Original Research

Correlation of Monoclonal Antibody Based Latex Agglutination Test with Haematological and Physiological Parameters for Diagnosis of Bubaline Trypanosomosis

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Abstract

Around 400 blood samples of buffaloes belonging to thirty villages in seven blocks of district Hisar, Haryana were screened for the presence of Trypanosoma evansi using Giemsa stained thin blood smears and monoclonal antibody based latex agglutination test (mAb-LAT). The haemoglobin (Hb; g/dL), packed cell volume (PCV; %) and the body temperature (^oF) were also examined. Blood examination did not reveal any sample positive for T. evansi infection, however, mAb-LAT showed 95 (23.7%), 101 (25.2%), 84 (21.0%), 38 (9.5%) and 82 (20.5%) samples showing strong, moderate, weak positive, doubtful and negative reactivity, respectively. Significant differences were observed in Hb and PCV values between positive samples when compared to suspected and negative samples inmAb-LAT test, while for temperature significant difference was seen between positive and negative samples only. These results pointed towards lower sensitivity of blood smear examination over mAb-LAT test for diagnosis of bubaline trypanosomosis.

Key words: Buffaloes, Haryana mAb-LAT, *Trypanosoma evansi*

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Introduction

The water buffalo (*Bubalus bubalis*) have been well domesticated in the Indian sub-continent by the era of medieval period. They are an important source of milk supply in today's period and yield nearly three times milk when compared to cows (TNAU Agritech Portal, 2014). More than half of the total milk produced (55%) in the country is contributed by 47.22 million milch buffalo heads. Among the various diseases reported in buffaloes, trypanosomosis caused by *Trypanosoma evansi* commonly known as '*surra'* is of foremost importance. It is an important vector borne disease of tropical and sub-tropical regions of the

world (Prasad, 2010). It is mainly transmitted mechanically by various biting flies like *Tabanus, Stomoxys, Lyprosia* and *Haematopota* species (Reid, 2002). The disease is pathogenic and widely distributed in Africa, South and Central America and Asia (Luckins, 1998; Pathak and Khanna, 1995; Wernery and Kadeen, 1995). The disease is often fatal in the absence of treatment and exhibits specific clinical signs (anaemia, loss of weight, abortion, stretching of neck, head pressing and death) which are variable from one host and one place to another (Desquesnes *et al.*, 2013). Bubaline trypanosomosis has often been observed as a chronic entity, however, under stress conditions it may become sub-acute or acute (Ray *et al.*, 1992). Recently, a case of *surra* in human has also been reported in India (Joshi *et al*, 2005), thereby, assuming its significance as a possible zoonotic threat in near future (Laha and Sasmal, 2007). In India, *T. evansi* infection is widely prevalent in different parts and is of significant economic importance in livestock production (Juyal *et al*., 2007). The disease is more common in Rajasthan, Punjab, Haryana, Uttar Pradesh, and Gujarat (Pathak *et al.*, 1995).

The conventional parasitological examinations frequently fail to detect any patent infections and often scanty parasitaemia is observed in peripheral blood even in chronic cases (Killick, 1968).The biochemical tests *viz*., formol-gel test, mercuric chloride test, flocculation test, turbidity test and concentration techniques like haematocrit centrifugation and Diethyl amino ethyl (DEAE) cellulose purification are reported to be less sensitive than animal inoculation methods which too are laborious, time consuming and unsuitable for large scale use in the field (Pegram and Scott, 1976). In the past, Rayulu (2007) developed a monoclonal antibody based latex agglutination test (mAb-LAT) to detect the circulating antigens of *T. evansi* in sera of domestic animals at LUVAS, Hisar, Haryana. The mAb-LAT test was also employed by Shyma (2009) and Verma (2010) giving high prevalence of 60.23% and 32.7%, respectively. Thus, the present study was designed to assess the prevalence of trypanosomosis in buffaloes in Hisar district using blood smear and mAb-LAT and correlate its results with haematological and physiological parameters for the confirmation of the disease.

Materials and Methods

Area of Study

The district Hisar is located in Haryana, India with 29° 5'5"N latitude and 75° 45'55"E longitudes in western Haryana with the average elevation of 215 m (705 ft) above mean sea level. The region is part of the alluvial Ghaggar-Yamuna plain.

