Bangalore Genie, India). The PCR protocol to amplify variable surface glycoprotein (VSG) gene as described by Sengupta *et.al.* (2010) was followed with few modifications. Extracted genomic DNA was stored at -20°C until needed. The genomic DNA (400 bp) was amplified using *Trypanosoma evansi* species specific primer pair DITRYF (5'

CGACCAGCCAGAACGAGCAGAAT 3') and DITRYR (5' CTT GTC GAT CGA GTT GAC GGT 3') as described by Sengupta *et al.* (2010).

RESULTS AND DISCUSSION

In the present study, buffaloes from Mumbai region irrespective of their health status, age, sex and breed were monitored for prevalence of *Trypanosoma evansi*.

The haemoflagellates detected in the study corresponded well with the morphology of *Trypanosoma evansi* as described by Soulsby (1982). The trypanosomes were 20 to 31 µm long with sub terminal kinetoplast, distinct undulating membrane and a free flagellum. The nucleus was conspicuous and centrally placed (Figure 3). Few dividing forms were also observed during Blood smear examination (BSE) at 100x objective.

Out of 253 buffaloes screened by both the methods (BSE and PCR) during the study, 75 buffaloes revealed *T. evansi* with prevalence rate of 29.64%. The conventional methods detected only 62 (24.50 %). The PCR method by amplifying the variable surface glycoprotein (VSG) gene at 391 bp, diagnosed 13 additional cases leading prevalence the exact rate of prevalence to 29.64%. As depicted in Table 1 and Figure 1, the prevalence of *Trypanosoma evansi* was found to be highest at Karjat (67.06%) followed by Wawarley (51.72%) and it was lowest at Govandi (14.28%). The higher prevalence at Karjat and Wawarley may be due to high population density of buffaloes and the ecological conditions favorable for vector development in the area as compared to other five centers included in the study.

Amongst the 62 buffaloes detected positive by BSE, 51 (82.26%) had mild, seven (11.29%)

Table 1. showing the pattern of exhibiting clinical signs by the buffaloes infected with *Trypanosoma evansi* in different regions of Mumbai.

Study Centers	Number Of Positive Buffaloes	Buffaloes Exhibiting Clinical Symptoms	Partial Anorexia	Pyrexia (>103°f)	Mucous Membranes /Anaemic	Oedema	Corneal Opacity
Karjat west	57/85 (67.05)	6 (10.90)	3 (0.05)	6 (10.90)	4 (7.27)	1 (1.81)	--
Govandi	3/21 (14.28)	1 (50.00)	--	--	--	1 (50.00)	1 (50.00)
Wawarley	15/29 (51.72)	3 (21.42)	3 (21.42)	1 (7.14)	1 (7.14)	--	--
Others	00/118 (00.00)	Nil	Nil	Nil	Nil	Nil	Nil
Total	75/253 (29.64)	10 (14.08)	6 (8.45)	7 (9.85)	5 (7.04)	2 (2.81)	1 (1.40)

Note: Figures in the parenthesis indicate the percentage of observed parameter to the respective fields.

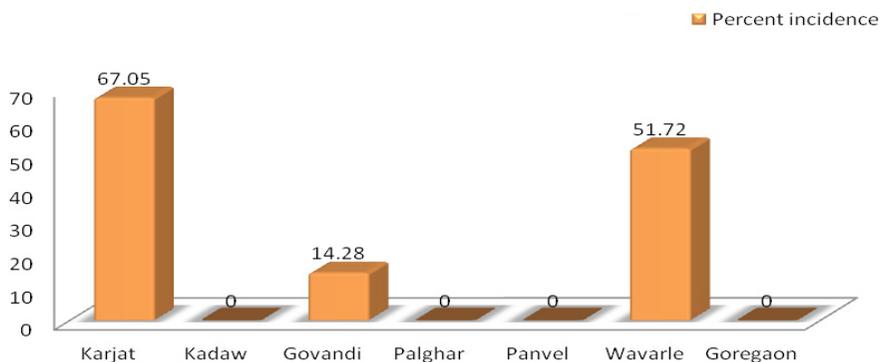


Figure 1. Graph showing the centre-wise incidence of *Trypanosoma evansi* in buffaloes of Mumbai region.



Figure 2. Corneal opacity in a buffalo tested positive for *Trypanosoma evansi*.

