



PREVALENCE OF TRYPANOSOMOSIS IN BUFFALOES OF MUMBAI REGION

S. Y. Shirale*, H. Y. Palampalle, B. S. Khillare S. B. Majee,
D. P. Kadam and S. S. Sole

*Assistant Professor & PhD Scholar, Department of Veterinary Parasitology
Bombay Veterinary College, Mumbai-12

Received: 25/07/2018

Edited: 31/07/2018

Accepted: 09/08/2018

Abstract: A study was undertaken to find out the prevalence of trypanosomosis in buffaloes of Mumbai region including nearby villages during May 2016 to April 2017. During this study, a total of 382 buffaloes were screened for presence of *Trypanosoma evansi* infection using blood smear examination and PCR technique. The prevalence of *T. evansi* in buffaloes by BSE was 11.52% and 20 samples negative by BSE were found to be positive by PCR method. 44 samples positive by BSE were also positive by PCR method. Thus, the overall prevalence of *T. evansi* was 16.75 per cent. The highest prevalence of trypanosomosis was observed in rainy season (28.44%) followed by winter (15.14%). In the present study *T. evansi* infection was not encountered during summer season. Rainy season was found to be the most favourable season for breeding and propagation of tabanus and stomoxys flies which plays the important role in the transmission of surra in animals. Prevalence of trypanosomosis in Murrah and Mehsana breed was 19.54% and 18.29% % respectively. *T. evansi* infection was not found in Jafrabadi buffaloes. Maximum prevalence was found in females (18.58%) as compare to males (6.78%). *T. evansi* infection was more prevalent in animals above 6 years (40.00%) followed by age group 3-6 years (14.05 %) and age below 3 years (5.42 %).

Keywords: Prevalence, Trypanosomosis, Conventional and Molecular technique.

Introduction

Trypanosoma evansi is a pathogenic haemoparasite with flagella which causes important disease called surra in animals. The disease in cattle and buffaloes is usually sub-clinical or chronic in nature which is characterised by anaemia, progressive loss of condition, loss of milk, abortion and immunosuppression. Surra has a seasonal distribution. It appears as a rule a few weeks after the onset of monsoon (Bhatia, B.B. and Shah H.L., 2001). The climate of Mumbai region is hot and humid which is more conducive for the propagation and breeding of Tabanus and Stomoxys flies. They act as a vector for transmission of *Trypanosoma evansi* infection in animals. Epidemiological data of any infection plays a vital role in treatment and control of the disease. The available literature mainly deals with few outbreaks which are not of much help in establishing true prevalence of the disease (Singh, 1975, Gill, 1977; Ponnudurai *et al.* 2015).

Materials and Methods

The prevalence study of trypanosomosis was conducted in Mumbai region including nearby villages during which 382 buffaloes from twelve different study centers were screened for *Trypanosoma evansi* infection for a period from May, 2016 to April, 2017. The centers included in this study were Safala, Agarwadi, Vasai, Taman, Nala-Sopara, Kalyan, Kolsewadi, Badlapur, Titwala, Karjat, Igatpuri and Bhiwandi. The peripheral blood smears from tip of ear pinna and whole blood samples from jugular vein were collected from suspected and pyrexia cases. The blood smears and the whole blood samples were brought to the laboratory and processed immediately for detection of *Trypanosoma spp.* The prevalence studies of trypanosomosis in buffaloes were carried out by analysing data in terms of overall prevalence; season wise prevalence, sex wise prevalence, breed wise prevalence and age wise prevalence were assessed.

Conventional diagnostic method:

A drop of blood was collected from the ear vein of suspected animals. Thin blood smears were prepared immediately after each blood collection. The blood smears were air-dried, fixed in methanol, subjected to Fields staining and examined microscopically for the presence of *T. evansi* as per method described by (Soulsby, 1982) for confirmation of trypanosoma species.

Molecular diagnosis:

Genomic DNA was extracted from the fresh blood samples by using the protocol of Genei™, Blood Genomic DNA Kit (Bangalore Genei, India). The isolated DNA samples in micro tubes were stored at -20°C until further use for PCR.