Experimental Design

The study was divided into two groups i.e. group 1 comprising of 300 blood samples collected from randomly selected buffaloes and group 2 of 100 blood samples from suspected cases reported at Teaching Veterinary Clinical Complex (TVCC), Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar

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or Government Veterinary Hospitals (GVH) at Hisar district from November, 2015 to May, 2016. The blood samples were collected from thirty villages located in seven blocks of district Hisar *i.e.* Hisar I, Hisar II, Hansi I, Hansi II, Naranaund, Barwala and Agroha of Hisar district. The rectal temperature (°F) was recorded from the presented animal before the collection of blood. The blood samples were collected aseptically and examined for the presence or absence of *T. evansi* by Giemsa staining of thin blood smears. The haemoglobin (Hb; g/dl) and packed cell volume (PCV; %) were estimated using Sahli's method and micro-haematocrit method, respectively as per Jain (1986). The serum was also isolated from the collected blood samples and monoclonal antibody based latex agglutination test (mAb-LAT) was performed as per method of Rayulu *et al*. (2009). The result was interpreted as per the time of agglutination i.e. time of agglutination is 0-5 min it was interpret as strong positive; 5-10 minutes as moderate positive; 10-13 minutes as weak positive; 13-15 min as suspected or doubtful and more than 15 min was negative result.

The other parameters (or risk factor) pertaining to the host like sex (male and female) and age i.e. heifer (upto 3 year), adult (age between 3-8 years) and old (above 8 years of age) were also recorded. The results of mAb-LAT were also compared with Hb, PCV and temperature values of the animals, to draw conclusions, if any. The results were analyzed using student's T-test and Dunken's Multiple Range Test (DMRT).

Results and Discussion

For the detection of *Trypanosoma evansi* in buffaloes, blood samples were collected from block Hisar I (villages: Balawas, Chirod, Dhansu, Dubeta, Gangwa, Harita, Ladwa), Hisar II (villages: Arya Nagar, Balsmandh, Dhiranwass, Dobhi, Kharia, Ludas), Hansi I (villages: Umra, Kanwari), Hansi II (villages: Bhaklana, Bhatoljatan, Bhatol Rangran, Jeetpara, Dharam Kheri, Khanda Kheri), Narnaund (villages: Rajpura, Shapura, Sulchani, Majra, Milakpur), Agraha (villages: Agroha, Kalirawan) and Barwala (villages: Bhabalpur, Barwala). A total of 400 blood samples which include 51 male, 349 female and 75 heifers, 272 adults and 53 old animals were examined. Maximum of 42 blood samples were collected from village Ludas, Hisar II. The blood smear examination using Giemsa staining did not reveal any case positive for *T. evansi* infection. Similar were the results of Haque *et al*. (2012) who found no blood sample positive by Giemsa stained blood smears out of 104 blood samples examined at different districts of Punjab. The lower efficacy of blood smear was also found by Dhami *et al*. (1999) from Punjab and Swarnkar *et al.* (1993) from Rajasthan. The results of mAb-LAT test for detection of *T. evansi* antigen on isolated serum samples of buffaloes from group 1 and group 2 are given in Table 1.

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Table 1: Results of mAb-LAT test for detection of *T. evansi* antigen

Group	mAb-LAT				
	Strong	Moderate	Weak	Suspected	Negative
Group 1 (n=300	60 (20.0)	69 (23.0)	60 (20.0)	34 (11.3)	77 (25.6)
Group 2 (n=100)	35 (35.0)	32 (32.0)	24 (24.0)	4 (4.0)	5 (5.0)
T-test value	3.05260**	1.79406^{NS}	0.85049 ^{NS}	2.16594*	4.43344**
Total	95(23.7)	101 (25.2)	84(21.0)	38 (9.5)	82(20.5)

**Significant at p<0.05; **Significant at p<0.01; NS: Non-significant; Figure in parenthesis shows per cent*

The mAb-LAT show 95 (23.7%) sample as strong positive, 101 (25.2%) moderate positive, 84 (21%) weak positive, 38 (9.5%) doubtful and 82 (20.5%) negative for trypanosomosis. A total of 280 (70%) samples were found positive for *T. evansi* as per mAb-LAT revealing a higher prevalence rate in buffaloes at Hisar. Similar findings have been reported in the past by Rayulu (2007) and Shyma (2009) who reported 37.19% and 78.2% prevalence of trypanosomosis, respectively in buffaloes at Hisar using mAb-LAT. As regards the physiological and haematological parameters, the haemoglobin progressively increased from strong (9.87) to negative (12.59) results in group 1 (randomly selected) and from strong (9.9) to negative (12.7) results of mAb- LAT in group 2 (suspected cases) (Table 2).

