Sero-prevalence and immunological characterization of *Trypanosoma evansi* infection in livestock of four agro-climatic Zones of Himachal Pradesh, India

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Research Article

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Abstract

Trypanosoma evansi, a hemoflagellate protozoan parasite causes wasting disease called surra in wide range of animals. Although, the organism has been reported from various parts of the India, data generated from organized epidemiological study is still in infancy in majority states of India. In the present study, livestock of Himachal Pradesh, India was targeted for epidemiological investigation of *T. evansi* infections. A total of 440 equines and 444 cattle serum samples were collected from four agro-climatic Zones. Further, serum samples of 280 buffaloes from three different agro-climatic Zones of Himachal Pradesh were also collected and evaluated for presence of *T. evansi* infection by indirect ELISA. Data generated showed higher prevalence in buffalo (23.57%) followed by cattle (22.52%) and equines (1.82%). Disease was found to be more prevalent ($P < 0.05$) in cattle of lower altitude as compared to those of higher altitudes. No significant variation was seen in prevalence of disease on the basis of age and sex of the animals. Serum biochemical analysis revealed increased levels of BUN in *T. evansi* infected equines. Levels of liver function enzymes such as ALT/GGT and AST were found to be significantly elevated ($P < 0.01$) in infected animals whereas glucose levels were significantly lower in surra infected animals as compared to non-infected animals. Western blot analysis of whole cell lysate (WCL) antigen of *T. evansi* using surra infected serum samples of equines showed immunodominant bands in the range of 100-25 kDa. Surra infected bovine serum samples recognized polypeptide bands in the range of 85-32 kDa, including protein clusters of 52-55 and 48-46 kDa. Poypeptide cluster of 62-66 kDa was found common to serum samples of bovines and equines from all agro-climatic Zones. Animal trypanosomosis was found to be highly prevalent in livestock of Himachal Pradesh and thus there is dire need for designing of proper control strategies against surra.

Introduction

Trypanosoma evansi, a unicellular, haemoflagellate parasite is responsible for causing a highly debilitating disease termed as surra in various host species and has a significant negative impact on livestock industry. *T. evansi* is considered to have developed from *T. brucei brucei*, due to alterations in its mitochondrial DNA, and has developed the ability to spread mechanically through diverse vector species such as *Tabanus* and *Stomoxys* (Desquesnes et al., 2013a; Tehseen et al., 2015). Economic losses due to surra in India were estimated to be US $ 671.1 million owing to several direct and indirect losses such as decrease in milk production, reduced growth, reduced draught power, reproductive losses, death and additional avoided expenses (Kumar et al., 2017). Human cases of *T. evansi* infections have also been reported from different parts of the world including India, adding a new dimension to its epidemiology as a rare zoonosis (Joshi et al., 2005; Chau et al., 2016). *T. evansi* is widely distributed across Northern parts of Africa, Middle East, Southeast Asia, Central and South America (Aregawi et al., 2019; Ereqat et al., 2020). The disease is present in different countries of Asia Including India, Myanmar, China, Vietnam, Malaysia, Indonesia, Philippines, Turkestan, Bhutan, Nepal, Laos, Thailand, Mongolia (Tuntasuvan and Luckins, 1998, Desquesnes et al., 2013a). Surra is endemic in India and has been reported from different parts of the country (Pathak and Chhabra, 2011). However, there are scant case reports of trypanosomosis from Himachal Pradesh, India. Himachal Pradesh is an agriculture-intensive state with 89.96% of its population being rural and dependent on agriculture for its livelihood (HP Economics and Statistics Department, Economic Survey 2017-18). However, due to a lack of organized epidemiological study of surra in the state, the prevalence status of the disease is still obscured. Therefore, the present study was designed to determine the epidemiology of Surra in Himachal Pradesh, which would help state veterinarians and policymakers in control of this disease.

Materials And Methods

Study area

The study was carried out in all the districts of Himachal Pradesh, India (Fig. 1). The state is situated between 30°22'40" to 33°12'40" N *latitude* and 75° 47'55" to 79°04'22" E *longitude* in northern India, at altitudes ranging from 350m to 6,975m above sea level (Jithendran and Bhat, 2000). The average annual rainfall of the state is 1111 mm (Asian Development Bank, 2010). Due to differences in elevation (450-6500 m), the climate of the state varies greatly from hot and sub-humid tropical in the southern low tracts to cold, alpine and glacial in the eastern and northern high mountain ranges. The state covers an area of 55673 km² and has a population of 1826290 cattle, 646570 buffalo, and 34070 equines according to 20th livestock census 2019.