The genomic DNA (400 bp) was amplified using *Trypanosoma evansi* species specific forward and reverse primer pair DITRYF (5' CGA CCA GCC AGA ACG AGC AGA AT 3' and DITRYR 5' CTT GTC GAT CGA GTT GACGGT 3') as described by (Sengupta *et al.* 2010). PCR Extracted genomic DNA samples were subjected to molecular technique PCR by using above primers. PCR amplification was carried out targeting variable surface glycoprotein VSG gene. The amplified product was analysed on 1.5% agarose gel (50 min at 70 V) by electrophoresis and visualized by using gel documentation system (Bio rad, USA).

Results and discussion:

Prevalence of *T. evansi* in buffaloes in Mumbai region was studied during May 2016 to April 2017. Out of 382 blood samples examined, 16.75 % (64/382) animals were found positive for *T. evansi*. The conventional method detected only 11.52% (44/382) while with PCR 16.75 % (64/438) cases were found positive for trypanosomosis during present study. The samples positive through blood smear examination were also positive by PCR while out of 64 samples, 20 samples positive by PCR technique were negative by Fields stained blood smear examination under 100X of compound microscope. Therefore PCR test showed higher sensitivity as compared with blood smear examination method. Molecular studies amplification

of DNA from whole blood yielded PCR product of 391 bp (Figure 1) which confirmed the species of parasite to be *Trypanosoma evansi* (Figure 2). PCR has been successfully used in detecting infection with *T. evansi* in buffaloes (Omanwar *et al.* 1999; Subi *et al.* 2011 and Shahzad *et al.* 2012).

The overall prevalence of bubaline surra was found to be 16.75 % which corroborates nearly with the previous study conducted by (Shetye 2000; Birajdar 2007) in Mumbai region. The infection rate recorded was also in accordance with (Parvider *et al.* 2015 Shazad *et al.* 2012 and Singh *et al.* 2017) who have reported prevalence of 9.58%, 11.11% and 18.56% trypanosomosis in Jalandhar of Punjab and Bahadurnagar, Okara, Pakistan and western Uttar Pradesh. On the contrary study conducted by Subi *et al.* (2011) reported the prevalence of 29.64% in the buffaloes of Mumbai. The difference may be attributed to environmental condition of the study year, constant vigilance and proper preventive measures adopted by the individual farmers.

Prevalence of trypanosomosis in buffaloes in relation to season, breed, sex and age of animal (Table 1).

Season: The highest prevalence of trypanosomosis was observed in rainy season 31(28.44%) followed by winter 33(15.13%). None of the blood smears examined in summer revealed *Trypanosoma evansi* organisms. Shetye (2000) and Birajdar (2007) reported similar trends in monsoon and winter in buffaloes of Mumbai region. Vahora *et al.* (2012) also reported high prevalence in cattle and buffalo during June to September. Hot and humid climate during rainy season favours the breeding of tabanus and stomoxys flies which leads to higher prevalence of infection in rainy season. The climatic conditions and geographical location of Mumbai region might be favourable for the growth and multiplication of different vectors.

Breed: As per breed-wise prevalence of trypanosomosis, was found to be approximately similar in Mehsana 18.29% (30/164) and Murrha buffaloes 19.54% (34/174) respectively. *T. evansi* infection was not found in Jafrabadi buffaloes. This finding is in line with Birajdar (2007), reported nearly equal prevalence in Mehsana (4.76%) and Murrah

(4.43%) breeds of buffaloes. The present findings are in agreement with the observations of Singh *et al.* (2017) and Nonga *et al.* (2009), who reported that the prevalence of trypanosomosis in buffaloes does not vary in different breeds. The difference in rate of infection among both the breeds is negligible which might be due to anatomical and physiological similarity among these breeds.

