



Spatial seroepidemiology, risk assessment and haemato-biochemical implications of bovine trypanosomiasis in low lying areas of Punjab, India



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ABSTRACT

A cross-sectional study was carried out on 594 bovines (341 buffalo adults, 31 buffalo calves, 163 cattle adults, and 59 cattle calves) to assess the exposure of native bovine population to *T. evansi* elicited trypanosomiasis in the low-lying areas of Punjab (India). We ruled out the endemicity of the disease with 10.77% (95%CI = 8.53–13.52) sero-positive and 23.56% (95%CI = 20.33–27.15) suspected cases by card agglutination assay. We have presented the spatial distribution of these cases as a guideline to local veterinary practitioners and policy-makers. The categorical assessment of risk factors revealed buffalo adults are the most susceptible group in the state despite insignificant differences in farm management practices. A significant increase in the WBC, platelet, AST and serum iron, and decrease in haemoglobin, haematocrit volume, and serum glucose was recorded in both *T. evansi* positive and suspected animals.

1. Introduction

Cattle and buffalos are the primary source of milk for human consumption in India. Bovine trypanosomiasis elicited by *Trypanosoma evansi* is an important tabanid fly-transmitted disease exacting a high public health burden and devastating impact on animal health, thereby decreasing the net milk yield. *T. evansi* causes deterioration of blood and tissues resulting in the condition termed as ‘surra’ (rotten). The presence of the disease in the state posing considerable economic losses due to weakness, abortion, and emaciation of infected animals [1,2].

This disease amasses to immense economic loss particularly during epidemic outbreaks [3], with an estimated 300 million cattle and buffaloes of Punjab exposed to infection [4], although a country-wide data is limited at risk of this disease. Prevalence of infection is influenced by various biotic and abiotic factors. Hot and humid regions are conducive for pupal and adult vector development, thereby enhanced the chance of infection [5]. Prophylactic and therapeutic measures are mandatory for premium livestock to control of this disease. Due to the paucity of data pertaining to the sero-epidemiology of the disease, we designed the current study which can guide local veterinary practitioners and policy-makers.

In this study, we use a rapid, sensitive and specific diagnostic technique to monitor sero-epidemiology of the disease in the low-lying

paddy growing areas of Punjab. Previous publication from our laboratory has defined these low-lying areas for the cross-sectional epidemiological studies of veterinary disease [6]. Disease spatio-epidemiological models based on remote sensing, machine-learning algorithms and Geographic Information Systems (GIS) platforms were obtained to predict areas of potential distribution of *Trypanosoma evansi*. The data was further analysed for correlation of seropositivity levels with host physiological conditions (haemato-biochemical alterations) and physical factors (climatic, edaphic and risk factors).

2. Materials and methods

2.1. Ethical statement

The ethics committee for animal experiments from the Guru Angad Dev Veterinary and Animal Sciences University approved the work proposal under the RKVY funded project. Blood samples from animals were collected with utmost care with the prior consent of the owners.

2.2. Study area and sampling index

Samples were collected from low-lying areas of Punjab province indicated in Fig. 1a. To study the status of seroprevalence of bovine

Abbreviation: CATT, card agglutination test for *T. evansi*; CPZ, central plain zone; SMZ, submountain undulating zone; UZ, undulating zone; WPZ, western plain zone; WZ, western zone

