

Japanese Encephalitis Virus in Equines: Serological Survey and Epidemiological Insights from Gujarat, India

ABSTRACT

Japanese encephalitis virus (JEV), a mosquito-borne zoonotic pathogen primarily transmitted by *Culex* species, is known to cause severe neurological disorders in horses. . The study was conducted to investigate the prevalence and potential risk factors associated with Japanese encephalitis virus (JEV) infection in equine populations. A total of 600 serum samples were collected from horses across 19 districts of Gujarat, including Ahmedabad, Anand, Amreli, Bhavnagar, and Kheda, between February and August 2024. The collected sera were analyzed using an indirect IgG ELISA, and statistical analyses were performed to evaluate potential risk factors. The overall seroprevalence of JEV was **26.0%**, with significant associations identified for geographic location ($p < 0.01$) and age ($p < 0.05$). The highest seropositivity was observed in Amreli district (61.53%) and among horses older than 20 years (46.15%). No significant differences were detected based on sex or breed. These findings emphasize the importance of region-specific surveillance and targeted control strategies to mitigate the risk of JEV transmission in equine populations.

Key words: Japanese Encephalitis Virus, Horses, ELISA, Risk factors

INTRODUCTION

Japanese encephalitis (JE) is a mosquito-borne viral disease affecting equines, caused by JEV, a *Flavivirus* belonging to the *Flaviviridae* family (Solomon and Whitley, 2004; CDCP, 2022). First human JEV reported in India in 1955, it spread from southern regions to West Bengal (1973), Assam (1976), and Uttar Pradesh (1978) (Dhillon and Raina, 2008), with Gorakhpur, Deoria, and Basti facing persistent annual outbreaks (Kabilan *et al.*, 2004; Parida *et al.*, 2006). The virus is maintained in a zoonotic transmission cycle involving *Culex* spp. mosquitoes, pigs, bats, and ardeid birds, while humans and equines act as dead-end hosts due to low viremia (Solomon, 2006; Cui *et al.*, 2008). JEV is enzootic in Asia, particularly in rice-growing regions that favor mosquito breeding, posing a significant risk to highly susceptible equines (OIE Terrestrial Manual, 2010). Infected horses may develop fatal encephalitis, exhibiting clinical signs such as anorexia, fever, lethargy, neurological dysfunction, paresis, paralysis, collapse, and coma, with mortality rates

ranging from 5 to 30 percent (Gulati *et al.*, 2011). Most infections remain subclinical, and in India, the absence of equine vaccination programs increases their susceptibility in endemic regions (Gulati *et al.*, 2011). Gujarat, geographically positioned between 20.1°N to 24.7°N latitude and 68.4°E to 74.4°E longitude, experiences a semi-arid to tropical climate with moderate annual rainfall, influencing vector activity and JE transmission dynamics. Considering the significance of JE as a major neurological disease in horses and the limited epidemiological studies in India, particularly in Gujarat, this study was undertaken to assess the seroprevalence of JEV in horses.

The research adhered to the guidelines of the Compendium of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under Project Approval No. 433/VMD/2024, granted by the College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand.

MATERIALS AND METHODS

In present study, a total of 600 horse serum samples were randomly collected from 19 different districts of Gujarat state, including Ahmedabad, Amreli, Anand, Aravali, Banaskantha, Bharuch, Bhavnagar, Gandhinagar, Junagadh, Kheda, Kutch, Mahisagar, Dang, Panchmahal, Rajkot, Sabarkantha, Surat, Surendranagar, and Vadodara. Furthermore, clinical cases presented at the Veterinary Teaching Complex, Zaveri Hospital, Anand, were also included and analyzed for the presence of antibody against JEV.

Collection and preservation of samples

A total of 600 blood samples from horses were collected in serum clot activator vials (Vacuette®) for the study on Japanese Encephalitis Virus (JEV). The Blood sample were allowed to clot at room temperature for 15-30 min and centrifuged at 1500 rpm at 4°C for 10 minutes (Eppendorf 5804 R, Germany). The harvested serum was then stored at -20°C for detection of antibodies against JEV virus through ELISA.

