

# Comparative seroprevalence and risk factor analysis of *Trypanosoma evansi* infection in equines from different agro-climatic zones of Punjab (India)

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## Summary

As parasitaemia is low and fluctuating during the chronic stage of infection, accurate detection of *Trypanosoma evansi* in blood is difficult. The primary aims of this investigation were to assess for the first time the seroprevalence of *T. evansi* in all agro-climatic zones of Punjab, by indirect enzyme-linked immunosorbent assay (iELISA) and card agglutination test (CATT/*T. evansi*), and to evaluate the risk factors associated with latent trypanosomosis. A total of 319 equine serum samples collected from 12 districts of Punjab (India) belonging to different agro-climatic zones revealed 39 (12.23%) and 9 (2.82%) samples to be positive by CATT/*T. evansi* and iELISA, respectively. The highest prevalence was recorded from the Ludhiana district (42.86% and 7.14% by CATT/*T. evansi* and iELISA, respectively) in the central plain zone (for which the overall prevalence was 15% and 4.17%, respectively). There was fair agreement between the tests for the detection of *T. evansi* ( $\kappa = 0.345$ ). Species was the most influential risk factor for infection, with odds ratios (ORs) of 2.81 and 5.63 for donkeys/mules, in comparison with horses, by CATT/*T. evansi* and iELISA, respectively. The female equine population (OR = 3.13, 95% confidence interval [CI] = 1.32–7.67 [CATT]) was found to be at a higher risk of seropositivity for *T. evansi*, particularly on 'unorganised' (inappropriately managed) farms (OR = 3.18, 95% CI = 1.53–6.65 [CATT]) and among animals used for commercial purposes (OR = 2.51, 95% CI = 1.20–5.21 [CATT]). In conclusion, to declare disease-free status, use of the iELISA followed by retesting of suspect samples by CATT/*T. evansi* is suggested.

## Keywords

Card agglutination test – Equine – India – Indirect enzyme-linked immunosorbent assay – Risk factor – Seroprevalence – Surra – *Trypanosoma evansi*.

## Introduction

*Trypanosoma evansi*, the most prevalent pathogenic kinetoplastid haemoprotozoan, causes a devastating immunosuppressive disease called 'surra' (from the Hindi word meaning 'rotten') in domestic, wild and laboratory animals throughout the tropical and subtropical areas of the world (1). It is endemic in most parts of the Indian sub-continent and is transmitted mechanically from infected carrier animals by haematophagous Dipteran insects belonging to the genera *Tabanus*, *Stomoxys*, *Haematopota*,

*Lyperosia* and *Hippobosca* (2). Although economic losses resulting from surra in India are believed to be large, particularly during epidemic outbreaks of the disease, the economic impact is difficult to assess because of incomplete epidemiological information and inaccurate data (3). On cattle ranches in the Brazilian Pantanal region, the estimated total losses due to *T. evansi* are about US\$ 2.4 million/year (4). Although the parasite infects a wide range of domestic and wild animals, and even humans (5), the effects of the infection in different geographical locations vary according to the strain of the parasite, and the species and genetic make-up of the host(s) infected (5, 6).

The control of the disease is mainly based on the recognition of infected animals by livestock keepers, who observe clinical signs and treat them on a herd basis or individually. This is an inaccurate and inefficient approach because many infected animals may remain undiagnosed and act as reservoirs of the parasite, given the periodically cryptic nature of the organism. Specific diagnosis of trypanosomosis is made on the basis of clinical evidence augmented by classical parasitological (blood smear examination), molecular (polymerase chain reaction [PCR] assays) or serological (card agglutination test [CATT/*T. evansi*], enzyme-linked immunosorbent assay [ELISA]) tests. Low and fluctuating parasitaemia renders the detection of the haemoparasite difficult on blood smear examination (7). In the diagnosis of natural infection in equines under field conditions, molecular testing may give false-negative results, especially when the level of parasitaemia is very low (8). This may be followed by a flare-up of the infection under conditions of stress (9). These suspected potential carriers can be confirmed by serological examination (10). Among the different serodiagnostic tests, the ELISA (immunoglobulin [Ig]G-based) is more likely to categorise truly uninfected animals correctly, whereas the CATT/*T. evansi* is more likely to classify correctly the truly infected animals (11). Camelids and equines are more susceptible to the infection than other species (cattle, buffalo, sheep and goats), and show high mortality (11, 12). An assessment of the risk factors may enhance the control of *T. evansi* infection because it can determine the factors associated with disease either at management or at host level.