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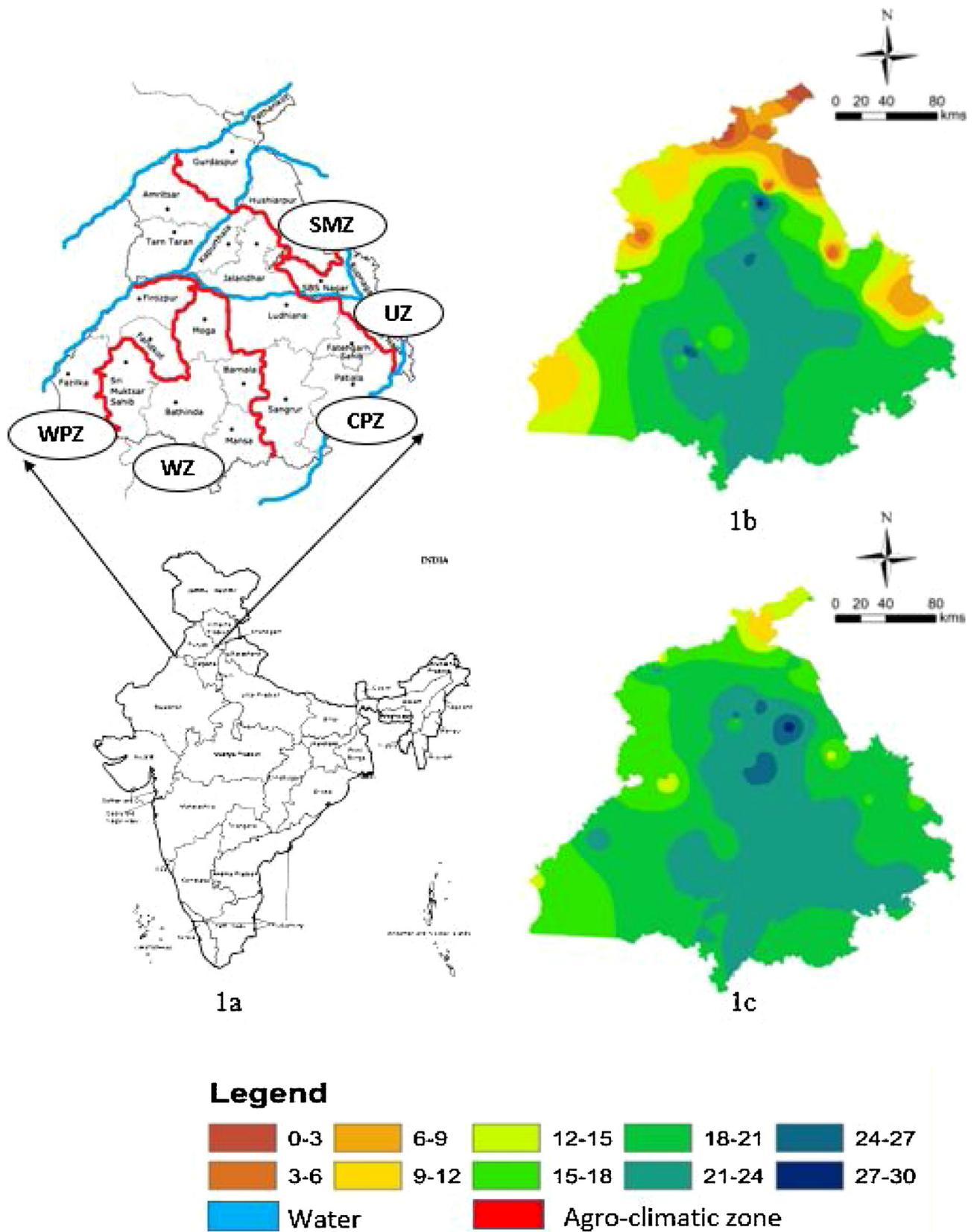


Fig. 1. (a) Map of Punjab (inset in map of India) showing with five agroclimatic zones (SMZ: Submountain Undulating Zone; UZ: Undulating Zone; CPZ: Central Plain Zone; WZ: Western Zone; WPZ: Western Plain Zone) and four water bodies running through (Sutlej, Ravi, Beas and Ghaggar). Predicted geographical distribution ranges of (b) high titre (+++/++) and (c) low titre (++/±) (right) *Trypanosoma evansi* by CATT/*T. evansi*.

trypanosomiasis, the expected prevalence of 50% with confidence limits of 95% and a desired absolute precision of 5% to collect maximum number of samples was considered [7]. The minimum sample size is reconfirmed by the method of Naing et al. [8] (where, n is the sample size, Z indicates Z statistic for a level of confidence (95%), P is the expected prevalence or proportion (on the scale of zero to one); d defines precision).

$$n = \frac{Z^2 P(1 - P)}{d^2}$$

The number of samples collected was adjusted for finite population and correlated with 594 samples (166 males, 428 females) of bovines and were collected from the areas adjoining the major rivers (Sutlej, Beas, Ravi and Ghaggar rivers: Fig. 1a) in five different agro-climatic zones of Punjab during the peak infection seasons (April to October of 2011–2013). Approximately five ml of blood sample from jugular vein was collected in EDTA coated (for haematology) and plain vacutainers (for serology) from each animal. Information regarding the herd size, age, species, sex, management practices, vaccination and/or treatment history of the animals was gathered in pre-designed epidemiological questionnaires. Sampled animals were thoroughly examined for the clinical signs of trypanosomiasis (intermittent fever, anaemia, weight loss, nervous signs, discharges from the eyes, and oedematous swellings under the skin).