Indirect IgG ELISA

The Indirect IgG ELISA protocol started by coating a 96-well Maxisorp flat bottom plate (Nunc, Denmark) with 50 µl of recombinant NS1 protein (10 µg/ml in carbonate buffer, pH 9.6) per well, kept for overnight incubation at 4°C. After 12 hours unbound antigen was removed by washing the plate three times with PBS-T (Phosphate Buffer Saline + Tween 20). To block nonspecific binding, 300 µl of 5% skimmed milk powder (SMP) made in PBS-T was added to each well and incubated at 37°C for 2 hours. After three washes, test serum samples (diluted 1:100 in 5% SMP) were added at 50 µl per well and incubated at 37°C for 1 hour, followed by another three washes were given through PBS-T. Next, 50 µl per well of Goat anti-Equine IgG (H+L) (HRP) conjugated secondary antibody (Novus, US), diluted at 1:10,000 in 5% SMP, was added and incubated at 37°C for 1 hour. After four washes, 100 µl of freshly prepared substrate solution (10 ml citrate buffer, pH 4.5, 10 mg o-phenylenediamine dihydrochloride, and 8 µl H₂O₂) was added per well and incubated at room temperature in the dark for 5 minutes. The reaction was stopped by adding 50 µl of 2M sulfuric acid per well. Absorbance was measured at 492 nm using an ELISA reader (Thermo Scientific, Multiskan Ex). The cutoff value was calculated as the average OD of negative controls plus 0.15, with OD values above the cutoff considered positive and those below considered negative. This entire ELISA protocol was carried out at VPH division of Indian Veterinary Research Institute (Bareilly, UP).



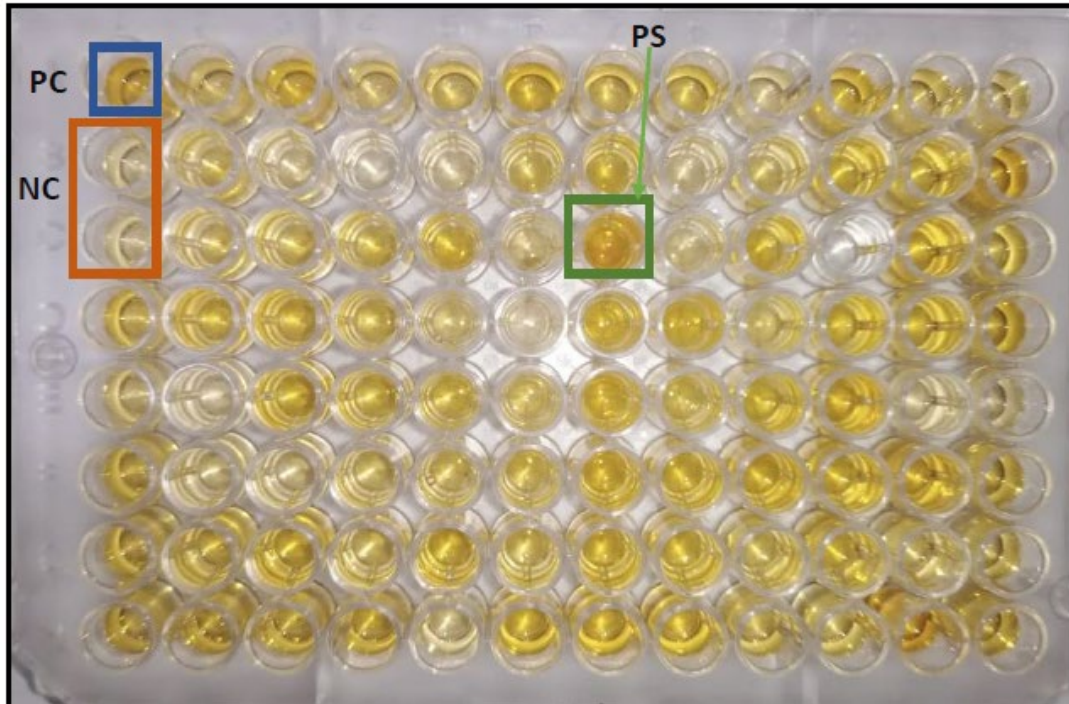


Figure 1.0: Screening of horse serum samples using Indirect IgG ELISA
PC= Positive Control, NC= Negative Control, PS=Positive Sample

Statistical Analysis

The data collected during the sampling of horses were categorized based on age, sex, breed, and district and were subjected to a Chi-square test using WASP-GOA 1.0 software. Values of $p > 0.05$ were considered non-significant, $p < 0.05$ were considered significant, and $p < 0.01$ were considered highly significant.

