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RESEARCH ARTICLE

Bovine Trypanosomosis in Punjab: Assessment of seroprevalence by CATT/ *T. evansi*

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Abstract

Trypanosomosis (Surra), caused by *Trypanosoma evansi*, is one of the most devastating mechanically transmitted vector-borne disease of domestic and wild animals in Asia, Africa, and South and Central America. To assess the seroprevalence of *T. evansi* in dairy animals, a total of 732 sera (282 cattle adult, 130 cattle calves, 212 buffaloes and 108 buffalo calves) samples collected from 20 districts of Punjab (India) belonging to different agro-climatic zones were subjected to card agglutination test for *T. evansi* (CATT/*T. evansi*). The overall seroprevalence of *T. evansi* as depicted by CATT was significantly higher (39.34%) than the parasitological prevalence by Giemsa stained based thin blood smears (2.18%). Through the argo-climatic zones, the parasitological prevalence ranged from 0% (Western zone) to 3.8% (Undulating zone) while seroprevalence ranged from 27.36% (Sub-mountain zone) to 43.02% (Undulating zone). The highest seroprevalence of *T. evansi* was observed in district Jalandhar (58.41%) of the Central Plain Zone; while lowest seroprevalence was observed in district Pathankot (16.67%) in Sub-mountain Zone. The seroprevalence of *T. evansi* was found to be higher in buffalo adults (50.47%) as compared to their calves (33.33%) or the cattle adults (36.88%). In conclusion CATT/*T.evansi* is a pen-site test and has higher field applicability for assessment of the serology based diagnosis. The risk factors were found to be most predominately associated with the location of farms as being inside the Sutlej basin (Odd's ratio=1.93).

Introduction

Trypanosoma evansi, a flagellated kinetoplastid haemoprotozoa, is the most prevalent pathogenic trypanosome having the largest geographical distribution in Asia, Africa, South and Central America (Laha and Sasmal, 2008; Dargantes *et al*., 2009). It causes a devastating disease named 'surra'. *T. evansi* infects a wide range of domestic and wild animals owing to its ability of mechanical transmission by biting flies like *Tabanus* and *Stomoxys* (Luckins, 1988; Desquesnes *et al.*, 2009; Kumar *et al.*, 2012). Effects of the infection in different geographical locations vary according to the strain of parasites and species of the host (Losos, 1980). Clinical signs are variable and non-specific (Killick-Kendrick, 1968). The disease in bovines is characterized by severe anaemia, nervous complications, weight loss, reduced productivity, infertility and abortion and death in some animals during the early phase of the disease (Juyal *et al*., 2005). The disease also interferes with immune system and causes immunosuppression paving the way for bacterial and viral infections (Holmes *et al.*, 1974; Onah *et al*., 1997; Holland *et al*., 2003; Singla *et al.*, 2009).

The standard parasitological method are routinely used to demonstrate and identify trypanosomes in the blood of the infected animal. The parasitological techniques have low sensitivity and cannot be relied upon for epidemiology and chemotherapy especially in bovines. Various serological tests like indirect immunofluorescent antibody test (IFAT), enzyme linked immunosorbent assay (ELISA) and card agglutination test for trypanosomiasis

(CATT) are used to identify the specific antibody responses. CATT/*T.evansi* is highly specific in detecting circulating antibodies as it targets variant surface glycoprotein *RoTat* 1.2. CATT/*T. evansi* for antibody detection was originally described by Bajyana and Hamers (1988) and converted into a test kit by the Institute of Tropical Medicine, Belgium. CATT/*T.evansi* being a pen-site diagnostic test can screen large number of samples, especially in the field conditions due to ease of use and can target specific animals for treatment with trypanocidal drugs. This test requires extensive evaluation in the various geographical regions where *T. evansi* is a problem. Although serial testing, which refers to ELISA followed by CATT, is recommended to help declare a disease-free condition (OIE, 2009), rapid card agglutination tests may be more field applicable.

The climate of the Punjab state is very favourable for the disease vector propagation, leading to increased mobility of *T. evansi* between susceptible and reservoir hosts (Juyal *et al.,* 2005; Gupta *et al.,* 2009). No previous systematic study on the seroprevalence and associated risk factors has been conducted for *T. evansi* infection in bovines throughout the state of Punjab This study on CATT/*T. evansi* was carried out to investigate the seroprevalence of *T. evansi* in dairy animals from different agro-climatic zones of Punjab and to compare the association of infection rates with hypothesized risk factors.

Materials and methods

Study areas

The study was carried out from May, 2011 to October, 2012 throughout the different agro-climatic zones of Punjab covering a total area of 50,362 square kilometres located between 29"30'N to 32"32'N latitude and 73"55'E to 76"50'E longitude. As per 18th Indian Livestock census 2007, the state has 6.8 million bovines with 5.0 million buffaloes and 1.8 million cattle.

