EPIDEMIOLOGICAL STUDIES AND DETERMINATION OF CLINICAL MARKERS OF TRYPANOSOMOSIS IN NATURALLY INFECTED BUFFALOES IN THE BRAJ REGION OF WESTERN UTTAR PRADESH, INDIA

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ABSTRACT

Present investigation was conducted with an aim to investigate epidemiology and clinical markers of trypanosomosis in naturally infected buffaloes. It was found to be prevalent throughout the year of study and maximum prevalence was observed in the wet months of monsoon and post monsoon season, September and October months and least in the dry months, May and June. It has been found that there was significant increase in prevalence of trypanosomosis in buffaloes with increase in age and parity. During present investigation the clinical markers of trypanosomosis were determined based on the degree of frequency distribution of clinical signs, which can be of great importance in field conditions where the diagnostic facilities are lacking.

Keywords: buffalo, *Bubalus bubalis*, trypanosomosis, clinical markers, epidemiology, TE-LAT

INTRODUCTION

Trypanosoma evansi is a widely-distributed haemo-flagellate of veterinary importance that infects a variety of mammals including horses, mules, camels, buffalo, cattle and deer. It causes epidemics of a disease called Surra, which is of great economic importance in Africa, Asia and South America, where thousands of animals die from *T. evansi* infection each year (Fernandez *et al.*, 2009). The infection is mechanically transmitted by blood-sucking insects of the genera *Tabanus, Stomoxys, Atylotus* and *Lyperosia* etc. but in India mostly by Tabanid biting flies (Vijay *et al*., 2002). Trypanosomosis ranks high in importance amongst bovine diseases due to its devastating effects on the livestock health leading to severe economic loss to the dairy industry due to loss of condition, reduction in milk yield etc. (Pholpark *et al*., 1999).

Prevalence of disease depends on rate of exposure, availability of infected animals, the insect reservoir and seasons (Mottelib *et al*., 2005). The disease is manifested by recurrent episodes of fever (105^{o}F to 106^{o}F) and parasitaemia which occur during the course of disease together with a progressive anemia and loss of condition. Abortions and infertility have been reported in buffaloes in Asia. Symptoms of high fever, muscle twitching, anorexia, increased salivation and acute abdominal pain manifested by kicking at

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the belly have recently been reported in (Luckins, 2004). Present investigation aimed to evaluate the epidemiology and to determine the clinical markers of trypanosomosis in naturally infected buffaloes in Braj region of western Uttar Pradesh.

MATERIALS AND METHODS

The study was performed at teaching veterinary clinical complex, Uttar Pradesh Pandit. Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura (TVCC, DUVASU, Mathura) from June 2013 to May 2014. Which is located in the Braj region of western Uttar Pradesh, and animals were brought for diagnosis and treatment of various ailments from different parts of Braj region. Out of 2494 buffaloes reported to the TVCC, a total of 890 buffaloes, suspected with trypanosomosis, with the history of suffering with fever, anorexia, loss of production and loss of body condition were selected for study. Total number of buffaloes coming to the TVCC for different ailments and number of buffaloes suspected, were recorded on monthly basis. In present investigation diagnosis of trypanosomosis was done by a monoclonal antibody-based latex agglutination test (TE-LAT), invented and provided Dr Ajit Singh, Professor Immunology, LLRUVAS, Hisar, Haryana, India. It involves the detection of *T. evansi* circulating antigens in sera samples from infected animals. Standard protocol was followed as proposed by inventor.

Epidemiological studies of trypanosomosis in buffaloes were done by analyzing data in term of overall prevalence, suspected prevalence, month wise prevalence, sex wise prevalence, age wise prevalence, breed wise prevalence, parity wise prevalence and pregnancy status wise prevalence were assessed as per formulas mentioned below, for more accurate assessment of epidemiology of trypanosomosis in the region buffaloes were further categorized as sex wise (Male and Female), age wise (<2 year, 2 to 5 years, and >5 years), breed wise Nondescript (ND), Murrah (M) and Bhadawari (B), parity wise (0, 1, 2 to 3 and >3), and pregnancy status wise (Pregnant and Non-pregnant).