RESULTS AND DISCUSSION

Overall seroprevalence of Japanese Encephalitis in horses of Gujarat

A total of 600 horse serum samples were analysed to study seroprevalence of Japanese Encephalitis (JE) antibodies using an Indirect IgG ELISA. Among these, 156 samples tested positive, resulting in an overall seroprevalence rate of 26.00% (156/600).

Risk factor analysis of JEV infection

Various risk factors, including spatial distribution, age, sex, and breed, were statistically analyzed for their significant effect on Japanese Encephalitis (JE) prevalence. The findings are presented below.

Spatial distribution of JEV infection

The district-wise seroprevalence of Japanese Encephalitis virus infection in Gujarat showed the highest seropositivity in Amreli (61.53%), followed by Bhavnagar (48.72%), Vadodara (44.44%), and Kheda (41.67%). Moderate seropositivity was observed in Kutch (27.91%), Surat (27.27%), Ahmedabad (25.81%), Panchmahal (25.00%), and Anand (22.92%). Lower seroprevalence rates were recorded in Aravali (18.52%), Banaskantha (18.52%), Gandhinagar (17.39%), Dang (15.38%), Junagadh (12.5%), Mahisagar (8.33%), and Rajkot (5.40%). Notably, no positive cases of Japanese Encephalitis Virus (JEV) were reported in Bharuch and Surendranagar. Statistical analysis revealed a highly significant effect ($p < 0.01$) of the geographical area on seropositivity of JEV.

Table 1. District wise seroprevalence of Japanese Encephalitis in horses of Gujarat using Indirect IgG ELISA

Districts	No. of horses tested	No. of positive horses	Prevalence (%)	p-value
Ahmedabad	93	24	25.81	0.001**
Amreli	26	16	61.53	
Anand	48	11	22.92	
Aravali	27	05	18.52	
Banaskantha	54	10	18.52	
Bharuch	08	00	00	
Bhavnagar	39	19	48.72	
Gandhinagar	23	04	17.39	
Junagadh	32	04	12.50	
Kheda	36	15	41.67	
Kutch	86	24	27.91	

Mahisagar	12	01	8.33
Dang	13	02	15.38
Panchmahal	20	05	25.00
Rajkot	37	02	5.40
Sabarkantha	14	03	21.43
Surat	11	03	27.27
Surendranagar	02	00	00
Vadodara	18	08	44.44
Aravali	27	05	18.52

*Significant at $p < 0.05$, ** Highly Significant at $p < 0.01$.

Age wise distribution of JEV infection

The significantly highest prevalence of JE was observed in horses aged >20 years (46.15%), followed by 0 to 5 years (30.20%). Horses aged >15 to 20 years showed a seroprevalence of 23.68%, those aged >10 to 15 years had a seroprevalence of 22.86%, and the lowest seroprevalence was recorded in the >5 to 10 years age group, at 19.67%. Statistical analysis revealed a significant effect ($p < 0.05$) of age on seroprevalence of JE in horses.

Table 2: Age wise seroprevalence of Japanese Encephalitis in horses of Gujarat using Indirect IgG ELISA

Age	No. of horses tested	No. of positive horses	Prevalence (%)	p-value
0 to 5 years	245	74	30.20	0.02*
>5 to 10 years	183	36	19.67	
>10 to 15 years	70	16	22.86	
>15 to 20 years	76	18	23.68	
>20 Years	26	12	46.15	

*Significant at $p < 0.05$, ** Highly Significant at $p < 0.01$.



Sex wise distribution of JEV infection

The prevalence of Japanese Encephalitis was higher in males at 27.79% (82/295) compared to females at 24.26% (74/305). However, statistical analysis indicated no significant difference ($p>0.05$) in sex-wise seroprevalence.

Table 3: Sex wise seroprevalence of Japanese Encephalitis in horses of Gujarat using Indirect IgG ELISA

Sex	No. of horses tested	No. of positive horses	Prevalence (%)	p-value
Male	295	82	27.79	0.32
Female	305	74	24.26	

*Significant at $p<0.05$, ** Highly Significant at $p<0.01$.

Breed wise distribution of JEV infection

The highest prevalence of JE was observed in non-descript horses at 36.84%, followed by Thoroughbred at 32.26% and Kachchhi-Sindhi at 29.92%. Kathiawari horses showed a seroprevalence of 23.53%, while the lowest prevalence was recorded in Marwari horses at 22.09%. Statistical analysis revealed a non-significant effect of breeds on the seropositivity of JEV.