Table 2: Physiological and haematological parameters in Group 1 (randomly selected) and Group 2 (suspected) buffaloes of Hisar, Haryana

mAb-LAT results	Haemoglobin (Mean ± S. E.)		
	Group 1	Group 2	t test value
Strong	9.87^{d}± 0.21	9.90^{d}±0.21	0.0839 ^{NS}
Moderate	10.97^{c}±0.11	10.87^{c}±0.18	0.4726 ^{NS}
Weak	11.75^{b}±0.13	12.27^{a}±0.20	2.2022*
Suspected	12.38^{a}±0.15	12.36^{a}±0.28	0.0785 ^{NS}
Negative	12.59^{a}±0.07	12.70^{a}±0.49	0.2202 ^{NS}
	PCV		
Strong	31.57^{d}±0.41	32.57^{d}±0.0.66	1.2925 ^{NS}
Moderate	34.94^{c}±0.35	34.61^{d}±0.52	0.5279 ^{NS}
Weak	36.78^{b}±0.35	37.55^{c}±0.65	1.0358 ^{NS}
Suspected	38.26^{a}±0.49	39.43^{c}±0.95	1.0930 ^{NS}
Negative	39.17^{a}±0.27	39. 00^{c}±1.14	0.1441 ^{NS}
	Body Temperature		
Strong	103.42^{a}±0.13	103.63^{a}±0.09	1.3832^{NS}
Moderate	102.73^{b}±0.11	102.92^{b}±0.07	1.4853 ^{NS}
Weak	101.95^{c}±0.13	101.83^{c}±0.10	0.7694 ^{NS}
Suspected	101.69^{cd}±0.18	102.21^{c}±0.26	1.6464 ^{NS}
Negative	101.52^{d}±0.11	101.32^{d}±0.26	0.7190 ^{NS}

Mean bearing different superscripts in a column differ significantly (p<0.05).

The PCV values increased progressively from strong (31.57) to negative (39.17) results in Group 1 and from strong (32.57) to suspected (39.4) and remained almost constant in negative (39) results in Group 2

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(suspected cases) (Table 2). In the past, Verma *et al*. (1976) and Singla *et al*. (1997) also revealed lower values of Hb and PCV in *T. evansi* infected calves. Regarding the temperature, there was decrease in the body temperature from strong (103.42ºF) to negative (101.52ºF) mAb- LAT in group 1and from strong (103.63ºF) to negative (101.32ºF) results in group 2 (Table 2). The values of body temperature in positive samples were significantly different from negative results in both samples collected from field as well as from TVCC/GVH. Payne *et al*. (1991) in the past have also reported marked effect on the temperature profiles of the buffaloes with *T. evansi* infection.

The statistical analysis of the results of Hb, PCV and temperature between Group 1 and Group 2 showed non-significant difference except the value of Hb in weak mAb-LAT results which was significant at $P<0.05$ by using 't test' (Table 2). Comparison between the values of Hb of animals at different results of mAb-LAT using Dunken's Multiple Range Test (DMRT) at 5% level showed significant difference of positive (strong, moderate and weak) results in comparison to suspected and negative results, however, the difference between suspected and negative results was non-significant. Similarly, values of PCV at different result of mAb-LAT using DMRT at 5% level showed significant difference between positive (strong, moderate and weak) when compared to suspected and negative results. The body temperature of animals with different results of mAb-LAT showed significant difference between the values of strong, moderate, weak and negative results using DMRT at 5% level showed significant.

Conclusion

In conclusion, the study revealed a higher prevalence (70.0%) of sub-clinical trypanosomosis among buffaloes of Hisar, Haryana by mAb-LAT test. The decreased values of Hb and PCV with increase in the temperature further supported the utilization of mAb-LAT test for diagnosis of bubaline trypanosomosis. The study confirmed the poor efficacy of blood smear examination in diagnosing bubaline trypanosomosis in field hospitals and suggested the use of other techniques for the diagnosis of trypanosomosis in domestic animals.

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References

1. Desquesnes M, Holzmuller P, Lai DH, Dargantes A, Lun ZR and Jittaplapong S. (2013). *Trypanosoma evansi* and *Surra*: A Review and Perspectives on Origin, History, Distribution, Taxonomy, Morphology, Hosts, and Pathogenic Effects. *Bio Medical Research International*, Article ID 194176, 22 pages. http://dx.doi.org/10.1155/2013/194176.

