PREVALENCE OF TRYPANOSOMOSIS IN BUFFALOES USING LATEX AGGLUTINATION TEST IN HISAR, HARYANA[#]

Pradeep Kumar[1], Sukhdeep Vohra, Krutanjal Iswain and Arun K. Sangwan
Department of Veterinary Parasitology, College of Veterinary Sciences
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, India

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ABSTRACT

Buffaloes from seven blocks of district Hisar, Haryana were examined for evidence of infection with *Trypanosom evansi* by Giemsa stained thin blood smear and monoclonal antibody based latex agglutination test (mAb-LAT) for detection of circulating antigen of *T. evansi.* A total of 400 blood samples were collected from seven blocks (Hisar-I, Hisar-II, Hansi-I, Hansi-II, Naranaund, Barwala and Agroha) of district Hisar, Haryana. The sex and age of the buffaloes under study were also recorded. Examination of blood smear did not reveal any positive case of *T. evansi*. However, mAb-LAT indicated 95 (23.7%), 101 (25.2%), 84 (21.0%), 38 (9.5%) and 82 (20.5%) samples as strong, moderate, weak positive, suspected and negative, respectively for *T. evansi* infection. A higher prevalence was observed in females and animals above 8 years of age as per mAb-LAT. The results confirm the poor efficacy of routine blood examination and recommend the use of new immunological tests for diagnosis of trypanosomosis in domestic animals.

Key words: Prevalence, *Trypanosoma evansi*, buffaloes, mAbLAT

Introduction

Buffaloes in Haryana are vital part of dairy farming and commonly known as "Black Gold". Buffaloes suffer from series of parasitic diseases in which trypanosomosisis caused by *Trypanosoma evansi* (*surra*) is most important disease of large ruminants. *Trypanosoma evansi* is responsible for anaemia, fever, abortions, body weight loss, decreased milk production and animal's draught capacity (Suliman *et al.,* 1989). Trypanosomosis is a vector borne disease of tropical and sub-tropical part of the world (Prasad, 2010). It is mainly transmitted mechanically by various haematophagus dipterans like *Tabanus, Stomoxy, Lyprosia* and *Haematopota* species (Reid, 2002). The disease in buffaloes, cattle and camels is usually chronic, though acute cases have also been reported (Ngaira *et al.,* 2003). Chronic infection may results in heavy production losses due to lowered milk and meat yield, abortions, premature births and also inability to feed the young ones apart from acting as carriers of the infection for healthy animals (Ngaira *et al.,* 2003). Trypanosomal infections are characterized by undulating parasitaemia and the host may not reveal parasites in their peripheral blood for several days (Vickerman, 1978). Conventional techniques of diagnosis trypanosomosis suffer from certain setbacks due to antigenic variation, variability in virulence and susceptibility of the host and hence none of the clinical signs can be counted as pathognomonic feature for routine diagnosis (Masake *et al.,* 2002).

The parasitological examinations frequently fail to detect any patent infections and often scanty parasitaemia is observed in peripheral blood in chronic cases (Killick-Kendrick, 1968). The monoclonal antibody based latex agglutination test (mAb-LAT) was developed to detect the circulating antigens of *T. evansi* in the sera of domestic animals by Rayulu (2007). The test is employed by Shyma (2009)) and Verma (2010) giving high prevalence of *T. evansi* i.e. 60.23% and 32.7%, respectively by mAb-LAT. Thus, the present study was planned to assess the prevalence of *Trypanosomaevansi* in field buffaloes of district Hisar using Giemsa stained thin blood smear and monoclonal antibody based latex agglutination test (mAb-LAT).

Materials and Methods

Hisar district is located in Haryana state with 29° 5'5"N latitude and 75° 45'55"E longitudes in western Haryana. The district consists of nine blocks with agriculture as the main livelihood and some person practicing animal husbandry with buffalo as their main livestock. In the present study a total of 400 blood samples of buffaloes were collected from 30 villages belonging to seven blocks (Hisar-I, Hisar-II, Hansi-I, Hansi-II, Naranaund, Barwala and Agroha) of Hisar from November, 2015 to May, 2016 (Table 1). The blood samples of these animals were examined using Giemsa stained thin blood smear for the presence of adult *T. evansi* protozoa. The serum form these blood samples were collected and monoclonal antibody based latex agglutination test (mAb-LAT) was performed as per method of Rayulu *et al.* (2009). The result of mAb-LAT was interpreted as per the time of agglutination as

S.No	Agglutination time	Interpretation (for *Trypanosoma evansi*)
1.	0-5 minutes	Strong positive
2.	5-10 minutes	Moderate positive
3.	10-13 minutes	Weak positive
4.	13-15 min	Suspected or doubtful
5	> 15 min	Negative for infection

The detail including sex (male and female) and age i.e. heifer (up to 3 year), adult (age between 3-8 yrs) and old (above 8 yrs of age) were also recorded in these animals. The results of mAb-LAT were also compared with sex and age of animals.