$$\text{Overall prevalence (\%)} = \frac{\text{No of positive cases (with TE – LAT) (Whole year)}}{\text{Total no buffaloes coming at the TVCC (N) (Whole year)}} \times 100$$

$$\text{Suspected prevalence (\%)} = \frac{\text{No of positive cases (with TE – LAT) (Whole year)}}{\text{Total no suspected buffaloes coming at the TVCC (Whole year)}} \times 100$$

$$\text{Month wise prevalence} = \frac{\text{No of positive cases (with TE – LAT) in specific month}}{\text{Total no of cases of suspected buffaloes coming at the TVCC in the same month}} \times 100$$

$$\text{Sex wise prevalence} = \frac{\text{No of positive cases of a specific sex (Whole year)}}{\text{Total no of cases of suspected buffaloes of same sex coming at the TVCC (Whole year)}} \times 100$$

$$\text{Age wise prevalence} = \frac{\text{No of positive cases of a specific category (age group) (Whole year)}}{\text{Total no of cases of suspected buffaloes in same specific category (age group) coming at the TVCC (Whole year)}} \times 100$$

$$\text{Breed wise prevalence} = \frac{\text{No of positive cases of a specific category (breed) (Whole year)}}{\text{Total no of cases of suspected buffaloes in same specific category (breed) group coming at the TVCC (Whole year)}} \times 100$$

$$\text{Parity wise prevalence} = \frac{\text{No of positive cases having specific category (parity) (Whole year)}}{\text{Total no of cases of suspected buffaloes in same specific category (parity) coming at the TVCC (Whole year)}} \times 100$$

$$\text{Pregnancy status wise prevalence} = \frac{\text{No of positive cases of buffaloes of specific category (Whole year)}}{\text{Total no of cases of buffaloes of same category at the TVCC (Whole year)}} \times 100$$

Clinical markers in the diagnosis of trypanosomosis were assessed by assessing frequency distribution of various clinical signs in each and every trypanosome positive buffaloes using TE-LAT. The frequencies of clinical signs were categorized as highest frequency (Clinical signs having frequency more than 60%), moderate frequency (Clinical signs having frequency between 30% to 60%) and lowest frequency (Clinical signs having frequency below 30%) Frequency distribution of different clinical signs and their combinations in trypanosome positive

animals were evaluated as % frequency as per below mentioned formula. Statistical analysis to test the level of significance among host related factors was done by chi-square test (Snedecor and Cochran, 1994).

$$\% \text{ Frequency} = \frac{\text{No of positive cases showing specific clinical sign (n)}}{\text{total no positive cases (N)}} \times 100$$

RESULTS AND DISCUSSION

Epidemiological study

In present study the maximum prevalence was recorded during month of September, 2013 followed by the month of October, 2013 and the least prevalence was recorded in the month of June, 2013 (Table 1). The order of decreasing prevalence was September followed by October, January, February, December, August, November, March, May, April, July, and lowest in the month of June. Trypanosomosis in buffaloes was found to be prevalent throughout the year of study and its increase in the wet months of monsoon and post monsoon season could be due to the abundance of vectors (Tabanid flies) at the highest level in the grazing areas and the incidence was lowest in dry months of May and June when there is very least presence of vector (Tabanid flies) in the grazing areas. The variations in the prevalence pattern of *T. evansi* infections in buffaloes could be due to the vector population increase in considerable number during rainy and post-rainy seasons than the winter and summer seasons of year. Inclement weather such as hot and humid climate in the months of monsoon and thereafter might have been incriminated to depress the body defense mechanism thereby resulting in the exacerbation of *T. evansi* infection in bovines. Similar findings were also reported by Muraleedharan *et al*. (2005)

Table 1. Percent prevalence of trypanosomosis in buffaloes in different months.