To date, no comprehensive assessment of the disease seroprevalence and risk factors has been conducted for *T. evansi* infection in equines in all agro-climatic zones of Punjab. Therefore, this study was designed to investigate the comparative seroprevalence of *T. evansi* using the indirect (i)ELISA and CATT/*T. evansi* and to determine potential risk factors associated with the prevalence of the disease among equines in Punjab.

## Materials and methods

### Ethical aspects (consent statements)

The Ethics Committee for Animal Experiments from the Guru Angad Dev Veterinary and Animal Sciences University granted approval (IAEC/2014/46-73) for this work to be conducted. Prior consent was obtained from the owners of the equines. Measures were taken to avoid any accidental injury to each animal while collecting the blood samples.

### Study areas and sampling frame

The province of Punjab covers a total area of 50,362 square kilometres between latitudes 29°30'N to 32°32'N

and longitudes 73°55'E to 76°50'E. There are about 34,000 horses and ponies at risk of infection with *T. evansi* in Punjab (13). The study was conducted in all the agro-climatic zones of Punjab (Table I) because one of the previous studies conducted in the authors' laboratory had revealed a high prevalence of trypanosomosis in bovines in the province (14). Blood samples were collected from representative equines in 12 districts of the five major agro-climatic zones of Punjab. A total of 319 samples (133 male and 186 female) were randomly collected to screen for *T. evansi* infection. Blood (~3 ml) was collected from the jugular vein of each animal into clot-activator vacutainers for serum extraction. To study the serological prevalence of the infection, an expected prevalence of 50% with confidence intervals (CIs) of 95% and a desired absolute precision of 5% were used when deciding on the required number of samples (15). The number of samples thus calculated was adjusted for a finite population and 319 samples were collected. A pre-designed epidemiological questionnaire, addressing the age (young: <2 years; adult: >2 years), sex, management and use of each equine, was used to analyse the risks associated with *T. evansi* transmission and was completed by the owner of each animal. The equine keepers following inappropriate management practices, such as rearing their stock in stables with kacha flooring, poor sanitation and unbalanced feeding programmes, were classified as 'unorganised', while those pursuing appropriate scientific management schedules were considered 'organised'.

### Blood films

Two thin blood films were prepared from each blood sample, dried on the spot, and then fixed in absolute methyl alcohol for 1–2 min in the laboratory. The smear was immersed in diluted Giemsa stain for 30–45 min, and then washed with distilled water to remove excess stain. The slides were left to dry and examined under an oil immersion lens (100× magnification) (16).

### Serological tests

#### Card agglutination test/*T. evansi*

The CATT/*T. evansi* for antibody detection was originally described and converted into a test kit by the Institute of Tropical Medicine, Belgium (17). Briefly, 25 µl of diluted serum was thoroughly mixed with about 45 µl of well-homogenised CATT antigen. The card was agitated in a circular motion using an electric rotator at 60–70 rpm at room temperature for 5 min. Samples showing blue granular agglutination were considered positive. The samples were read in comparison with the control wells according to the instructions supplied. Agglutination patterns were scored as – (negative), ± or + (suspected), and ++ or +++ (positive).