2.3. Blood film

Two methanol fixed thin blood films of each blood sample were Giemsa stained and examined under oil immersion lens [9]. During examination of slide, erythrocytes in ~15 microscopic fields (approximately 9000 cells) were observed.

2.4. Serological test

CATT/*T. evansi* for antibody detection was originally described and converted into a test kit by the Institute of Tropical Medicine, Belgium [10]. Samples were scored as – (negative), ± or + (suspected), and + + or + + + (positive) according to the manufacturer's instructions based on the appearance of blue granular agglutination.

2.5. Spatial analysis of disease and correlation with environmental variables

The total number of animals sampled in village was linked with digital village boundary in Geographic Information System (GIS) and the centroid of each village was generated in ArcGIS 10.2. The spatial depiction of disease determined by CATT/*T. evansi* were prepared by Inverse Distance Weighting (IDW), an interpolation technique, assuming that a value of an attribute at an unsampled location is a distance-weighted average of known data points within a local neighbourhood surrounding the unsampled location (Fig. 1a, b). Prevalence of disease was correlated by Pearson correlation (r) with environmental factors which may affect *T. evansi* prevalence such as mean of average temperature, precipitation, potential evapotranspiration and cloud cover (data of last five years retrieved from: http://www.indiawaterportal.org/met_data/).

2.6. Haemato-biochemical parameters

The haematological parameters of the whole blood were studied on fully automated analyzer, ADVIA 2120 Haematology System (Siemens Health Care Diagnostic Inc. Deerfield, IL, U.S.A). Differential leucocytes were counted manually under oil immersion of light microscope in Giemsa stained blood smears. Biochemical parameters were estimated as per the protocol of Agappe Diagnostic Ltd. on semi auto analyzer RA-50. For haemato-biochemical analysis, of all positive cases, only the

animals with no other haemoparasitic infection were selected for the comparison, to eliminate the alterations implicated by protozoans other than *T. evansi*.

2.7. Statistical analysis

The prevalence of *T. evansi* was assessed by detecting parasitemia in blood smear and by analysing seropositivity for anti-*T. evansi* antibodies. The findings were correlated by Pearson's chi-square test at $P \leq 0.05$; 95% confidence interval using SPSS 16.0 software. Confidence interval was calculated by formula given below [7], where n is the sample size, p is the expected prevalence.

$$p \pm 1.96 \times \sqrt{\frac{p(1 - p)}{n}}$$

One-way analysis of variance (ANOVA) of haematological and biochemical parameters was analysed on SPSS 16.0 software. Animals with no clinical sign, or history of any treatment were selected as non-infected controls (group III).

3. Results

3.1. Spatio-epidemiological distribution

Rigorous sampling ($n = 594$) was done from at least three distant low-lying sites from each district of different agroclimatic zones. Parasitemia was not observed in any blood smear. In serology, 64 (10.77%, 95%CI = 8.53–13.52) animals displayed high titre (positive: + + + / + +) and 140 (23.56%, 95%CI = 20.33–27.15) low positive titre (suspected: + / ±). Highest frequency of positive cases was reported in district Gurdaspur (24%, 95%CI = 11.50–43.43) of sub-mountain undulating zone, and suspected cases were highest in district Amritsar (38.5%, 95%CI = 22.43–57.47) of central plain zone. Lowest incidence of positive cases was recorded from district Roopnagar (1.7%, 95%CI = 0.25–8.86), and of suspected cases from district Pathankot (9.5%, 95%CI = 2.65–28.91). There was statistically insignificant difference in the preponderance rate of both higher ($\chi^2 = 21.94$) and lower ($\chi^2 = 18.38$) titre samples among the different districts of Punjab falling in low lying areas (Fig. 1b and c). The maps of spatial distribution of *T. evansi* determined by CATT/*T. evansi* showed that the variation in occurrence of the disease ranged from 1 to 24% for high titre (+ + + / + +) and from 9 to 39% for low titre (+ / ±). Spatial analysis of all premises revealed that chances of *T. evansi* infection were lower toward the central zone of the province and higher towards periphery (as seen in both Fig. 1b and c).