Study design and sample collection

Blood samples were randomly collected state-wide from 732 dairy animals (282 cattle adults, 130 cattle calves, 212 buffalo adults, and 108 buffalo calves) from 20 different district of five major agro-climatic zones *viz.,* Central Plain Zone, Undulating Zone, Western Zone, Western Plain Zone, and Sub-mountain Zone of Punjab (India). Dairy animals under study included indigenous (*Bos indicus*) and cross bred cattle, and buffaloes (*Bubalus bubalis)*. Five ml of blood samples were collected in EDTA coated and plain vacutainers from the jugular vein of the animals. Information was collected about the description and health status of animals, and location of the farm to evaluate the risk factors.

Giemsa stained thin blood smears (GSTBS)

Thin blood smears were prepared immediately after each blood collection. The blood smears were air dried, fixed in methanol, stained with Giemsa and examined under oil immersion lens of microscope for the presence of *T. evansi*.

Card agglutination test for T. evansi (CATT/T. evansi)

Sera was separated from collected blood samples by centrifuging at 3000rpm for 5 min and then tested for the presence of anti-*T. evansi* antibodies using the CATT/*T. evansi* kit (Institute of Tropical Medicine, Antwerp, Belgium). Approximately, 45 µl of the antigen was poured into the circular reaction zones of the supplied plastic card and mixed with 25 µl of the test sera diluted at 1:4 with PBS (pH 7.2) according to the manufacturer's instructions. The dropper was held vertically and drops were allowed to fall freely without touching the card to maintain a constant volume. The antigen/serum mixture was mixed and spread properly using a clean plastic stirring rod to approximately 1mm from the rim of the test area and the stirring rod was wiped with sterile filter paper after each use. Each plastic card contained two reaction zones for the positive and negative controls.

The card was agitated for 5 minutes at 70 rpm after positioning on a flat bed of electric orbital rotator (Ika Labortechnik, KS 130 basic, Germany). After five minutes the results were read before removing the card from the rotator. A reaction was considered positive when agglutination is visible with the naked eye (Verloo *et al*., 2000). Animals positive for anti-trypanosome antibodies by CATT showed different degrees of agglutination graded as +++ (very high positive), ++ (high positive), + (moderate positive), ± (mild positive) and – (negative).

Statistical analysis

χ^2 test was employed to compare parasitological and seroprevalence of *T. evansi,* in various agro-climatic zones of Punjab. Odd's ratio was calculated to assess the risk factors.

Results

Out of 732 blood samples subjected to CATT/*T. evansi* agglutination test and GSTBS examination, the overall parasitological and seroprevalence of the infection in Punjab state was recorded to be 2.18 % by GSTBS and 39.34% by CATT/*T. evansi*, respectively. All parasitologically positive samples by GSTBS were also positive by CATT/*T.evansi*. Seroprevalence of *T. evansi* depicted by CATT was significantly higher than the parasitological prevalence based on GSTBS method. The incidence rate by conventional technique GSTBS ranged from 0% (Western zone) to 3.8% (Undulating zone).

The highest seroprevalence of *T. evansi* by agglutination assay was recorded in Undulating Zone (43.02%) followed by Central Plain Zone (42.69%), Western Zone (41.38%), and Western Plain Zone (28.26%) and lowest in Sub-mountain Zone (27.37%) (Table1). The prevalence based on CATT/*T. evansi* differed significantly among five different agro-climatic zones under study (P = 0.025). The district wise seroprevalence was highest in Jalandhar (58.41%) in Central plain zone; while the lowest Pathankot (16.67%) in Sub-mountain Zone.

The overall seroprevalence among cattle was 35.19% (145/412) of which 31.54% (41/130) was recorded in cattle calves and 36.88% (104/282) in adults; the age wise difference being non significant. The overall prevalence of trypanosomosis in buffaloes was 44.68% (143/320), out of which 33.33% (36/108) was recorded in buffalo calves and 50.47% (107/212) in adults; the age wise difference being significant (P=0.003547). The seroprevalence of *T. evansi* was found to be higher in buffalo adults (50.47%) as compared to their calves (33.33%) or the cattle adults (36.88%) (Table 2). The species wise seroprevalence was significantly higher in buffaloes (44.68%) as compared to cattle (35.19) (P=0.009107).

Based on the level of agglutination, 2.73%, 15.16%, 7.37%, 14.08% and 60.66% animals were graded as very high (+++), high (++), moderate (+), mild positive (±) and negative (-) respectively. The risk factors (odds ratio, OR) associated with the presence of the infection were: age, >1 year/ < 1 year (OR = 1.56); sex, male/ female (1.04); species, buffaloes/ cow (1.48); and location in/ outside Sutlej basin (1.93) (Figure1) (Table 3).