**Table 1**  
**Seroprevalence of *Trypanosoma evansi* in equines in five agro-climatic zones of Punjab**

Zones	Districts	Samples		CATT/ <i>T. evansi</i>						iELISA		
				+++ / ++ (%)		+ / ± (%)		95% CI		Positive (%)		
				(A)	(B)	(C)	(D)	95% CI	(C)	(D)	95% CI	(C)
SMZ	Hoshiarpur	55	38	6 (10.91)	4 (10.53)	4.17–24.13	16 (29.09)	14 (36.84)	23.38–52.72	1	0 (0)	∞ (0.0–∞)
	Pathankot		17		2 (11.76)	3.29–34.34		2 (11.76)	3.29–34.34	(1.82)	1 (5.88)	1.05–26.98
UZ	SBS Nagar	53	22	7 (13.21)	4 (18.18)	7.31–38.52	11 (20.75)	6 (27.27)	13.15–48.15	2	1 (4.55)	0.80–21.80
	Mohali		31		3 (9.68)	3.35–24.90		5 (16.13)	7.09–32.63	(3.77)	1 (3.23)	0.50–16.19
CPZ	Amritsar	120	23	18 (15.00)	1 (4.35)	0.77–20.90	42 (35.00)	8 (34.78)	18.81–55.11	5	1 (4.45)	0.70–20.90
	Jalandhar		10		1 (10.00)	1.79–40.40		4 (40.00)	16.82–68.73	(4.17)	1	1.79–40.42
	Ludhiana		14		6 (42.86)	21.38–67.41		6 (42.86)	21.38–67.41	(10.00)	1 (7.14)	1.27–31.47
	Patiala		73		10 (13.70)	7.61–23.41		24 (32.88)	23.19–44.27		2 (2.74)	0.70–9.45
WZ	Moga	28	20	4 (14.29)	3 (15.00)	5.24–36.04	10 (35.71)	5 (25.00)	11.19–46.87	1	1 (5.00)	0.80–23.61
	Bathinda		8		1 (12.50)	2.24–47.09		5 (62.50)	30.57–86.32	(3.57)	0 (0)	∞ (0.0–∞)
WPZ	Ferozepur	63	37	4 (6.35)	2 (5.41)	1.50–17.70	10 (15.87)	6 (16.22)	7.65–31.11	0	0 (0)	∞ (0.0–∞)
	Fazilka		26		2 (7.69)	2.14–24.14		4 (15.38)	6.15–33.53		0 (0)	∞ (0.0–∞)
	<b>Total</b>		<b>319</b>		<b>39 (12.23)</b>	<b>9.07–16.28</b>		<b>89 (27.90)</b>	<b>23.26–33.06</b>		<b>9 (2.82)</b>	<b>1.49–5.27</b>
	<b>Chi-squared</b>			<b>3.14</b>	<b>17.03</b>		<b>8.46</b>	<b>18.96</b>		<b>3.06</b>	<b>7.38*</b>	
	<b>Kappa</b>					<b>0.345</b>						

CATT: card agglutination test  
 CI: confidence interval  
 CPZ: Central plain zone  
 iELISA: indirect enzyme-linked immunosorbent assay  
 SBS: Shaheed Bhagat Singh

SMZ: Sub-mountain undulating zone  
 UZ: Undulating zone  
 WPZ: Western plain zone  
 WZ: Western zone  
 \* $p \leq 0.05$

(A): zone-level samples  
 (B): district-level samples  
 (C): zone-level prevalence  
 (D): district-level prevalence