3.2. Risk factors analysis

The assessment of relative risk ratio revealed the incidence of *T. evansi* was uniformly distributed through the geography of the region (Table 1). The difference was significant between animals of different age, species and sex. Among the various animal groups understudy, the preponderance of higher titers was of the order, buffalo adults > buffalo calves > cattle adults > cattle calves; while that of lower titers was buffalo adults > cattle adults > buffalo calves > cattle calves (Fig. 2). The sero-prevalence of *T. evansi* didn't have a strong correlation with any of the considered climatic factors (Table 2). The farm management conditions also seem to have no effect on the disease occurrence (Table 1) (Fig. 3).

3.3. Associated haemato-biochemical alterations

For haemato-biochemical studies, only 36 animals with higher titre (group I) and 49 animals with lower titre (group 2) free from tick infestation or any other haemoprotozoan were selected. A significant

Table 1

Distribution of variables identified to determinate the risk factors associated with seroprevalence of bovine trypanosomiasis in Punjab, India.

Factors	Variables	Total sample	Positive (%)	High titre	Low titre	χ ² -square	df	P- value	Relative risk (RR)
Eastern area	SMU-UN	182	52 (28.58)	20	32	3.99	2	NS	a
Central area	CPZ	294	107 (36.39)	26	81				1.27
Western area	WPZ-WZ	118	45 (38.14)	18	27				1.33
Age	Adults	504	189 (37.50)	59	130	18.49	3	S	3.34
	Calves	90	15 (16.66)	05	10				a
Species	Buffalo	372	143 (38.44)	36	107	10.08	1	S	1.53
	Cow	222	61 (27.47)	28	33				a
Sex	Male	166	36 (21.69)	16	20	16.37	1	S	a
	Female	428	168 (39.25)	48	120				1.81
Management	Organized	233	73 (31.33)	25	48	1.5	1	NS	a
	Unorganized	361	131 (36.29)	39	92				1.59
Total		594	204 (34.34)	64	140				

a: indicates the reference factor for relative risk analysis; df: degree of freedom.

increase in WBC and platelet count with statistically insignificant but apparent increase in lymphocyte count in both sero-positive and suspected animals. These animal groups also depicted lower levels of haemoglobin and higher serum iron levels. Statistically, serum glucose (GLU) level varied significantly at P ≤ 0.05 in group I as compare to group II and III animals (Fig. 4). Sero-positive samples also has increased levels of aspartate aminotransferase, a liver function enzyme.

4. Discussion

Punjab is heavily dependent on agriculture and animal husbandry for its economy. In this scenario, disease surveillance and epidemiological reporting are mandatory specifically for the veterinary diseases that remain undecipherable by the gold standard techniques of thin blood smear examination [11]. Such cases can be the nidus of infection which tends to flair during stress conditions. Serological assays help map out areas with higher risk, which otherwise get an underwhelming attention.

In the present study, we did not encounter any animal with teaming parasitaemia observable in blood smears, thus supporting the fact that microscopic detection is not feasible until the parasitemia reaches 2.5 × 10⁶/ml [12]. In milder or chronic infection, the intermission phase may be prolonged and, even when symptoms appear, trypanosomes may still be undetectable blood, thus dithering the treatment, thereby raising the chance of morbidity and mortality in animal population [13]. CATT/*T. evansi* can be a promising tool for timely

Table 2

Correlation of seroprevalence of *T. evansi* with various environmental variables in Punjab (India).

Environment variables	Overall seroprevalence	High titre seroprevalence
Maximum temperature	-0.17607	-0.31431
Minimum temperature	-0.25392	-0.40095
Average temperature	-0.21409	-0.31431
Diurnal temperature range	-0.25369	-0.31805
Precipitation	-0.18057	-0.00914
Potential evapotranspiration	0.040814	-0.09737
Cloud cover	-0.56653	-0.29653

diagnosis and treatment interventions curtailing covert disease conditions in the field and this survey will help to make better control measure.

In the present study, 140 animal sera with low agglutination were considered suspected as in non-infected horses, some time slight reactions at CATT/*T. evansi* test can be observed, thus the cut-off at + + reactions [14], accounted for 64 high titre sero-positive animals.