Agro-climatically, state is divided into four Zones viz. Sub-Mountain and Low Hills Subtropical Zone (Zone-I), Mid Hills Sub-humid Zone (Zone-II), High Hills Temperate Wet Zone (Zone-III), and High Hills Temperate Dry Zone (Zone-IV) (Jithendran and Bhat, 2000). Out of these four Zones, distribution of Zone 2 and 3 could not be separated into different districts and thus samples were collected considering both the Zones as a single Zone i.e. Zone 2+3. Equine and cattle serum samples were collected from all four agro-climatic Zones, whereas due to negligible population of buffaloes in Zone 1, their samples were collected from remaining three Zones only.

Study Animals

Animals included in the study were cattle, buffaloes and equines of different age sex and breeds. Equines comprised indigenous breeds of horses, mules and donkeys in the study area, while bovines included both crossbred and indigenous types of cattle and buffaloes of all ages and sex categories. In the study area, equines were kept in the traditional extensive system in all four agro-climatic Zones, whereas bovines were mostly reared in the intensive system.

Study design, sampling, and sample size

A cross-sectional study was carried out from August, 2020 to March 2021 to assess the prevalence of equine and bovine trypanosomosis in Himachal Pradesh. The study animals were chosen using a simple random sampling procedure (Thrusfield, 1995). The required sample size for epidemiological studies was calculated according to Thrusfield (2005) taking expected prevalence as 10% on the basis of previous study (Kumar et al, 2013), at confidence interval (CI) of 95% and 5% desired level of precision. The calculated sample was adjusted for a finite population of samples which correlated with 278 buffalo, 412 horses and 417 cattle samples.

Sample and Data collection

Approximately 5 ml of blood was collected in plain vials from jugular vein of each animal under septic conditions for extraction of serum which was stored at -20°C for subsequent analysis. The species, age, and sex of selected animals were recorded to assess the risks associated with the prevalence of surra in bovines and equines in the study area. Equines were divided into two age groups: young (<2 years) and adult (>2 years), while bovines were divided into three age groups: young (<2 years), adult (2-5 years), and old (>5 years).

Laboratory examination

Indirect ELISA

The collected Serum samples were examined for presence of Anti *T. evansi* antibodies by Indirect ELISA using whole cell lysate (WCL) antigen prepared from purified trypanosomes. ELISA was performed as per the method standardized by Kumar et al. (2013). The samples were taken in duplicate and absorbance readings were taken at a wavelength of 450nm in multiscan plus ELISA reader (Thermo Fisher Scientific, Finland). Relative percent positivity (RPP) value was estimated by analyzing one positive and one negative control serum in each plate in duplicate. Serum samples that showed RPP value higher than cut off value were considered positive for presence of *T. evansi* antibodies. An RPP value of 15% was used as a cut-off value to evaluate sero-positivity of any of the test serum samples of equines and bovines against *T. evansi* infection (Kumar et al., 2013).

Immunoblot assay

For characterization of immunodominant antigens, immunoblot analysis was carried out using WCL antigen of *T. evansi* and serum samples of equines, cattle and equines. SDS–PAGE was performed under reducing conditions according to the method of (Laemmli, 1970), using Dual Mini Gel Cast Electrophoresis System (Atto Corporation, Japan) to know the polypeptides profile of WCL antigen. Denatured samples of WCL and protein markers were electrophoresed through stacking gel and subsequently through resolving gel at 50 V and 100 V, respectively.

Immunoblotting was done as per method defined by Towbin et al. (1979) using serum samples found positive in antibody ELISA. SDS–PAGE (10%) electrophoresed WCL antigens were trans-blotted on the PVDF membrane using tris–glycine–methanol transfer buffer in semi-dry transfer apparatus (Atto Corporation, Japan) at a constant current of 0.8 mA/cm^2 for a period of 90 minutes. The membrane was blocked overnight using skimmed milk 7% (w/v) (SM-PBST) and the blocked membrane was then incubated for 1 h with 1:20 dilution of respective serum samples in SM-PBST at a temperature of 37 °C. Further, the membrane was made to react with anti-species secondary IgG (1:250 dilutions) at 37 °C for 1 h. Immuno-reactive bands developed on reaction with positive serum were visualized using 3, 3' Diaminobenzidine (DAB) substrate. The substrate reaction was then terminated by washing the membranes with distilled water.