**Indirect enzyme-linked immunosorbent assay**

The iELISA was conducted at the National Research Centre on Equines (NRCE), India. Briefly, the optimum dilutions of whole cell lysate antigen, conjugate (rabbit anti-horse IgG, whole molecule, horse radish peroxidase [Sigma Aldrich Co., St Louis, USA]) and known positive reference serum were determined. Each serum sample was tested in duplicate. The ELISA plates were coated with 50-µl aliquots containing 500 ng protein antigen in 0.1 M carbonate/bicarbonate buffer (pH 9.6) per well. After overnight incubation at 4°C, the plates were washed three times with phosphate-buffered saline containing 0.05% Tween-20 (PBST). The wells of the ELISA plate were blocked with 100 µl 5% skimmed milk in PBST for 1 h at 37°C. After three further washes in PBST, 50 µl of 1:100 diluted sera with 5% skimmed milk in PBST was added to each well. After an incubation phase of 1 h at 37°C, the plates were again washed three times and 50 µl of a 1:10,000 dilution of rabbit anti-horse IgG–peroxidase conjugate (Sigma) was added and the plates were incubated

for 1 h at 37°C. Lastly, after washing three more times, 50 µl per well of 1:20 dilution tetra-methylbenzidine substrate (TMB/hydrogen peroxide, 20× concentration) was added. The reaction was stopped by adding 50 µl of 1 M sulphuric acid to each well. The plates were read at 450 nm on an ELISA reader (Bio Tek, Winooski, USA) and the results were reported as the average optical density at 450 nm (OD<sub>450</sub>) of duplicate samples (18).

**Statistical analysis**

The prevalence of *T. evansi* with respect to various physical and biological factors was statistically analysed, employing Pearson’s Chi-squared test at  $p \leq 0.05$ . Potential risk factors were analysed using WinEpiscope software v. 0.1 and online software (statpages.info/ctab2x2.html). For the iELISA, any sample showing an OD<sub>450</sub> above the mean + 4 standard deviations (SDs) of three negative wells was considered positive. The SD of the OD<sub>450</sub> of three negative wells was also calculated. Cohen’s kappa was calculated to assess agreement between the tests.

## Results

### Blood film examination

Out of 391 animals screened, *T. evansi* was found in only one animal by classical thin blood smear examination.

### Serological tests

#### Card agglutination test/*T. evansi*

Using this serodiagnostic assay, 39 samples showing +++/++ titres on the CATT/*T. evansi* were considered positive, while 89 samples showing +/- titres were considered to be suspected cases (Table I). Among the various districts under

study, the highest prevalence of positive titres was reported from the district of Ludhiana (42.86%, 95% CI = 21.38–67.41) in the central plain zone (overall prevalence: 15%, 95% CI = 11.46–26.18), about which the farmers were informed immediately (Table I, Fig. 1). The prevalence, based on the presence of anti-trypanosome antibodies, was found to differ non-significantly among the various districts as well as zones under study.

#### Indirect enzyme-linked immunosorbent assay

Out of 319 sera examined, only 9 samples showed a positive titre on the iELISA (Table I). Among the various districts under study, the highest prevalence of positive titres was again reported from the district of Ludhiana



CATT: card agglutination test  
ELISA: enzyme-linked immunosorbent assay

**Fig. 1**

**Relative prevalence of seropositive and suspected cases of *Trypanosoma evansi* infection among equines in the districts of Punjab under study**

(7.14%, 95% CI = 1.27–31.47) in the central plain zone (overall prevalence: 4.17%) (Table I, Fig. 1). The prevalence, based on the presence of anti-trypanosome antibodies, did not differ significantly among the various districts and zones under study.

**Risk factor analysis**

The assessment of the odds ratio (OR) revealed the prevalence of *T. evansi* to be uniformly distributed among the equine population with respect to various risk factors (Table II). However, the difference in prevalence was significant in terms of the management (OR = 3.18, 95% CI = 1.53–6.65; OR = 7.8, 95% CI = 1.45–55.43) and use of the animals (OR = 2.51, 95% CI = 1.20–5.21; OR = 4.89, 95% CI = 1.06–25.28) by CATT/*T. evansi* and iELISA, respectively. The highest prevalence values were found in ‘unorganised’ farms (21.36%, 7.29%), and in equines used for commercial purposes (19.79%, 6.67%). The species of the host animal was apparently the most influential risk factor for infection, with ORs of 2.81 (positive) and 5.63 (suspect) by CATT/*T. evansi* and a prevalence of 26.09% in horses and 65.22% in donkeys/mules. The prevalence differed significantly between male and female animals (OR = 3.13, 95% CI = 1.32–7.67 [CATT/*T. evansi* for females). With regard to age, adult equines had a higher prevalence of *T. evansi* infection, and the difference was significant for the CATT/*T. evansi* (Table II). There was fair