Table 4: Breed wise seroprevalence of Japanese Encephalitis in horses of Gujarat using Indirect IgG ELISA

Breed	No. of horses tested	No. of positive horses	Prevalence (%)	p-value
Kathiawari	136	32	23.53	0.11
Marwari	249	55	22.09	
Kachchhi-Sindhi	127	38	29.92	
Thoroughbred	31	10	32.26	
Non-Descript	57	21	36.84	

*Significant at $p<0.05$, ** Highly Significant at $p<0.01$.

The samples were collected mainly from horses of Gujarat police and from small private owned establishments. These horses are traditionally used for patrolling, breeding, transportation, and for recreation. The study revealed, overall seroprevalence of JEV is 26.00% in horses of Gujarat.



This finding are similar to Kapdi *et al.* (2017) (25.11%) but lower than Sahu *et al.* (2022) (33.73%), while being higher than Kapdi *et al.* (2022) (19.38%) and Begum *et al.* (2022) (7.87%). The variations in Japanese Encephalitis (JE) prevalence in horses can be attributed to factors such as geographical differences, environmental conditions (rainfall and temperature), the season of sample collection, variations in vector populations, the presence of reservoir hosts like pigs and birds, and differences in sample analysis methods (Gingrich *et al.*, 1992, Van den Hurk *et al.*, 2009; Litzba *et al.*, 2010, Mulvey *et al.*, 2021). The present findings indicate natural infection with the virus, as vaccination against Japanese Encephalitis is not performed in the region.

The high JEV seropositivity in Amreli, Bhavnagar, Vadodara, and Kheda may be driven by ecological factors such as warm, humid conditions, heavy monsoon rainfall, extensive rice farming, and wetlands that facilitate mosquito breeding and avian host populations (Walsh *et al.*, 2022; Anonymous 2022). In contrast, the absence of reported cases in Bharuch and Surendranagar may be due to the limited number of samples collected, which could have affected seropositivity detection (Thrusfield, 2018).

This study shows higher JE prevalence in the youngest (0–5 years) and oldest (>20 years) horses. Older horses are more susceptible due to declining immunity and reduced care (Saxena & Dhole, 2008). In endemic areas, prior exposure helps to develop antibodies, reducing infection rates with age (Saxena and Dhole, 2008). The small sample size in the >20 years group (26 horses) may have inflated prevalence estimates, affecting reliability (Thrusfield, 2018).

Non-descript horses showed higher JE prevalence, likely due to their labor-intensive use and less care is given, increasing mosquito exposure. In contrast, Marwari horses had the lowest prevalence, benefiting from superior care due to their economic and breeding value. However, no statistically significant association was observed.

The higher JE prevalence in males may be due to increased exposure to vectors, as they are more frequently engaged in outdoor activities such as riding, horse shows, labor tasks, and distinct management practices. However, the findings were not statistically significant.

Changing agricultural practices, such as expanded irrigation systems that create mosquito breeding sites, along with the growing range of migratory birds (cattle egrets and night heron) and the rise in pig farming, have contributed to the emergence of JEV infections in new regions of India, Nepal, and Australia over the past decade (Gulati *et al.*, 2011). Additionally, JEV transmission through mosquito vectors poses a risk of spreading to humans and other animal



populations in 10 out of the 13 states where JEV seropositivity in horses has been noted (Parida *et al.*, 2006; Misra and Kalita, 2010).

CONCLUSION

The observed JEV seroprevalence in horses highlights the potential risk of future outbreaks within equine populations, which could lead to increased morbidity and mortality. To better understand and mitigate this risk, further research is needed to model and predict the transmission dynamics of vector-borne viral diseases between horses and mosquitoes. The high seropositivity detected in horses across Gujarat confirms that JEV is endemic in the region. Therefore, implementing a comprehensive 'One Health' approach is essential. This initiative should involve collaboration between governmental and non-governmental organizations, along with key stakeholders from relevant ministries, departments, and agencies, to develop national, sub-regional, and regional strategies for routine surveillance and reporting of JEV. Establishing such measures as part of a public health preparedness plan is crucial to controlling the spread of this zoonotic disease among equids and other susceptible species.

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