Biochemical examination

Serum biochemical Parameters such as Total Protein (TP), Albumin (ALB), Globulin (GLO), Total Bilirubin (TBIL), Direct and Indirect Bilirubin (DBIL and IBIL), Glucose (GLU), Blood Urea Nitrogen (BUN), Aalanine aminotransferase (ALT), Aspartate Aminotransferase (AST) and Gamma GlutamylTransferase (GGT) were analyzed using ERBA biochemical diagnostics kits (standard protocol as per supplier). Biochemical alnalysis was done at standard wavelength using the Automated Clinical Chemistry Analyzer-EM 200 (Transasia Bio-Medicas Ltd., India).

Statistical analysis

Statistical analysis of all quantitative data was done using Graph Pad Prism software version 8.0.2 (San Diego, California, USA). Different risk factors associated with prevalence of *T. evansi* were statistically compared using the Chi square test. Differences in the serum biochemical levels of control and affected groups were compared using the t-test. The p values below 0.05 were considered statistically significant.

Results

Sero-prevalence of surra

Out of 1165 (440 equine, 444 cattle and 280 buffalo) serum samples examined 174 (14.95%) were found to be positive for antibodies against *T. evansi* (Fig. 2). Overall sero-prevalence of surra was found to be 1.82% (08/440) in equines (Table 1, Fig. 2). No significant difference was reported in prevalence between horses (3.14%) and mules (1.07%) (Table 1). Prevalence was found to be higher in cattle and buffaloes as compared to equines. Out of 444 cattle tested 100 (22.52%) were found to be positive for *T.* evansi antibodies by Indirect ELISA. In buffaloes, 66 were found to be positive among 280 examined with sero-prevalence rate of 23.57% (Table 1). In cattle population, significantly higher prevalence was observed in Zone 4 (31.13%) and Zone 2+3 (26.39%) as compared to Zone 1 (10.07%). In buffaloes, prevalence was found to be 20.14% in Zone 2+3 and 26.95% in Zone 4. However, in equines, no significant difference was reported in prevalence among different Zones (Table 2, Fig. 2). Also, age and sex of the animals were not found to affect prevalence of *T. evansi* in equines, bovines and cattle.

Table 1
Species wise comparison of prevalence of trypanosomosis in livestock of Himachal Pradesh, India.

Species	**No. examined**		**No. found positive**		**Prevalence range (95% CI)**		**Chi square value, df**	**p-value**
E	H	M	H	M	H	M	2.67, 1 ^{NS}	0.1021
	149	281	05	03	**3.14%** (0.44-5.86)	**1.07%** (0.0-2.27)		
	440		08		**1.82%** (0.57-3.07)		74.93, 1****	<0.0001
Bo (C/B)	724		166		**22.93%** (19.87-25.99)			
C	444		100		**22.52%** (18.64-26.41)		0.06690, 1 ^{NS}	0.7959
B	280		66		**23.57%** (18.60-28.54)			
Total (Bo, E)	1164		174		**14.95%** (12.90-17.00- %)			
E = Equine, C= Cattle, B= Buffalo, Bo= Bovine								
NS- non significant (P>0.05), *significant (P<0.05), **highly significant (P<0.01)								

Table 2
Comparative statement of the prevalence of trypanosomosis in equine, cattle and buffaloes of different agro-climatic zones of Himachal Pradesh, India.

	Zone	Number examined	Number found positive	Prevalence % (Range (95% CI)	Chi square value, df	P-value
E	Zone 1	139	02	**1.43%** (0-3.42)	1.207,2 NS	0.5470
	Zone 2+3	140	4	**2.86%** (0.10-5.62)		
	Zone 4	161	02	**1.24%** (0-2.95)		
C	Zone 1	149	15	**10.07%** (5.24-14.90)	13.87, 2 **	0.0010
	Zone 2+3	144	38	**26.39%** (19.19-33.59)		
	Zone 4	151	47	**31.13%** (23.74-38.51)		
B	Zone 2+3	139	28	**20.14%** (13.48-26.81)	1.115, 1 NS	0.2911
	Zone 4	141	38	**26.95%** (19.63-34.27)		
E = Equine, C= Cattle, B= Buffalo						
NS- non significant (P>0.05), *significant (P<0.05), **highly significant (P<0.01)						

Immunological characterization

SDS-PAGE

SDS-PAGE analysis of WCL antigen of *T. evansi* revealed multiple visible polypeptides bands in range of 60-18.5 kDa. Among all these, major polypeptides bands were observed in the range of 66.2-38 kDa. Minor polypeptides bands were seen in the range of 18.5-30 kDa. Closely migrated clusters of polypeptides that were not separated by SDS-PAGE were also found in the range of 40-45 kDa.