Highest incidence of sero-positive cases from district Gurdaspur region (Fig. 1b, c). may be corroborated with hot and humid conditions conducive climate of low-lying Sutlej basin [6] areas and preponderance of vector in the region. Similar trend was observed in plain areas of this region in a previous report from our laboratory [15]. The disease is endemic throughout India particularly in low-lying areas

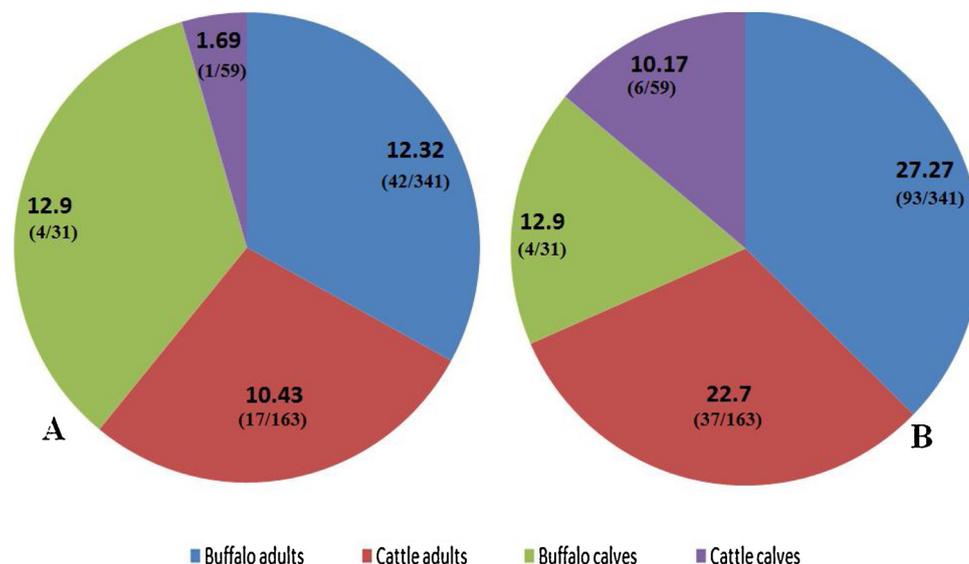


Fig. 2. Relative preponderance of higher (A i.e. left) and lower (B i.e. right) titres for CATT/*T. evansi* among various animal groups.