DOI 10.5455/ijlr.20180803031953

2. Dhami DS, Juyal PD and Singla LD. (1999). Seroepidemiology of *Trypanosomaevansi* infection by using CATT. *TheIndian Veterinary Journal*, 76: 842-844.
3. Haque M, Joyti, Singh H, Singh NK and Rath SS. (2012). PCR based detection of cryptic *Trypanosoma evansi* infection in cattle. *Indian Veterinary Journal*, 89 (3): 19-21.
4. Jain N C. (1986). Schalm's Veterinary Haematology. 4thedn. La and Febiger, Philadelpia, USA.
5. Joshi PP, Shegokar VR, Power RM, Herder S, Katti R, Saiket HR, Dani VS,Bhargara A, Jannin J and True P. (2005). Human Trypanosomiasis caused by *T. evansi* in India. *American Journal of Tropical Medical and Hygiene*, 73: 491-495.
6. Juyal PD, Kaur P and Singh NK. (2007). Control strategies against trypanosomosis (*surra*) due to *Trypanosoma evansi* in India. In: Proceedings of the National Seminar on "Recent diagnostic trends and control strategies for haemo-protozoan infections in livestock", Feb, 9-11, 2007, held at S.D. Agricultural University, Sardar Krushinagar, Gujarat. pp. 4-7.
7. Killick KR. (1968). The diagnosis of trypanosomiasis of livestock. A review of current techniques. *Veterinary Bulletin*, 38: 191-197.
8. Laha R and Sasmal NK. (2007). Advancement in diagnosis of *surra* and its dissemination for the field. In: Proceedings of National Seminar on "Recent diagnostic trends and control strategies for Haemo-protozoan infections in livestock", Feb' 9-11, 2007 at S.D. Agricultural University, Sardar Krushinagar, Gujarat, India. pp., 59-65.
9. Luckins AG. (1998). *Trypanosoma evansi. Asian Journal of Parasitology*, 4: 137-142.
10. Pathak KML and Khanna ND. (1995). Trypanosomosis in camel (*Camelus dromedarius*) with particular reference to Indian subcontinent: a review. *International Journal Animal Science*, 10: 157-162.
11. Payne RC, Suanto IP, Djauhari D, Partoutomo S, Wilson AJ, Jones TW, Boid R and Luckins AG. (1991). *Trypanosoma evansi* infection in cattle, Buffalo and horses in Indonesia. *Veterinary Parasitology*, 38: 109-119.
12. Pegram RG and Scott JM. (1976). The prevalence and diagnosis of *Trypanosoma evansi* infection in camels in southern Ethiopia. *Tropical Animal Production and Health*, 8: 20-27.
13. Prasad KJ. (2010). Emerging and Re-emerging parasitic Diseases. *International Journal of Medical Sciences*, 23: 45-50.
14. Ray D, Biswas G and Sen GP. (1992). *Trypanosoma* infection in cattle and buffalo. *Indian Journal of Animal Sciences*, 62: 420.
15. Rayulu VC. (2007). Characterization of surface antigens of *Trypanosoma evansi* by monoclonal antibodies and development of suitable assays. PhD. Thesis, CCS Haryana Agricultural University, Hisar, India.
16. Rayulu VC, Chaudhri SS and Singh A. (2009). Evaluation of parasitological and monoclonal antibody based assays in detection of *Trypanosoma evansi* infection in animals. *Indian Journal of Animal Sciences*, 79: 978-981.
17. Reid SA. (2002). *Trypanosoma evansi* control and containment in Australia. *Trends in Parasitology*, 18(5): 219-224.
18. Shyma KP. (2009). Comparison of monoclonal antibody based latex agglutination test with PCR for diagnosis of *Trypanosoma evansi* in domestic animals. MVSc thesis, CCS Haryana Agricultural University, Hisar, Haryana.
19. Singla LD, Juyal PD, Roy KS and Kalra IS. (1997). Host response of cow-calves against levamisole as immune-potentiator in *T. evansi* infection: Haemato-pathological study.
20. *Journal of Veterinary Parasitology*, 11: 55-63.
21. Swarnkar CP, Raisinghani PM, Kumar D and Bhan AK. (1993). Detection of circulating antigen of *Trypanosoma evansi* in *surra* suspected cattle and buffaloes. *Indian Journal of Animal Health*, 32: 177-178.
22. TNAU Agritech portal (2014). Animal Husbandry: Livestock: Buffalo: Breeds

DOI 10.5455/ijlr.20180803031953

23. Verma BB, Gautam OP and Malik PD. (1976). *Trypanosoma evansi*: Therapeutic efficiency of diminazene aceturate in cross-bred calves *Bos tarsus* and *Bos indicus*. *Experimental Parasitology*, 40: 406-410.
24. Verma R. (2010). Cross reaction between *Trypanosoma evansi* and other haemoprotozoan in cattle. MVSc thesis, CCS Haryana Agricultural University, Hisar, Haryana.
25. Wernery U and Kadeen OR. (1995). Infectious Diseases of Camelids. Blackwell Wissenschafts-Verlag, Berlin. 42: 411-414.

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