Immunoblot assay

On immunoblot analysis immunodominant bands identified in serum samples from *T. evansi* infected equines were in the molecular weight range of 100- 25kda. Polypeptide cluster of 62-66 kDa was recognized by serum samples of equines from all the Zones. Other polypeptide bands recognized were of 57, 62 and 68 kDa from Zone 2+3 and 7 polypeptide bands of 72, 57, 50, 47, 40, 45 and 25 kDa from Zone 4.

Serum samples of cattle and buffaloes from all regions also recognized immunodominant polypeptide cluster of 62–66 kDa. Polypeptide cluster of 55-52 kDa was identified by serum samples of cattle of Zone 1 and Zone 2+3. Serum samples of cattle from Zone 2+3 and Zone 4 detected another polypeptide cluster in the range of 48-46 kDa and 68-70 kDa respectively. Individual polypeptides recognized by cattle were of approximately 70, 66, 55, 36, 32 kDa from Zone 1, 70 and 40 kda from Zone 2+3 and 55, 40, 38, 35 kDa from Zone 4.

Immunodominant polypeptides of 85, 80, 55, 48, 35, 38, 15 kDa were detected in buffaloes of 2+3 Zone, whereas, only 39, 38 and 35 kDa bands were observed in serum sample of Zone 4 buffaloes. In addition, polypeptide cluster in the range of 52-55 kDa and 68-70 kDa were also recorded in serum samples of buffaloes from Zone 2+3 and Zone-4, respectively.

Serum biochemistry

Serum biochemical values of control and *T. evansi* sero-positive horses, cattle and buffaloes are shown in the Table 3. The mean values of ALT and AST in *T. evansi* sero-positive group were found be significantly higher as compared to non infected group of cattle, buffaloes and equines. Mean serum glucose levels in *T. evansi* infected animals was found to be reduced significantly in comparison to control animals. In equines,

significant drop in mean albumin level was observed in *T. evansi* infected animals. In comparison to control group, mean serum globulin levels of infected cattle group was found to be significantly higher. Mean BUN value showed a significant rise in *T. evansi* sero-positive equines as compared to the non infected group.

Table 3
Comparative statement of biochemical parameters between infected and serological negative samples.

	Equine				Cattle				Buffalo			
Parameters	**Control**	**Infected animals**	**t- test**	**P value**	**Control**	**Infected animals**	**t- test**	**P value**	**Control**	**Infected animals**	**t- test**	**P value**
Total protein	7.09 ± 0.09	7.61 ± 0.42	1.21^{NS}	0.2918	7.21 ± 0.13	8.18 ± 0.29	2.71^{**}	0.0092	6.88 ± 0.13	7.09 ± 0.37	0.52^{NS}	0.6101
Albumin	3.02 ± 0.05	2.56 ± 0.16	3.03^{**}	0.0050	2.74 ± 0.06	2.73 ± 0.13	0.03^{NS}	0.9756	2.58 ± 0.06	2.51 ± 0.11	0.62^{NS}	0.5379
Globulin	4.07 ± 0.08	5.05 ± 0.41	2.34^{NS}	0.0789	4.48 ± 0.106	5.45 ± 0.37	3.20^{**}	0.0023	4.29 ± 0.11	4.58 ± 0.30	0.87^{NS}	0.4008
Total bilirubin	0.27 ± 0.03	0.24 ± 0.07	1.60^{NS}	0.1606	0.124 ± 0.00	0.20 ± 0.08	1.01^{NS}	0.3491	0.07 ± 0.00	0.10 ± 0.04	0.80^{NS}	0.4397
Direct bilirubin	0.09 ± 0.01	0.14 ± 0.07	0.62^{NS}	0.5567	0.04 ± 0.00	0.05 ± 0.01	0.12^{NS}	0.9019	0.03 ± 0.00	0.02 ± 0.00	0.27^{NS}	0.7884
Indirect bilirubin	0.17 ± 0.03	0.19 ± 0.06	1.75^{NS}	0.1300	0.07 ± 0.00	0.15 ± 0.06	1.15^{NS}	0.2924	0.04 ± 0.00	0.08 ± 0.04	0.86^{NS}	0.4074
Blood urea nitrogen	21.0 ± 2.00	50.9 ± 10.5	2.76^{*}	0.050	18.78 ± 1.70	61.3 ± 19.02	2.22^{NS}	0.0674	18.89 ± 2.51	23.75 ± 4.44	1.01^{NS}	0.3206
GGT	20.42 ± 0.60	32.96 ± 1.75	8.04^{**}	0.0000	21.87 ± 1.76	63.57 ± 8.01	5.07^{**}	0.0014	25.40 ± 2.30	60.9 ± 4.90	7.30^{**}	0.0000
AST	138.62 ± 4.75	209.98 ± 17.61	5.48^{**}	0.0000	81.9 ± 4.70	143.2 ± 8.00	6.38^{**}	0.0000	106.81 ± 5.77	150.40 ± 8.36	4.19^{**}	0.0002
Glucose	92.47 ± 1.69	57.34 ± 4.63	8.18^{**}	0.0000	77.85 ± 2.84	48.7 ± 4.76	5.03^{**}	0.0000	82.93 ± 4.05	56.15 ± 6.20	3.61^{**}	0.0011