agreement between the CATT/*T. evansi* and the iELISA for the detection of *T. evansi* (kappa = 0.345).

**Discussion**

Blood smear examination is the gold standard technique for detecting haemoprotozoan infection but has low sensitivity (19). In this study, only one blood sample tested positive, with very low parasitaemia, thus supporting the fact that microscopic detection is not feasible until  $2.5 \times 10^6$  parasites per millilitre of blood are present (20). In low-dose infection, the intermission phase may be long and, even when symptoms are present, trypanosomes may still not be detectable in blood. This delays treatment and thereby increases the rates of morbidity and mortality in the animal population (21).

Regarding an efficient pen-side test, the literature provides contradictory opinions about the use of the CATT/*T. evansi* targeting the RoTat 1.2 antigen (22). Prior to any international movement or during quarantine, the IgG-based iELISA would be appropriate for verifying that animals are free from infection (9), but in situations where there is overt disease, and to monitor the treatment of animals with trypanocidal drugs, the CATT/*T. evansi* can be used. For declaring disease-free status, the use of

**Table II**  
**Distribution of variables used to investigate the risk factors associated with CATT/*T. evansi* and iELISA seroprevalence in equines in Punjab, India**

Factors	Variables	Samples	CATT +++/++ (%)	Odds ratio (95% CI)	CATT +/- (%)	Odds ratio (95% CI)	iELISA (%)	Odds ratio (95% CI)
<b>Sex</b>	Male	133	8 (6.02)		26 (19.55)		2 (1.523)	
	Female	186	31 (16.67)	3.13 (1.32–7.67)	63 (33.87)	2.11 (1.21–3.69)	7 (3.91)	2.56 (0.48–18.15)
	$\chi^2$		8.20*		7.91*		1.44	
<b>Age</b>	More than 2 years	267	35 (13.11)		81 (30.34)		8 (3.09)	
	Less than 2 years	52	4 (7.69)	1.81 (0.58–6.31)	8 (15.38)	2.40 (1.03–5.79)	1 (1.96)	1.58 (0.19–34.31)
	$\chi^2$		1.19*		4.84*		0.18	
<b>Species</b>	Horses	296	33 (11.15)		74 (25)		5 (1.72)	
	Donkeys/mules	23	6 (26.09)	2.81 (0.92–8.30)	15 (65.22)	5.63 (2.13–15.18)	4 (21.05)	12.25 (2.50–58.94)
	$\chi^2$		4.44		17.16*		0.05	
<b>Management</b>	‘Organised’	216	17 (7.87)		58 (26.85)		2 (0.93)	
	‘Unorganised’	103	22 (21.36)	3.18 (1.53–6.65)	31 (30.10)	1.17 (0.68–2.03)	7 (7.29)	7.80 (1.45–55.43)
	$\chi^2$		11.83*		0.37		8.77*	
<b>Use</b>	Recreational	223	20 (8.97)		47 (21.08)		3 (1.36)	
	Commercial	96	19 (19.79)	2.51 (1.20–5.21)	42 (43.75)	2.91 (1.68–5.04)	6 (6.67)	4.89 (1.06–25.28)
	$\chi^2$		7.33*		17.15*		5.89*	
<b>Total</b>		<b>319</b>		<b>39</b>		<b>89</b>		<b>9</b>