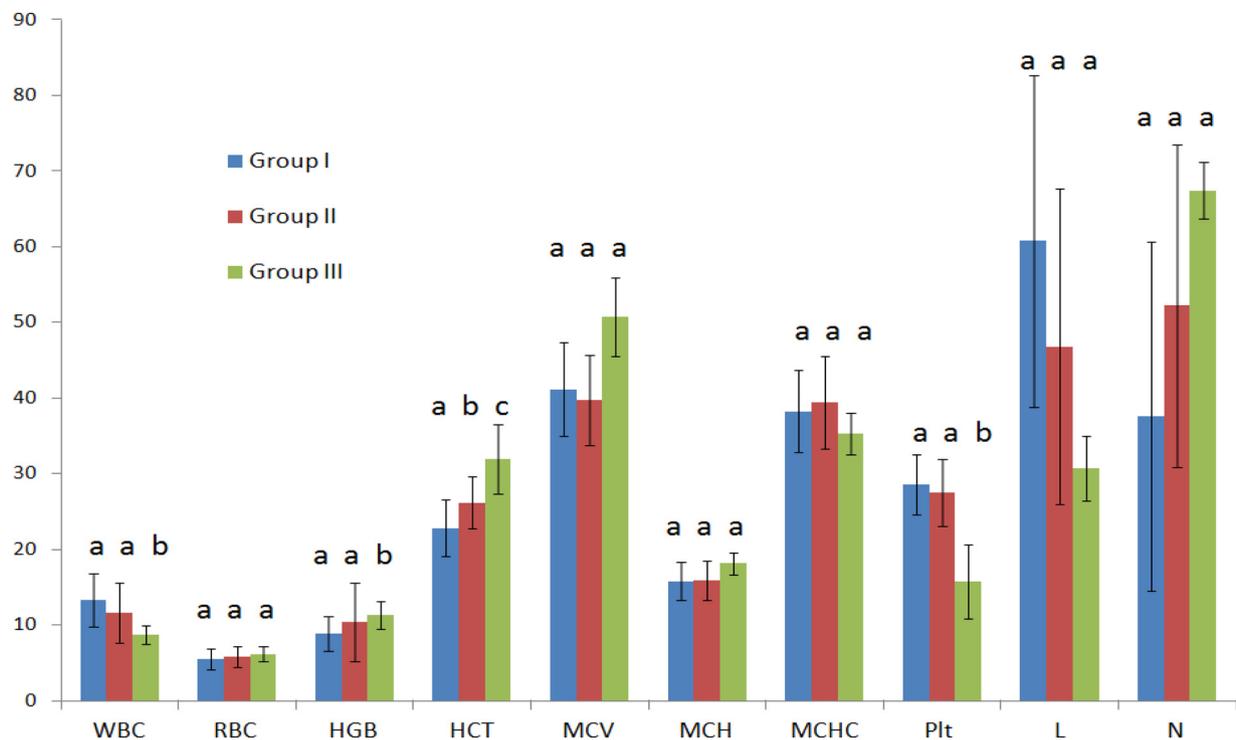


Fig. 3. Blood cellular changes including WBC (white blood cells, $\times 10^3/\mu\text{L}$), RBC (red blood cells, $\times 10^6/\mu\text{L}$), HGB (haemoglobin concentration, g/dL), HCT (haematocrit value, %), MCV (mean corpuscular volume, fL), MCH (mean corpuscular haemoglobin, pg), MCHC (mean corpuscular haemoglobin concentration, g/dL), Plt (platelet count, $\times 10^4/\mu\text{L}$), L (lymphocyte count/ 100 WBC), and N (neutrophil count/ 100 WBC) of animals with high positive (Group I), and low positive (Group II) titres compared to non-infected healthy controls (Group III). Superscript ^{a,b} and ^c indicates values differing significantly at $P \leq 0.05$ by one-way ANOVA.