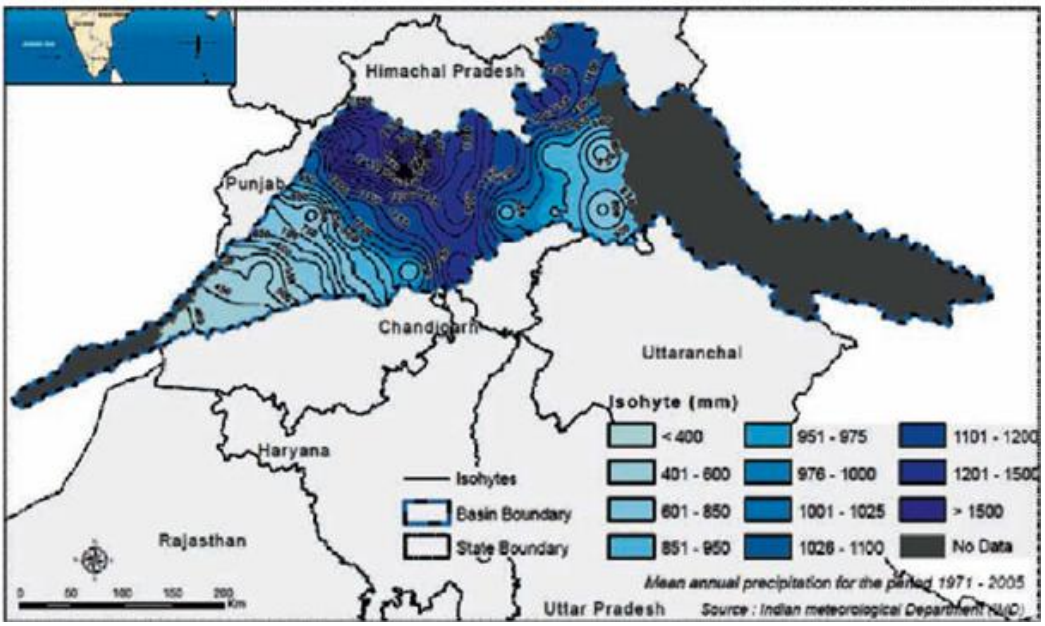

Figure 1: The Sutlej basin and spatial variation of rainfall within. (*Source: ADB, 2011 with data from IMD*)

Table1: Zone and District-wise prevalence of *T. evansi* infection detected by blood smear examination and seroprevalence by CATT/*T.evansi*

Agro-climatic Zone	District	Samples	GSTBS positive	CATT positive (%)
Central Plain Zone	Amritsar	16	0	3(18.75)
	Jalandhar	113	6 (5.31)	66 (58.41)
	Kapurthala	17	0	5 (29.41)
	Ludhiana	126	3 (2.38)	42 (33.33)
	Patiala	46	0	23 (50)
	Fatehgarh sahib	18	0	5 (27.78)
	Tarn taran	13	0	5 (38.46)
	Total	**349**	**9 (2.57)**	**149 (42.69)**
Undulating Zone	Nawanshehr	48	2(4.16)	22 (45.83)
	Ropar	38	1 (2.63)	15 (39.47)
	Total	**86**	**3 (3.48)**	**37 (43.02)**
Western Zone	Bathinda	15	0	6 (40)
	Moga	42	0	19 (45.24)
	Sangrur	20	0	7(35)
	Mansa	51	0	20 (39.22)
	Muktsar	7	0	2 (28.57)
	Barnala	10	0	6 (60)
	Total	**145**	**0**	**60 (41.38)**
Western plain Zone	Faridkot	12	1 (8.33)	4 (33.33)
	Ferozepur	34	1 (2.94)	9 (26.47)
	Total	**46**	**2 (4.34)**	**13 (28.26)**
Sub-mountain Zone	Gurdaspur	35	0	9(25.71)
	Hoshiarpur	59	2 (3.38)	18 (30.51)
	Pathankot	12	0	2 (16.67)
	Total	**106**	**2 (1.88)**	**29(27.36)**
Total		**732**	**16 (2.18)**	**288 (39.34)**
			P = 0.371352*	P= 0.025157**

*Differing non-significantly (P≤0.05); **Differing significantly (P ≤0.05)

Table 2: Species and age-wise seroprevalence of *T. evansi* detected by CATT/*T.evansi*

Species		Score of Agglutination					Total	Positive (%)
		+++	++	+	±	-		
Cattle	Adult	11	51	17	25	178	282	104 (36.88)
	calves	1	14	7	19	89	130	41 (31.54)
	Total	12	65	24	44	167	412	**145 (35.19)**
Buffalo	Adult	6	39	21	41	105	212	107 (50.47)
	Calves	2	7	9	18	72	108	36 (33.33)
	Total	8	46	30	59	179	320	**143 (44.68)**
Total		**20**	**111**	**54**	**103**	**444**	**732**	**288 (39.34)**