Results and Discussion

The Giemsa stained thin blood smear examination of 400 blood smear did not revealed any positive for *Trypanosoma evansi*. The results of serum samples of these buffaloes using mAb-LAT are given in Table 2. The results show 95 (23.7%) sample as strong positive, 100 (25.0%) moderate positive, 82 (20.5%) weak positive, 41 (10.25%) and 82 (20.5%) were

[1#]Part of M.V.Sc. Thesis of the first author and Corresponding author email:sukhdeepvohra@gmail.com

Table 1: Detail of the area of collection of samples in seven blocks of Hisar

Effects	Moderate ambience Mean ± SEM(120)	Extreme Hot ambience Mean ± SEM(120)	Per cent Change In Hot ambience	Extreme Cold ambience Mean ± SEM (120)	Per cent Change In Cold Ambience
Ambience Overall Value(120)	8.00 ^{b}± 0.10	15.70 ^{b}± 0.17	96.25	9.75 ^{b}± 0.10	21.87
Male (60)	8.54 ^{bc}± 0.008	16.72 ^{bc}± 0.008	95.78	10.79 ^{bc}± 0.009	26.34
Female (60)	7.46 ^{bc}± 0.008	14.68 ^{bc}± 0.009	96.78	8.71 ^{bc}± 0.009	16.75
3-8 Months age (40)	6.42 ^{bd}± 0.009	13.69 ^{bd}± 0.009	113.20	7.70 ^{bd}± 0.009	19.93
8-13 Month sage (40)	7.98 ^{bd}± 0.009	15.68 ^{bd}± 0.009	96.49	9.75 ^{bd}± 0.009	22.18
13-18 month sage (40)	9.60 ^{bd}± 0.008	17.73 ^{bd}± 0.008	84.68	11.80 ^{bd}± 0.009	22.91

Figure in parenthesis shows per cent

Table 2: Results of mAb-LAT for 400 serum samples of buffaloes of Hisar

Group	mAb-LAT result				
	Strong positive	Moderate positive	Weak positive	Suspected/ doubtful	Negative
Total	95 (23.7)	100 (25)	82(20.5)	41 (10.25)	82(20.5)

Figure in parenthesis shows per cent

Table 3: Results of mAb-LAT in relation to sex of buffaloes of Hisar

mAb-LAT results / sex	Male	Female	Total
Strong positive	16 (31.37)	79 (22.63)	95 (23.75)
Moderate positive	8 (15.68)	92 (26.36)	100 (25)
Weak positive	9 (17.64)	73 (20.91)	82 (20.5)
Suspected / doubtful	7 (13.72)	34 (9.74)	41 (10.25)
Negative	11 (21.56)	71 (20.34)	82 (20.5)
Total animals	51	349	400

Figure in parenthesis shows per cent

doubtful and negative for trypanosomosis, respectively. A total of 277 (69.25%) samples were found positive for *T. evansi* as recorded by mAb-LAT. The mAb-LAT results from the sera samples of buffaloes as per their sex are shown in Table 3. A higher prevalence of infection was observed in females i.e. 69.91% (244/349) as per mAb-LAT compared to male i.e. 64.70% (33/51) buffaloes of Hisar. Only 41 (10.25%) and 82 (20.5%) buffaloes were suspected and negative for *T. evansi* infection as per mAb-LAT. The mAb-LATresults from the sera samples of buffaloes as per their age is shown in Table 4. The results of buffaloes as per their age for *T. evansi* infection showed a highest prevalence in old i.e. 75.47% (n= 40/53) followed by heifer i.e. 73.68% (n=56/76) and lowest in adult animals i.e. 66.78% (n=181/271). Only 41 (10.25%) and 82 (20.5%) buffaloes were suspected and negative for *T. evansi* infection as per mAb-LAT.

In our study a higher prevalence of *Trypanosoma evansi* (69.25%) was observed by the examination of the sera of buffaloes in Hisar, Haryana. Rayulu (2007) and Shyma (2009) also reported 37.19% and 78.2% prevalence in buffaloes in Hisar by mAb-LAT, respectively. Verma (2010) reported 32.7% sera samples of cross bred calves positive by mAb-LAT.

In the present study a higher prevalence of trypanosomosis (69.91%) was reported in female as compared to male (64.70%). Singh *et al.* (2012) also reported a higher prevalence of infection in female buffaloes of Ludhiana. The study also revealed a higher prevalence of infection in older animals (75.47%), followed by heifer (73.68) and lowest in adult animals (66.78%). Similar type of prevalence was observed by Singh *et al.* (2012). Payne *et al.* (1991) in Indonesia also reported an ascending age-related prevalence of infection of *T. evansi* in buffaloes with highest serological infection rates in older animals.

The present research revealed a higher prevalence of subclinical form of trypanosomosis among buffaloes i.e. without showing any clinical signs of disease. The study also supports the previous finding of poor efficacy of blood examination for diagnosis of trypanosomosis. Thus the present results recommend the use of new immunological techniques for the diagnosis of trypanosomosis in domestic animals.

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