NS- non significant (P>0.05), *significant (P<0.05), **highly significant (P<0.01)

Discussion

Surra is one of the most important hemoprotozoan diseases of livestock with varying prevalence between countries and regions. The study involved cattle, buffaloes and equines of different agro-climatic Zones of Himachal Pradesh. Overall sero-prevalence of *T. evansi* shown by indirect ELISA in equine was 1.82%. Among equines, prevalence between mules and horses was not found to differ significantly in the present study. Similar finding has been reported in previous study (Yadav et al., 2019). However, there are reports of significantly higher prevalence of trypanosomosis in mules as compared to horses (Kumar et al., 2013; Prashar et al. 2018). In cattle and buffaloes, the prevalence of Surra was observed to be 22.52 and 23.57% respectively, with no significant difference, although the prevalence was significantly lower in equines. Similarly, higher sero-prevalence of Surra was reported in cattle 42.7 and buffaloes 48.0 %, in comparison to horses (1.7 %) in Indonesia (Payne et al., 1980). Abera (2016) also reported significantly higher sero-prevalence of surra in cattle (37.3%) in contrast to equines (10.7%) in Northern Ethiopia. Cattle and buffaloes are usually kept together in a tie-stall housing system and due to the interrupted feeding habit of tabanid flies, usually, the whole of the herd gets affected which can be related to the higher prevalence of the disease. On the other hand, the equine population is scattered and mostly employed for transportation, resulting in less contact with other affected animals. In addition, equines are kept loose, and their defensive behavior deters tabanids from biting (Muzari et al., 2010). Furthermore, *T. evansi* infection in equines is thought to be very fatal, with death of most of the infected animals in early stages (Desquesnes et al., 2013a), potentially resulting in the reduction in number of sick animals and the creation of an equine population with minimal signs of infection.

Prevalence of surra in equines from Zones 1 (1.43 %), 2+3 (2.86 %), and 4 (1.24%) showed no significant differences. Similarly, no significant variation was observed in prevalence of surra in equines between the Western and Central Plain Zone of Punjab (Sumbria et al., 2014). In cattle and buffaloes, no significant difference was found in prevalence between Zone 2+3 and Zone 4; however prevalence was found to be significantly lower in cattle from Zone 1 as compared to Zone 2+3 and Zone 4. Lower prevalence in Zone 1 can be attributed to its temperate and dry conditions which are unfavorable for proliferation of different vectors responsible for transmission of surra. A study on effect of climate variables on vector and prevalence of bovine trypanosomosis revealed higher prevalence of flies of genus *Tabanus* and *Stomoxys* in

lowland in comparison to midland and highland regions of Ethiopia (Zekarias et al., 2017). The finding of this study was consistent with previous study showing higher seroprevalence of bovine trypanosomosis in Zones with higher temperatures (43.02% in the undulating Zone) compared to those with lower temperatures (27.36% in the Sub-mountain Zone) in Punjab (Singla et al., 2013).

In the present study, prevalence of *T. evansi* infection was not found be affected by sex of animals which is in agreement with previous studies (Tafese et al., 2012; Singla et al., 2013; Singh et al., 2016; Parashar et al., 2018). The possibility of both the sexes being equally exposed to the bites of disease-carrying vectors may explain the similar prevalence between them. In contrary, several authors have reported higher prevalence of surra infection in female than male animals and was linked to numerous stress factors such as pregnancy and lactation in female animals, rendering them more susceptible to infection (Agrawal et al., 2013; Sumbria et al. 2017; Sharma et al. 2019; Dodiya et al. 2020).