S.N.	Month	Total no of buffaloes	No of cases examined (suspected)	Positive no of cases	Prevalence (%)	Overall prevalence (%)
1	June (2013)	254	74	28	37.83	11.02
2	July (2013)	176	78	34	43.58	19.31
3	August (2013)	190	82	42	51.22	22.10
4	Septerber (2013)	269	139	95	68.34	35.32
5	October (2013)	201	100	63	63	31.34
6	November (2013)	143	54	25	46.29	17.48
7	December (2013)	159	50	26	52	16.35
8	January (2014)	160	50	28	56	17.50
9	February (2014)	162	51	27	52.94	16.67
10	Mach (2014)	258	69	31	44.92	12.02
11	Appril (2014)	239	65	29	44.61	12.13
12	May (2014)	283	78	35	44.87	12.37
Total		**2494**	**890**	**463**	**52.02**	**18.56**

who reported that *T. evansi* infection rate was high in south west monsoon followed by north east monsoon and the lowest incidence was recorded in hot weather followed by cold weather. Lohr *et al*. (1986) observed the high rate of infection during the rainy season. Sinha *et al.* (2006) evaluated the highest incidence during monsoon followed by winter and least during summer season. Awandkar *et al*. (2004) reported a prevalence of trypanosome spp. in cattle and buffaloes and found it to be highest in monsoon followed by post-monsoon, winter and summer seasons. Rundassa *et al.* (2013) found relatively higher incidence rate during the wet seasons of the year. The incidence of surra in bovines recorded by Dhami *et al*. (1999) opined that rainy and post rainy seasons showed high incidence due to high prevalence of the vectors.

Prevalence study according to sex

In present study it has been found that prevalence of trypanosomosis in buffaloes does not vary with the sex (Table 2). The findings are in agreement with the findings of Eyasu *et al*. (2013), Mbahin *et al*. (2013), Rundassa *et al.* (2013) and Chanie *et al*. (2012) earlier reported that the associations between the infection rates of trypanosomosis within different sex groups are statistically insignificant.

Table 2. Percent prevalence of trypanosomosis in buffaloes according to various host related factors (sex, age, breed, parity and pregnancy).

Host related factors	No of examined (890)	No of positives (463)	Prevalence (%) (52.02)	Chi-square value
Sex				
Male	24	12	50	$\chi^2_{1\,d.f.} = 0.04^{NS}$
Female	866	451	52.08	
Age, years				
< 2	84	6	7.14	$\chi^2_{2\,d.f.} = 84.22^{*}$
2-5	121	62	51.24	
> 5	685	395	57.66	
Breed				
N.D.	748	390	52.14	$\chi^2_{2\,d.f.} = 0.03^{NS}$
M	90	46	51.11	
B	52	27	51.92	
Parity				
Zero	98	8	8.16	$\chi^2_{3\,d.f.} = 95.51^{*}$
1	100	45	45	
2 to 3 times	340	192	56.47	
More than 3 times	328	206	62.80	
Pregnancy status				
Pregnant	396	204	51.51	$\chi^2_{1\,d.f.} = 0.00^{NS}$
Non pregnant	470	247	52.55	

Prevalence study according to age

It has been found that there was significant increase in prevalence of trypanosomosis in buffaloes with increase in age (Table 2). The highest prevalence was found in category 3 (>5 years age) followed by category 2 (2 to 5 years of age) and lowest prevalence was recorded in category 1 (<2 years age group). Therefore, in present study it had been found that prevalence of trypanosomosis in buffaloes increases with age. It may be due to the stress factors like lactation and pregnancy which will make the animals predisposed for the infection apart from exposure to the biting flies which are primary source for the transmission of the infection among the animals. It may also be due to the fact that the old animals are usually allowed to feed by grazing outside, so there might be more chance of exposure to biting flies than the young ones that's are managed by intensive feeding. These findings of present investigation are in agreement with the findings of Chanie *et al*. (2012); Bhutto *et al*. (2010); Muraleedharan *et al*. (2005); Sinha *et al*. (2006).

Prevalence study according to breed

In present study it has been found that prevalence of trypanosomosis in buffaloes does not vary in different breeds (Table 2). The present findings are in agreement with the findings of Nonga *et al*. (2009) who reported that sex, breed, grazing system, farm size, acaricide application and chemoprophylaxis were not the risk factors for the trypanosomosis infection.