\* $p \leq 0.05$   
CATT: card agglutination test

CI: confidence interval  
iELISA: indirect enzyme-linked immunosorbent assay

the iELISA followed by retesting of suspect samples by the CATT/*T. evansi* is recommended (9). In this study, 89 samples with agglutination scores of +/- were only considered suspicious for infection because slight reactions on the CATT/*T. evansi* can be observed in uninfected horses; therefore, the cut-off was set at a reaction score of ++ (23). Using both tests, the seroprevalence of *T. evansi* was found to be highest in the Ludhiana district of the central plain zone; the farms in this zone are in the vicinity of paddy fields which are conducive to the breeding of tabanid flies. This may have led to the high prevalence of *T. evansi* in this area, which is in agreement with other studies (24).

The assessment of various risk factors demonstrated that the prevalence of infection in female equines (OR = 3.13, 2.11 by CATT/*T. evansi* and iELISA, respectively) was greater than that in their male counterparts, probably due to their use as both draught and breeding animals (19, 25). In this study, a markedly lower prevalence was observed in equines less than 2 years of age, when compared with adults. Maternal antibodies provide passive immunity to the young until the age of 3–6 months (26); this immunity diminishes from 6 months to 2 years of age, and their chance of encountering the infection also increases (OR = 1.81, 2.40) when the animals are used for sports and/or work as draught animals. Similar findings have been reported in camels, showing a higher prevalence of surra in the age group above 4.5 years when compared with the age group of 1.5–4.5 years (27). As donkeys and mules are kept mainly outdoors under poor conditions during daily work, their chance of exposure to vectors increases, resulting in an increased risk of haemoparasitic infection in these species (in this study, OR = 2.81, 5.63) (25, 28). Owing to sub-optimal management practices, animals on 'unorganised'

farms had a higher risk of infection (OR = 3.18, 7.80) with *T. evansi* because the likelihood of direct contact with vectors is higher on these farms (19, 25). Advanced management and disease control programmes reduce the chance of infection in equines kept for recreational purposes, while open grazing practices in equines used for commercial purposes increase the risk of *T. evansi* infection (OR = 2.51, 4.89) (29).

## Conclusions

This investigation indicated that, in the early stage of infection, both techniques (CATT/*T. evansi* and iELISA) may be used to determine the seroprevalence of *T. evansi* and to evaluate the effectiveness of drugs. In order to declare disease-free status, use of the iELISA followed by retesting of suspect samples by CATT/*T. evansi* is suggested.

## Competing interests

The authors declare that they have no competing interests.

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# L'infection par *Trypanosoma evansi* chez les équidés de différentes zones agro-climatiques du Pendjab (Inde) : prévalence sérologique comparative et analyse des facteurs de risque

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### Résumé

La détection minutieuse de *Trypanosoma evansi* dans le sang est difficile en raison du nombre faible et fluctuant de parasites pendant la phase chronique de l'infection. L'étude présentée par les auteurs vise, d'une part, à réaliser une première évaluation de la prévalence sérologique de *T. evansi* dans chacune des zones agro-climatiques du Pendjab en utilisant une épreuve immuno-enzymatique