Similar to sex, age of the animals was not found to have any significant effect on prevalence of surra in equines, cattle and buffaloes suggesting that all age groups of animals are equally exposed to and affected by *T. evansi*. This corroborates previous observations, which demonstrated similar prevalence of surra in all age group animals (Tehseen et al., 2017; Batu et al., 2017; Alanazi et al., 2018 and Singh et al., 2019). Previous researchers have revealed varied results regarding the effect of age on the incidence of surra, which contradicts the current data. Prevalence was reported to be higher in older as compared to younger animals (Payne et al., 1991; Singla et al., 2013; Prashar et al., 2016; Gangwar et al., 2019; Sharma et al., 2019; Dodiya et al., 2020). In contrast, young equines were found to be at significantly higher risk of infection as compared to the adults (Sumbria et al., 2017). In bovines, increasing sero-positivity with age could be attributable to antibody persistence due to the chronic nature of disease, rather than an age-specific component.

In *T. evansi* infected equines, the BUN value was observed to be significantly higher when compared with the non-infected group. This finding is in agreement with earlier reports (Singh et al., 2011, Chavda et al., 2016, Yadav et al., 2016). BUN levels rise as a result of parasite damage during pathogenesis, which causes mononuclear infiltration of the renal glomeruli, interstitial nephritis, tubular degeneration, and tissue deterioration in the visceral organs (Hilali et al., 2006; Bal et al., 2014). The mean levels of both AST and ALT/GGT enzymes were found to be significantly higher in *T. evansi* infected group of animals. These findings in the present study are in consonance with those reported in previous studies (Sivajothi et al., 2015; Amin et al., 2020). The increase in AST levels might be attributed to tissue damage induced by trypanosomes, as well as the destruction of the parasite by the host immune system, resulting in the release of trypanosomal AST (Takeet and Fagbemi, 2009; Pandya et al., 2018a). This rise in ALT levels could be due to hepatic damage caused by the parasite (Akinseye et al., 2020). The glucose levels in infected animals were considerably lower than those in the non-infected group. Significant reduction in glucose levels in infected as compared to the control group is also reported by several workers (Cadioli et al., 2006), Singh et al. (2011), Sivajothi et al. (2015), and Pandya et al. (2018b). This phenomenon of hypoglycemia can be attributed to direct utilization of glucose by the trypanosomes. In addition, fever and hepatocellular damage associated with trypanosomes infection which causes an increase in metabolic rate and therefore greater utilization of glucose by the host (Von Brand, 1973; Stephen, 1986; Opperdoes et al., 1987). Contrary, Aquino and coworkers (2002) observed no significant reduction in glucose levels of infected animals. The polypeptide pattern of WCL antigen prepared was identical to earlier reports (Yadav et al. 2013). Major polypeptides found were in molecular weight range of 66.2-38 kDa in addition to some high and low molecular weight polypeptides. These observations are similar to those reported in previous study in which a comparable polypeptide pattern of *T. evansi* was observed with WCL antigen of buffalo, horse, and cow isolates (Laha and Sasmal, 2008). Similarly, major polypeptides of *T. evansi* of equine isolate were observed in the range of 62–66 kDa, 52–55 kDa and 41–43 kDa (Yadav et al., 2013). Immunoblot analysis of *T. evansi* positive field serum of equines revealed major immunogenic polypeptides present in molecular weight range of 62-66 kDa and some minor polypeptide band in the range of 72- 25 kDa. The polypeptide clusters identified in our study were similar to those observed by previous researchers (Aquino et al., 2010; Yadav et al., 2013). In bovines, immunodominant polypeptides bands ranging from 85 to 32 kDa were detected. However, the main poypeptide bands seen in all of the bovine serum samples were between 62-66, 52-55 kDa and 48-46 kDa range. Immunodominant proteins of molecular weight range of 178–24 kDa were observed with antigen prepared from the *T. evansi* isolate of horse, cattle and buffalo (Laha and Sasmal, 2008). The 48-46 and 38 kDa bands were recognized in both cattle and buffalo which are similar to those reported by Aquino et al. (2010) mainly in later stages of infection suggesting the chronic nature of the disease in bovines. The presence of these polypeptides in the chronic stages of infection might be due to the release of internal antigens following the destruction of parasites by VSG-specific antibodies (Aquino et al., 2010). The 62-66 kDa polypeptide cluster was found to be recognized by the immune serum of all the infected animals including cattle, buffalo, and equines. Immunodominant bands in a similar molecular weight range have been reported previously in different studies (Giardina et al., 2003; Laha and Sasmal, 2008; Aquino et al., 2010; Yadav et al., 2013).