Prevalence study according to parity

During present investigation it has been observed that prevalence of trypanosomosis in buffaloes increases with parity (Table 2). The possible cause may be same as that with increase in age that is due to demands of production (lactation) and reproduction (pregnancy) acts as a stress factors to the animals which might leads to Immunological and nutritional stress, therefore, predisposed the animals for infection. These findings of present investigation are in agreement with the findings of Chanie *et al*. (2012); Bhutto *et al*. (2010); Sinha *et al*. (2006); Muraleedharan *et al*. (2005).

Prevalence study according to pregnancy status

It has been observed in present study that prevalence of trypanosomosis in buffaloes does not vary between pregnant and non-pregnant buffaloes (Table 2). Although pregnancy acts as stress to the animals but it is also a fact that more care and good nutrition is given to the pregnant animals than non-pregnant ones which minimizes the stress in the pregnant animals. Therefore, no variations in the prevalence of trypanosomosis in buffaloes in present study could be recorded between pregnant and non-pregnant buffaloes. The findings of the present investigation are contrary to the findings reported by cheah *et al.* (1999) that higher prevalence of trypanosomosis in late and early pregnancy in cattle.

Frequency distribution of different clinical signs (Clinical markers of trypanosomosis)

In present study it had been found that predominant clinical signs observed in trypanosomosis in buffaloes (Table 3 and Figure 1) were anorexia, fever, depression, reduced milk yield, congested mucous membrane, nasal discharge, emaciation, respiratory distress, pallor mucous membrane, anemia and edema of dependent parts with highest frequency (>60%). Therefore, these signs can be considered in concert can be relied upon to diagnose trypanosomosis in field conditions as a clinical markers of trypanosomosis

where laboratory diagnosis facility are lacking.

Clinical signs observed in present investigation (Table 3) with moderate degree of frequencies (30 to 60%) are superficial lymph node swelling, salivation, ocular discharge, Ileus, head pressing. These signs can also facilitate in the diagnosis of trypanosomosis in the field conditions along with predominant clinical signs mentioned

Table 3. Percent frequency distribution of various clinical signs (Clinical markers) in buffaloes suffering from trypanosomosis.

Sl. No.	Clinical signs	No of + ve cases	% Frequency	
1.	Anorexia	415	89.63	Frequency more than 60%
2.	Fever	389	84.02	
3.	Depression	345	74.51	
4.	Reduced milk yield	329	71.06	
5.	Congested mucous membrane	310	66.95	
6.	Nasal discharge	304	65.66	
7.	Emaciation	291	62.85	
8.	Respiratory distress	288	62.20	
9.	Pallor mucous membrane	287	61.99	
10	Anaemia	283	61.12	
11	Edema of dependent parts	278	60.04	
12.	Superficial lymph node swelling	221	47.73	
13.	Salivation	204	44.06	Frequency between 30 to 60%
14.	Ocular discharge	200	43.2	
15.	Ileus	166	35.85	
16.	Head pressing	144	31.10	
17	Frequent urination	138	29.80	
18.	Excitement	125	26	Frequency below 30%
19.	Circling	112	24.19	
20.	Abortion	84	18.14	
21.	Abdominal pain	76	16.41	
22.	Muscular twitching	70	15.12	
23.	Diarrhoea	70	15.12	
24.	Bloat	63	13.61	
25.	Localized paralysis	23	4.97	
26.	Generalized paralysis	4	0.86	
27.	Exophthalmia	3	0.65	
28.	Corneal opacity	2	0.43	

Note: No of trypanosome positive buffaloes were 463.

above therefore they may also be considered as a clinical markers of trypanosomosis in field conditions.

Clinical signs observed (Table 3) with lowest frequencies (<30%) are frequent urination, excitement, circling, abortion, abdominal pain, muscular twitching diarrhoea, bloat, corneal opacity, localized paralysis, generalized paralysis and exophthalmia. Therefore, these signs can also be sometime considered in the diagnosis of trypanosomosis in field conditions in sporadic cases as clinical markers of trypanosomosis.