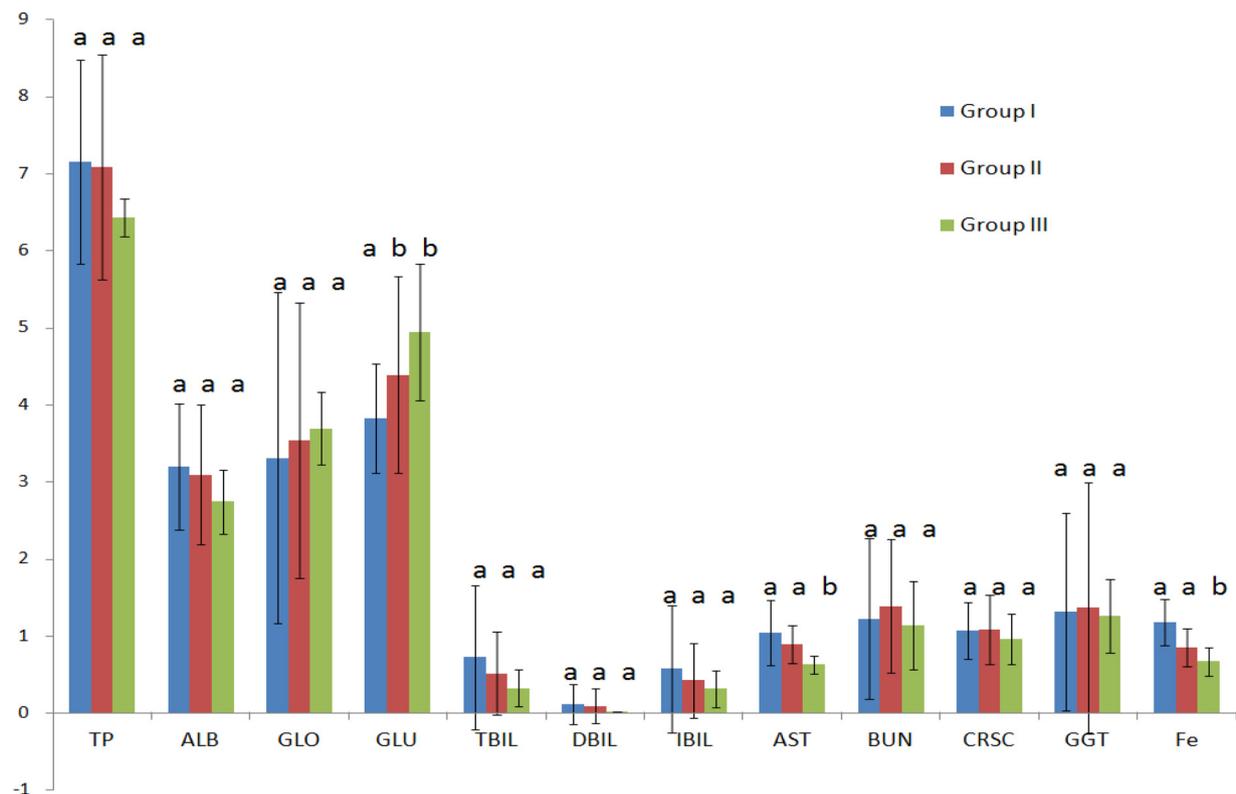


Fig. 4. Biochemical changes in serum parameters including TP (Total Protein, g/dL), ALB (Albumin, g/dL), GLO (Globulins, g/dL), GLU (Glucose, mg/dL x10), TBIL (Total bilirubin, mg/dL), DBIL(Direct bilirubin, mg/dL), IBIL (Indirect bilirubin, mg/dL), AST (Aspartate aminotransferase, U/L x100), BUN (Blood urea nitrogen, mg/dl x10), CRSC (Creatinine, mg/dL), GGT (Gamma glutamyltransferase, U/L x10), and Fe (serum iron concentration, $\mu\text{g}/\text{dL} \times 100$) of animals with high positive (Group I), and low positive (Group II) titres compared to non-infected healthy controls (Group III). Superscript ^{a,b} and ^c indicates values differing significantly at $P \leq 0.05$ by one-way ANOVA.

[16]. For our purpose, serological assay for the detection of anti-trypanosome circulating antibodies was economical and applicable at large scale on site-screening [17].

The assessment of various risk factors depicted that the adult buffalo population is more prone to the infection in contrast to cattle. This observation can be linked to the fact that older animals travel long distance for grazing and draught as well as harvesting crops in tabanid flies challenging areas. Moreover, young animals are also naturally protected to some extent by maternal antibodies [18]. In India, many dairy farmers prefer rearing females for the dual purpose of reproduction and milk yield. During pregnancy, immune system of females is compromised, thus making them predisposed to infection, which explains higher exposure risk of female animals.

Spatial data analysis of all premises revealed that chances of *T. evansi* infection were higher in region like Kapurthala, Gurdaspur, and some part of Jalandhar. This trend of spatial distribution correlated with the fact that *T. evansi* is transmitted by tabanid flies found predominantly in the vicinity of paddy fields of the central region of Punjab. The central region of Punjab is rich in water bodies which provide irrigation resource for the paddy field. These areas have favorable environmental conditions for breeding the vector tabanid flies [19]. Sero-epidemiology aids in gauging individuals that might maintain the reservoir of the infection and contribute to further dissemination of the disease in the animal population [2].

The haemo-biochemical examination revealed significant leucocytosis and increase in platelet count associated with *T. evansi* infection which may be due to the multiplicity of the antigens resulting into enhanced stimulation of immune system [20]. Elevation of AST enzyme indicates hepatic tissue damage including coagulative necrosis, distortion of hepatic cords and heavy infiltration of the hepatic tissues with lymphocytes; these observations are similar to other mammal hosts parasitized by *T. evansi* [21,22]. This further indicates the consequences of hypoxia resulting from anaemia and jaundice. In infected animal, *T. evansi* aggrandizes the systemic antigen-antibody immune complexes and their subsequent deposition in the vital organs may play a role in tissue harm thereby causing increase in AST level [23,24]. Significant decrease in the level of haemoglobin, haematocrit volume, and serum glucose level was recorded in sero-positive and suspected animals as compared to non-infected controls indicating anaemic condition associated with *T. evansi* infection [15,25]. The consumption of glucose by the parasite for self sustenance leads to an overall decrease in serum glucose level; these finding were in concordance with Singh et al. [26]. For all other parameters, no significant difference was recorded in the animals of the three groups. This correlation of physiology of animals with their sero-positivity for anti-*T. evansi* antibodies, validates the findings of CATT assay.

In conclusion, the present research endeavour reveals the endemicity of *T. evansi* in Punjab with prominence of various odds of infection, emphasising on the physiopathological manifestation associated with latent infection of *T. evansi* detectable by CATT/*T. evansi*. Prominently, over 10.7% of the bovine had higher titre by CATT/*T. evansi*. The haemato-biochemical alterations in non-symptomatic bovines with minimal clinical evidences insinuated more such cases in this area owing to the established endemicity of the disease. As per our knowledge, this is the first study of spatial distribution of *T. evansi* in Punjab by serological technique which can potentially help inform policy and decision making concerning large-scale trypanosome control strategies.

Conflict of interests

The authors declare that there is no professional or financial conflict of interests related to this paper.

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