Conclusion

Overall sero-prevalence of surra of 14.95% in livestock of Himachal Pradesh suggested that the disease is endemic in the region and, consequently, proper control strategies should be designed and implemented in order to prevent further spread and economic losses caused by this disease. Also, the additional studies for characterization of immunodominant antigens of *T. evansi* using antibodies of different host species may help in better understanding of the host-parasite relationship and provide some clarity regarding immunogenicity and

pathogenicity of this parasite. The specific immunogenic polypeptides discovered from the parasite could be tested further for application in the diagnosis of animal trypanosomosis.

Declarations

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Author Contribution RK and SK designed the research proposal, DS and KS conceived the research. SG helped in preparation of the draft of the manuscript. All authors have read and approved the manuscript**.**

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Ethics declarations Prior approval was taken for animal experimentation in the present study from Institutional Animal Ethics Committee of ICAR-NRCE, Hisar.

Conflict of interest The authors declare that they have no conflict of interest.

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Figures

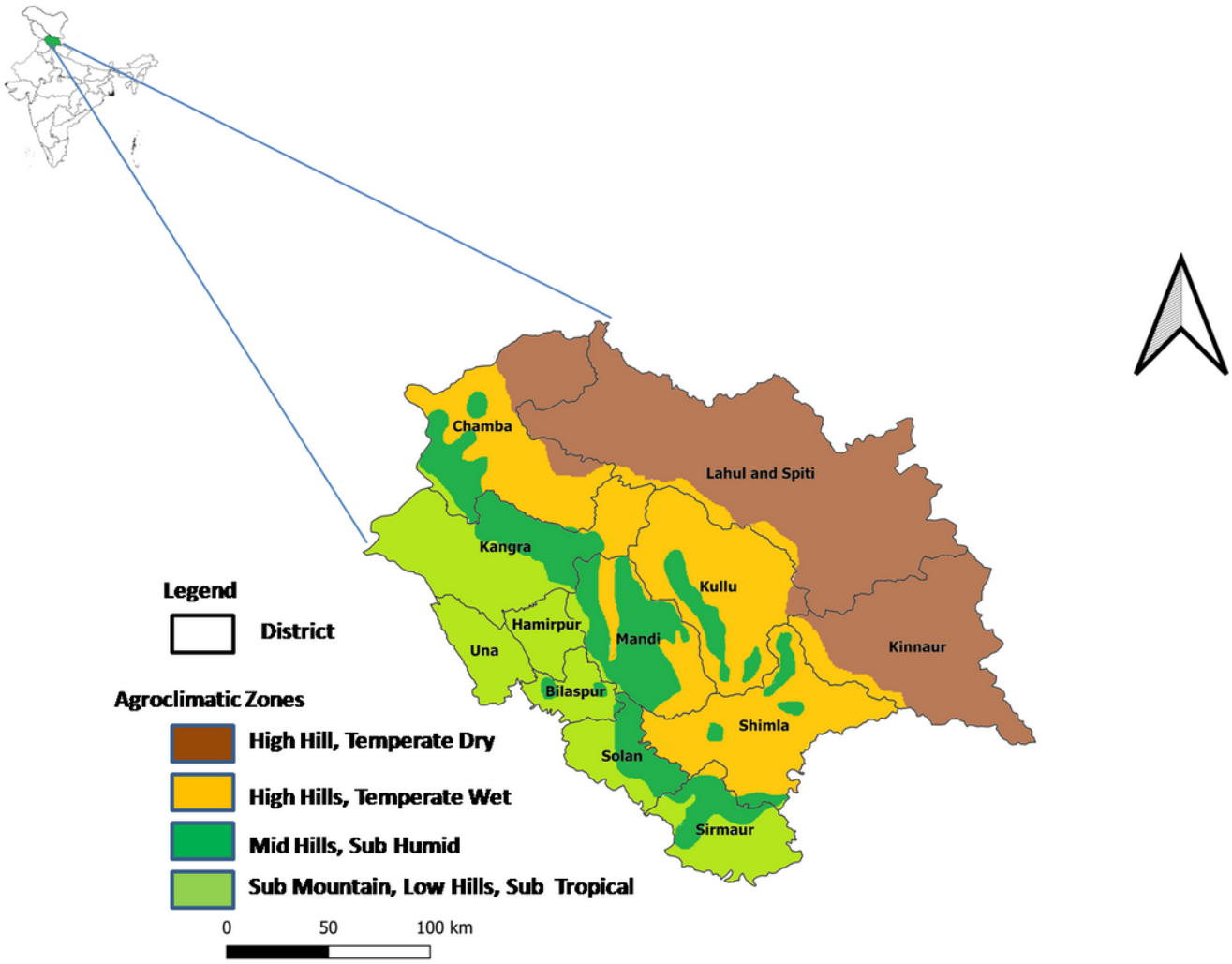

Figure 1

Different agro-climatic zones of Himachal Pradesh, India.

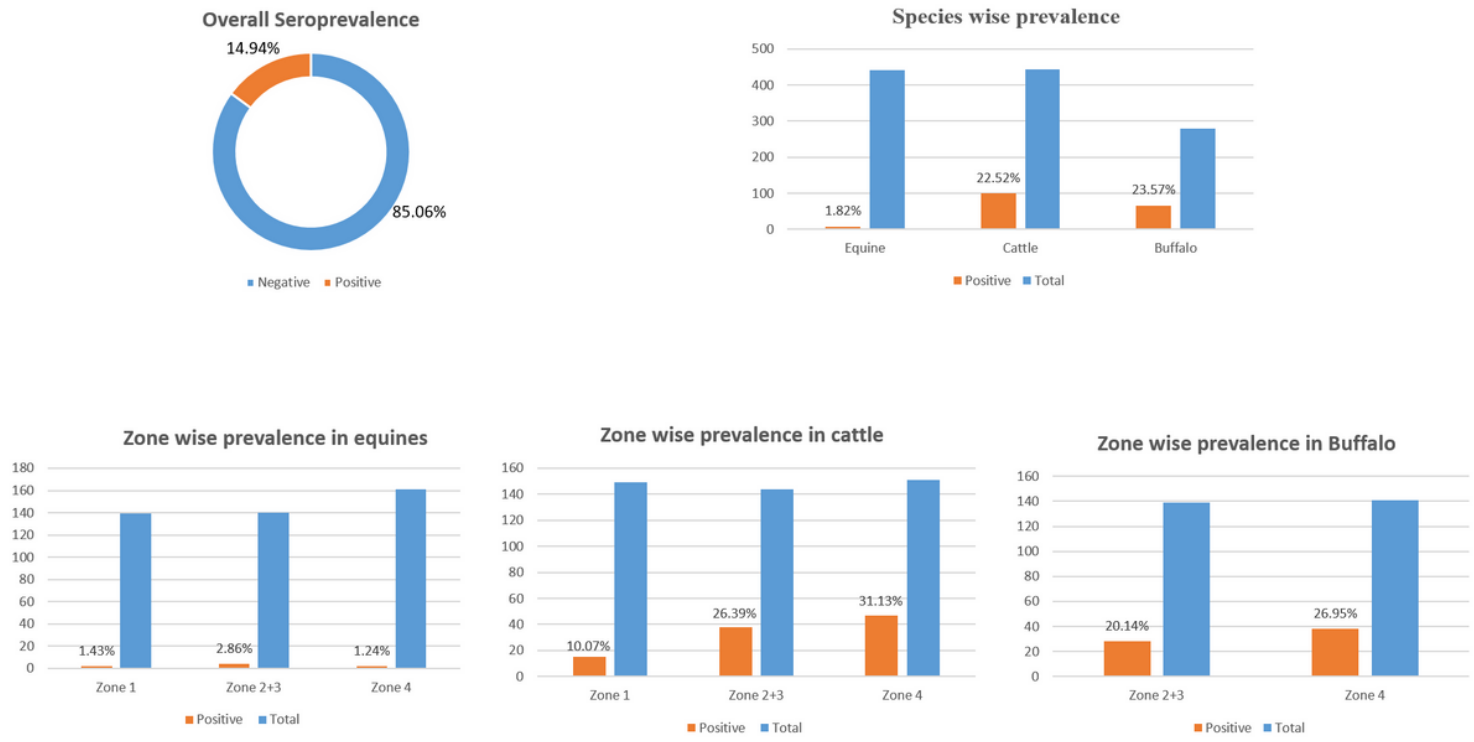

Figure 2

Sero-prevalence of trypanosomosis in different agro-climatic zone of Himachal Pradesh, India.