Table 3: Relative Risk factors with respect to the incidence of Trypanosomosis

Risk Factor		sample size (n= 732)	Positive cases (n =288)	Percent positive	Odd's ratio
Age	>1	494	211	43.11	1.56
	<1	238	77	31.51	
Sex	Male	187	75	40.11	1.04

	Female	545	213	39.08	
Species	Buffalo	320	143	44.68	1.48
	Cow	412	145	35.19	
Location	Sutlej Basin	580	246	42.41	1.93
	Out of Sutlej basin	152	42	27.63	

Discussion and Conclusion

The present study was designed to determine the seroprevalence of anti-*Trypanosoma evansi* antibodies in cattle and buffaloes of Punjab, India based on CATT/*T.evansi* targeting the variant surface glycoprotein *RoTat* 1.2 gene. Serological assays for the detection of anti-trypanosome circulating antibodies have high measure of validity, are economical and applicable at large scale screening (Lejon *et al*., 2003). CATT/*T. evansi* is a quick and easy test which can be performed under laboratory and field conditions (Magnus *et al.,* 1978; Gutierrez *et al.*, 2000). High test accuracy has been reported for CATT/*T. evansi* in India (Pathak *et al*., 1997), Brazil (Franke *et al*., 1994), Mauritania (Dia *et al*., 1997), Indonesia (Davison *et al.,* 1999) and Kenya (Ngaira *et al*., 2003). As many cases remain undetected and untreated even after the completion of parasitological investigations. This situation is disappointing for the infected individual as well as the other animals in the herd. These individuals might maintain the reservoir of the infection and contribute to further dissemination of the disease in the animal population.

The prevalence of surra in Punjab, India, was reported to be 7.92%, with more sub-clinical cases in buffaloes than in cattle (Singla *et al.*, 2004). The statistically significant difference in the seroprevalence among the Central Plain Zone, Undulating Zone and Western Zone was observed. The difference between the seroprevalence values of these zones was found to be statistically significant with Sub-mountain Zone. This can be attributed to the fact that the sample collection sites in Central Plain Zone, Undulating Zone and Western Zone lies within Sutlej Basin (*Source: ADB, 2011 with data from IMD*) where climate and rainfall precipitation (> 950mm) during the study period created hot and humid conditions. The areas in the vicinity of Sutlej basin mainly low lying and disease is endemic throughout India particularly in low-lying areas (Juyal, 2011).

In contrast to this, Sub-mountain region also lies in this basin, but have lower temperature, thus attributing to lower prevalence of surra. Western plain Zone have lower precipitation (~400mm) corroborating to consequential lower prevalence of the disease. By and large the relevant data signifies that the trypanosomosis is very much prevalent and wide spread in the Punjab state. *T. evansi* is also prevalent in other parts of India like Eastern region (42.1% in cattle and 43.8% in buffaloes) (Laha and Sasmal, 2009). The assessment of risk factors indicated that the animals lying in Sutlej basin area have approximately double propensity of carrying anti trypanosome anti bodies than those lying outside (Table 3).

In terms of species related variation in seroprevalence values, buffaloes showed higher prevalence than cattle though the difference was non-significant (Table 2). It may be due to the fact that buffaloes act as reservoirs most frequently (Juyal, 2011).The difference in risk for subclinical infection of the disease on the basis of sex was almost negligible (Table 3).

Regarding age of the animal, adult buffalo population revealed significantly higher seroprevalence of anti-trypanosome antibodies than the other groups. Odd's ratio for the risk assessment of the latent trypanosomal infection (serologically detected by CATT/*T. evansi*) on the basis of age was 1.56 indicating that adult animals were more prone (Table 3). Similar to present study findings, earlier reports in camels showed higher prevalence of surra in age group above 4.5 years as compared to age group of 1.5 to 4.5 years (Gutierrez *et al*., 2000, Swai *et al.,* 2011). However, no significant difference in prevalence of trypanosome infection between different age groups of cattle (Delafosse *et al.,* 2006). As in the present findings, young boran calves were more resistant to trypanosomosis than older animals (Dolan, 1998). One hypothesis for this pattern simply reflects the number of exposure years, which are less in young ones than adults (Dolan, 1998). Similar trend has been observed in earlier studies in Burkina Faso (Desquesnes *et al.,* 2009) and Ghana (Mahama *et al.,* 2004) involving cattle.

To conclude, Surra is the one of the deadly disease of bovines, which acts as the reservoir host**.** Owing the economic losses in terms of morbidity and mortality in bovines the seroepidemiology of the diseases is very important. CATT/*T.evansi* is a pen-site test and has higher field applicability and able to access large number of sample. The seroprevalence varied which was highest in undulating zone of Punjab and in buffaloes.

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