Sex: In present study it has been found that prevalence of trypanosomosis in buffaloes was higher in females 18.58% (60/323) than in males 6.78% (4/59). Higher infection recorded in female might be due to stress during pregnancy and lactation, which could decrease resistance in females and render them more susceptible to infection. Agrawal (2016), also reported highest prevalence of *T. evansi* infection in female buffaloes 20.20 %, followed by male buffaloes 8.62 %. The present findings corroborate with the study of Das *et al.* (1998), who reported high prevalence in adult she buffaloes (3.25%) as compare to the heifers (1.16%) and buffalo bulls (0.36%). However, Singh *et al.* (2017) found that prevalence of trypanosomosis in buffaloes does not vary with the sex.

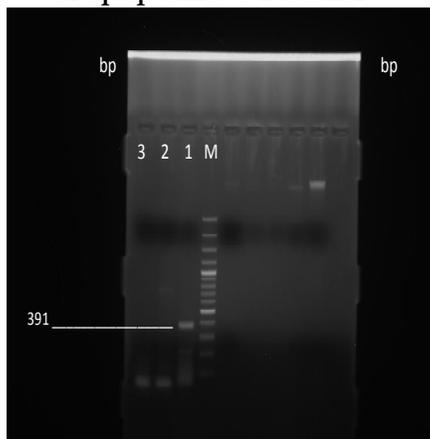
Age: As regards the age-wise occurrence of *T. evansi*, it was observed that in adult buffaloes above 6 years of age, the prevalence rate was higher (40.00%) than the buffaloes ageing between 3 to 6 years (14.05%) and buffaloes below 3 years of age (5.42%). In almost every survey conducted in India, the efforts

were made to note the influence of age on the prevalence of *T. evansi* by (Roy *et al.* 2004; Singh *et al.* 2017; Rani *et al.* 2015) and found that the prevalence of trypanosomosis increases with increase in age of the animals. Higher infection in adult animals may be due to the stress factors like lactation and pregnancy which could make the animals predisposed for the infection apart from exposure to the vectors which play a vital role in transmission of the infection among the animals.

Conclusion:

In the present study 11.52% samples were positive by BSE, while 16.75% samples were positive by PCR, indicating the high sensitivity of PCR for surveying the disease in epidemiological studies. Buffaloes of Mumbai region are highly endemic for trypanosomosis and occurrence of the disease was high during rainy season than winter and null in summer season. It has been found that prevalence of trypanosomosis in buffaloes does not vary in different breeds. The rate of infection in females is higher than males. It is observed that prevalence of trypanosomosis in buffaloes increases with age due to the stress factors like lactation pregnancy and decline in immune status. Further research should be targeted towards understanding the other factors which could influence the prevalence of trypanosomosis in buffaloes in order to evolve the strategic control measures.

Figure 1: PCR amplified product 391 of bp specific to *T. evansi*



Lane: M: 100 bp DNA Ladder

Figure 2: *T. evansi* in blood smear infected buffalo.



Table 1: Prevalence of trypanosomosis in buffaloes of Mumbai region in relation to season, breed, sex and age

Parameter		No. Examined	No. positive	% Prevalence
Season	Summer	55	00	00
	Rainy season	109	31	28.44
	Winter season	218	33	15.14
Breed	Murrah	174	34	19.54
	Mehsana	164	30	18.29
	Jaffrabadi	44	00	00
Sex	Male	59	04	6.78
	Female	323	60	18.58
Age	< 3 years	166	9	5.42
	3-6 Years	121	17	14.05
	>6 years	95	38	40.00

References:

- Agrawal, V. (2016). Haemoprotozoan infections with special reference to trypanosomosis and its molecular diagnosis in dairy animals. A part of PhD thesis submitted to Nanaji Deshmukh Veterinary Science University, Jabalpur.
- Bhatia B. B. and Shah H. L. (2001). Protozoa and Protozoan Diseases of Domestic Livestock. pp.19, Indian Council of Agricultural Research. Pusa New Delhi.
- Birajdar Prashant (2007). Epidemiology, Clinical and chemotherapy of trypanosomosis in buffaloes (*bubalus bubalis*) in Mumbai region. A part of thesis submitted to Maharashtra Animal and Fishery Science University, Nagpur.
- Cheah, T.S., Sani, R. A., Chandrawathani, P., Bahri, S. and Dahlan, I. (1999). Epidemiology of *Trypanosoma evansi* Infection in Crossbred Dairy Cattle in Malaysia. Tropical Animal Health and Production 31:25-31.
- Gill, B. S., (1977). Trypanosomes and Trypanosomiasis of Indian Livestock. Indian Council for Agricultural Research, New Delhi, India.
- Nonga, H. E. and D. M. Kambarage (2009). Prevalence of bovine trypanosomosis in Morogoro, Tanzania. *Pakistan Journal of Nutrition*, 8(3): 208-213.
- Omanwar, S., J. R. Rao, S. H. Basgoudaanvar, R. K. Singh and G. Butchaiah (1999). Direct and sensitive detection of *Trypanosoma evansi* by polymerase chain reaction. Acta. Vet. Hung., 47: 351- 359.
- Parvider Kaur Vipan Kumar, Heigo Pal, Hanish Sharma and Wadawan, V. M. (2015). Seasonal Incidence of Haemoprotozoan Diseases in Cattle and Buffalo. *International Journal of Current Research Vol. 7, Issue, 03, pp.13457-13459*
- Ponnudurai G., Sivaraman, S. N., Rani and Veerapandian, C. (2015). An outbreak of trypanosomosis in buffaloes caused by diminazene resistant *Trypanosoma evansi*. Buffalo Bulletin Vol. 34, No. 1.
- Rani, N .L., Suresh, K. and Rajesh, K. (2015). A retrospective study on clinico-epidemiological aspects of trypanosomiasis in buffaloes. Inter. J. Vet. Sci., 4(2): 97-100.
- Sengupta, P. P., M. Balumahendiran, V.S., Suryanaryana, A.G., Raghavendra, B.R., Shome, Gajendragad, M.R. and K. Prabhudas (2010). PCR-based diagnosis of surra-targeting VSG gene: Experimental studies in small laboratory rodents and buffalo. Vet. Parasitol., 171(1-2): 22-31.
- Shahzad, W., Munir, R., Khan, M. S., Ahmad, M. U. D. and Iqbal M. (2012). MOLECULAR DIAGNOSIS AND CHEMOTHERAPY OF *TRYPANOSOMA EVANSI* IN NILI-RAVI BUFFALOES AT

DISTRICT OKARA (PAKISTAN). The Journal of Animal And Plant Sciences, 22(3 Suppl.) Page: 212-216

- Shetye Yogesh (2000). Studies on the prevalence of blood parasitic infection in cattle and in & around Mumbai. A part of M.V.Sc. thesis submitted to Konkan Krishi Vidyapeeth, Dapoli.
- Singh, A. (1975). Epizootiological study of haematozoa and *Toxoplasma gondi* in bovines of Punjab and relationship of piroplasms of buffaloes with those of cattle. Ph. D. Thesis, Punjab Agricultural University, Ludhiana., India.
- Singh, A. P. Tripathi, A.K. Verma, A.K. Srivastava , A. and Singh, R.K. (2017). Epidemiological Studies and Determination of Clinical Markers of Trypanosomosis in Naturally Infected Buffaloes in the Braj Region of Western Uttar Pradesh, India. Buffalo Bulletin (July-September, 2017). Vol.36 No.3
- Subi Migri, G. P. Bharkad* and M.L. Gatne (2016). Prevalence of Clinical and Subclinical forms of *Trypanosoma evansi* Infection in Buffaloes of Mumbai Region (M.S.) of India. Buffalo Bulletin Vol.35 No.4
- Vahora ,S. P. Patel, J. V. Patel, B. B. Patel, S. B. and Umale, R. H.(2012). Seasonal incidence of Haemoprotozoal diseases in crossbred cattle and buffalo in Kaira and Anand districts of Gujarat, India. Vet. World, Vol.5 (4): 223-225.