(ELISA) indirecte et le test d'agglutination sur carte pour la trypanosomose (CATT/*T. evansi*) et, d'autre part, à évaluer les facteurs de risque associés à une présence inapparente de la trypanosomose. Au total, sur les 319 sérums d'équidés prélevés dans 12 districts du Pendjab (Inde) appartenant à des zones agro-climatiques différentes, 39 échantillons (12,23 %) ont donné des résultats positifs avec le CATT/*T. evansi* et 9 échantillons (2,82 %) ont donné des résultats positifs à l'ELISA indirecte. La prévalence la plus élevée a été enregistrée dans le district de Ludhiana (42,86 % de résultats positifs avec le CATT/*T. evansi* et 7,14 % de résultats positifs avec l'ELISA indirecte) dans la zone des plaines centrales (où la prévalence globale s'élevait, suivant les méthodes de test, à 15 % et 4,17 %, respectivement). La détection de *T. evansi* par les deux tests a été concordante ( $\kappa = 0,345$ ). Le facteur de risque ayant le plus d'influence sur la probabilité d'infection était l'espèce, ce risque étant plus élevé chez les ânes et les mulets que chez les chevaux (rapport de cotes [*odds ratio*, OR] de 2,81 [CATT/*T. evansi*] et de 5,63 [ELISA indirecte]). Les femelles présentaient également un risque plus élevé de posséder des anticorps anti-*T. evansi* que les mâles (OR = 3,13; intervalle de confiance [IC] à 95 % : 1,32–7,67 [CATT]), en particulier dans les élevages « informels » (sans gestion sanitaire) (OR = 3,18 ; IC à 95 % : 1,53–6,65 [CATT]) ainsi que parmi les animaux utilisés à des fins commerciales (OR = 2,51 ; IC à 95 % : 1,20–5,21 [CATT]). En conclusion, pour la démonstration de l'absence d'anticorps, les auteurs recommandent d'utiliser l'ELISA indirecte puis de soumettre les échantillons douteux à un test de confirmation au moyen du CATT/*T. evansi*.

#### Mots-clés

Cheval – Épreuve immuno-enzymatique indirecte – Facteur de risque – Prévalence sérologique – Test d'agglutination sur carte – *Trypanosoma evansi*.



## Seroprevalencia comparada y análisis de los factores de riesgo de la infección por *Trypanosoma evansi* en equinos de distintas zonas agroclimáticas del Punjab (India)

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#### Resumen

La detección precisa de *Trypanosoma evansi* en la sangre resulta difícil porque en la fase crónica de la infección la parasitemia es baja y fluctuante. Los autores describen una investigación encaminada principalmente a determinar por primera vez la seroprevalencia de *T. evansi* en todas las zonas agroclimáticas del Punjab por ensayo inmunoenzimático indirecto (ELISAi) y por aglutinación en placa, así como los factores de riesgo asociados a la tripanosomosis latente. De un total de 319 muestras de suero equino procedentes de 12 distritos del Punjab (India) situados en diferentes zonas agroclimáticas, la aglutinación en placa deparó resultado positivo en 39 de ellas (un 12,23%) y el ELISAi en 9 (2,82%). El máximo nivel de prevalencia se registró en el distrito de Ludhiana (42,86% y 7,14% por aglutinación en placa y ELISAi, respectivamente), sito en la zona de la planicie central (que en conjunto deparó una prevalencia del 15% y el 4,17%, respectivamente). Ambas pruebas resultaron bastante coincidentes por lo que respecta a la detección de *T. evansi* (coeficiente kappa = 0,345). El factor de

riesgo más influyente resultó ser la especie: en comparación con los caballos, los asnos o mulas presentaban una razón de probabilidad (RP) de 2,81 y 5,63 para la aglutinación en placa y el ELISAI respectivamente. Se observó que la población de yeguas (RP = 3,13; intervalo de confianza [IC] al 95% = 1,32–7,67 [aglutinación en placa]) presentaba un riesgo más elevado de seropositividad para *T. evansi*, especialmente en explotaciones «desorganizadas» (mal gestionadas) (RP = 3,18; IC 95% = 1,53–6,65 [aglutinación en placa]) y entre los animales utilizados con fines comerciales (RP = 2,51; IC 95% = 1,20–5,21 [aglutinación en placa]). Los autores concluyen proponiendo que, a los efectos de declarar la ausencia de enfermedad, se utilice en primer lugar el ELISAI, seguido de la prueba de aglutinación en placa para las muestras sospechosas.

#### Palabras clave

Ensayo inmunoenzimático indirecto – Equino – Factor de riesgo – Prueba de aglutinación en placa – Seroprevalencia – *Trypanosoma evansi*.



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