There are no any previous reports in the literature describing frequency distribution of different clinical signs in trypanosomosis in bovines; however some reports are available describing only few clinical manifestations noticed by individual workers. The most commonly reported clinical signs are pyrexia, parasitaemia, progressive emaciation, dullness, generalized edema, recurrent episodes of fever, anemia, respiratory distress, continuous lacrimation, sudden drop in milk yield, deep and prolonged breathing creating noise, nervous symptoms such as circling movement or beating head to mangers, shivering of muscles, coma, collapse and death, abortions and infertility in trypanosomosis in cattle and buffaloes by various workers (Manohar, 1984; Lohr *et al*., 1986; Baracos *et al*., 1987; Gill, 1991; Davison *et al*., 1999; Luckins, 2004; Pathak and Singh, 2005; Singh *et al*., 2011).

In present study presence of fever may be due to the toxins liberated by the parasites which were present in the blood, irrespective of concentration of the parasite this leads to the change in the body temperature set point in the hypothalamus under the influence of pyrogenic stimuli released during infection. Respiratory distress, anemia, pallor mucous membrane may be due to the significant decrease in Hb, and TEC that was found during the present study in trypanosome positive buffaloes this might had happened due to

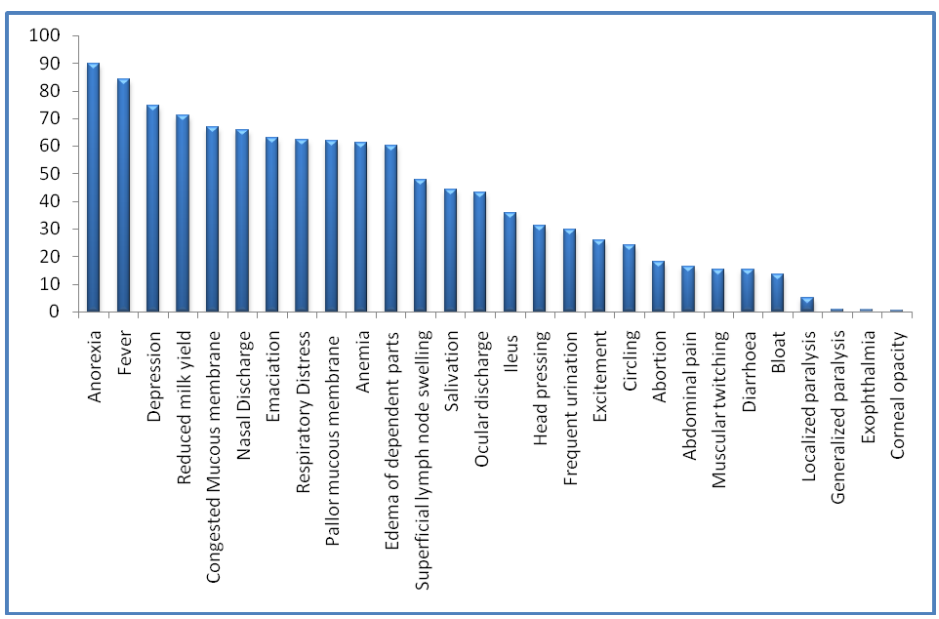

Figure 1. Percent frequency of different clinical signs (Clinical markers) of trypanosomosis in buffaloes.

the destruction RBC and reduction in hemoglobin concentration due to inhibitory effect of toxins released by the trypanosomes (Singh *et al*., 2011). Edema of dependent parts, and depression noticed in the present investigations are might be due to the severe anemia (Pathak and Singh, 2005). Loss of appetite, fever and pale mucous membranes, severe anemia, jaundice, abortion, hyperemia and petechiation of the conjunctiva, keratitis, cervical lymph node enlargement, nasal discharges, diminished milk production, diarrhea, and accentuated weight loss neurological symptoms of dysmetria, ataxia and muscle weakness, salivation, and edema and profuse diarrhea had been earlier reported in bovine trypanosomosis (Fabiano *et al*., 2012; Welled *et al.*, 1989; Brown and Leak, 1999). Ocular discharge and corneal opacity in trypanosomosis had been earlier reported by Saror (1980) but literature could not reveal any citations related to presence of signs of ileus in trypanosomosis. Nervous signs like incordination of gait, circling movements, head pressing, and excitement were seen which may be due to long standing chronic infection resulting in invasion of brain by *T. evansi* (Tuntavusan, 1997).

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