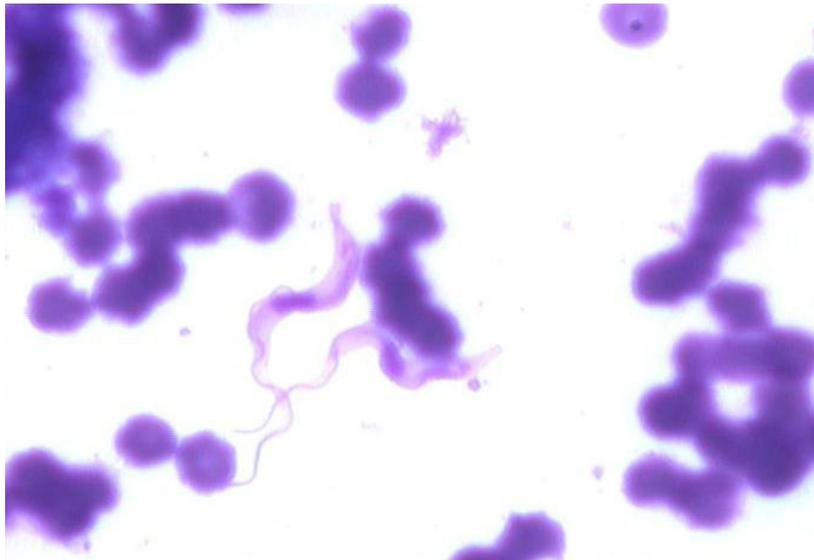


Figure 3. *Trypanosoma evansi* in stained thin blood smear under high power field (100x).

moderate and four (6.45%) had high level of parasitaemia. In an endemic area and particularly in buffaloes, similar trend was also reported by Birajdar (2007) in this region. The degree of parasitaemia varies with the susceptibility of host, clinical phase of infection, stress due to physiological conditions viz., lactation and pregnancy and other factors such as concurrent infection, adverse climatic conditions, malnutrition *etc.*

In the present study, out of 75 positive animals only 10 (13.33%) buffaloes were exhibiting one of the typical clinical signs suggestive of trypanosomosis. Of which 7 (9.33%), buffaloes had pyrexia (>102°F rectal temperature), 5 (6.66%), were anaemic, 2 (2.66%), had oedema of knee joints, one (1.33%) and had corneal opacity (Figure 2) and (8.0%) exhibited partial anorexia.

Many of the scientists recorded various clinical signs like anorexia, rise in body temperature (103 to 105°F), nervous signs, dullness, salivation, Congested conjunctivae, lacrymation, nasal discharge in early stages of the disease (Singla *et al.*, 1996) and anaemia, emaciation, staggering gait, oedema of joints, corneal opacity in chronic phase (Singh and Joshi, 1991; Birajdar, 2007 and Mervet *et al.*, 2010). There is very wide variation in the clinical signs of *T. evansi* infection in buffaloes reported by various scientists (Singh and Joshi, 1991 and Birajdar, 2007) across the globe. However, clinical characterization in the present study revealed only 13.33% cases showing either of the clinical sign and not all together.

The majority of the infected buffaloes (88.66%) were found apparently healthy without any clinical signs thus indicating the high reservoir potential of the buffaloes.

Mallick and Dwivedi (1981) reported body temperature of infected cases was ranging

from 99°F to 104°F which strongly supports the findings of present study as 90.66% of infected buffaloes showed rectal temperature in normal range. This indicates that parasitemia doesn't coincide with pyrexia. Bhatia *et al.* (2006) had also reported possibility that the population may have asymptomatic animals with parasitemia reservoir status animal.

The prevalence of bubaline surra was found to be 29.64% which is distinctly higher than the previous study conducted by Birajdar (2007) and Shetye (2000) in the same region. Patel *et al.* (1983); Laha (2009); Rao and Hafeez (2005); Sivajothi *et al.* (2011) also reported comparatively lower rate of prevalence (below 10%) using conventional technique from Gujarat, Eastern part of India and Andhra Pradesh respectively.

However, the prevalence rate reported in this study is in line with the findings of Mandal (1977) and Singh and Joshi (1999) from Andhra Pradesh and Maharashtra region respectively. The discrepancy in prevalence rate could be attributed to environmental factors influencing vector population (Soulsby, 1982 and Bhatia *et al.*, 2006), parasite related factors such as strain of *T. evansi*, host related factors such as clinical form of the disease, managerial factors such as cleanliness and hygiene (OIE, 2010), physiological factors such as stress of pregnancy and lactation (Dargantes *et al.*, 2005), intercurrent diseases (Stephen, 1986), malnourishment (Gill, 1991; Mallick and Dwivedi, 1981) and diagnostic procedures (Sengupta *et al.*, 2010).

Thus, it is concluded that there was alarming high prevalence of *Trypanosoma evansi* (Bubaline surra) in Mumbai region along with high level (86.66%) of subclinical status of the disease in apparently healthy buffaloes indicating their high reservoir potential of surra.

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