RESEARCH ARTICLE

Sero-Diagnostic Concordance of Equine Infectious Anaemia in Saurashtra Region of Gujarat

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Abstract

Equine Infectious Anaemia (EIA) is a vector-borne retroviral disease of equids with significant implications for equine health, movement, and trade due to its lifelong persistence and lack of curative treatment. A sero-surveillance study was conducted in the Saurashtra region of Gujarat from January to June 2025, to generate baseline epidemiological data, assess the seroprevalence of Equine Infectious Anaemia Virus (EIAV), evaluate indirect ELISA (iELISA) in comparison with Agar Gel Immunodiffusion (AGID), and document associated clinicopathological alterations. A total of 514 horses of different age, sex, and breed were clinically examined and subjected to haematological, biochemical, and serological analyses. Based on suggestive clinical and clinicopathological findings, 47 horses (9.14%) were screened using RP-26-based iELISA, of which two (0.39%) were seropositive; however, none was confirmed by AGID, indicating an extremely low apparent EIAV prevalence. District-wise analysis showed that both seropositive horses originated from Bhavnagar district (2/117; 1.71%), with no positive cases in other districts of Saurashtra, resulting in an overall seroprevalence of 0.39% (2/514). Age-wise seropositivity was observed in the 1-5 years (1/185; 0.54%) and >5-20 years (1/293; 0.34%) groups. Both seropositive horses were females (2/383; 0.52%), with no positive case in male (0/131; 0%). Breed-wise seropositivity was detected in Kathiawadi (1/231; 0.43%) and Thoroughbred horses (1/44; 2.27%). Month-wise detection was limited to January (2.85%) and May (1.82%). No significant predisposition was found with respect to district, age, sex, breed and season. Indirect ELISA showed high specificity (99.61%), overall accuracy (99.61%), and a negative predictive value of 100%. Agreement between iELISA and AGID was good (κ = 0.705). Overall, EIAV circulation in the Saurashtra region was minimal and sporadic, likely reflecting favorable climatic conditions, low vector density, and improved management practices. The study provides valuable baseline data and underscores the need for continued surveillance to maintain the region's low-risk status for EIA.

Key words: Agar Gel Immunodiffusion (AGID), Epidemiology, Equine Infectious Anaemia Virus (EIAV), Indirect ELISA (iELISA), Serosurveillance, Saurashtra region.

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Introduction

Horses (*Equus caballus*) have long played an important role in human society as transport and draft animals, particularly in regions of India such as Haryana, Punjab, Rajasthan, J & K and Gujarat, with indigenous breeds including Marwari, Kathiawari, Manipuri, Spiti, Bhutia, and Zanskari. According to the 20thLivestock Census, India's horse population declined to 3.4 lakh in 2019 (45.6% decrease), whereas Gujarat recorded a population of 0.22 lakh, showing a 19.42% increase compared to the previous census (BAHS, 2022).

Equine Infectious Anaemia (EIA) is a chronic and potentially fatal disease of equids caused by Equine Infectious Anaemia Virus (EIAV), a macrophage-tropic lentivirus of the family *Retroviridae*. EIAV is an enveloped, positive-sense single-stranded RNA virus (~9 kb) that integrates into the host genome, causing lifelong infection, and is classified under the genus *Lentivirus*, often referred to as the "country cousin" of HIV (Craigo and Montelaro, 2011). EIAV is transmitted mainly through mechanical transfer of infected blood by biting flies, particularly stable flies (*Stomoxys calcitrans*) and horse/deer flies (*Tabanus*, *Hybomitra*, *Chrysops* spp.), with transmission influenced by host viremia and vector density, while mosquitoes are not proven effective vectors (Kemen and Coggins, 1972).

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Equine Infectious Anaemia (EIA) can present as an acute, occasionally fatal illness but more often occurs as a chronic disease with recurring fever, anaemia, thrombocytopenia,

diarrhoea, weakness, and ventral edema, without marked immunosuppression due to the virus's macrophage tropism (Sellon *et al*., 1994; Cordes and Issel, 1996). Definitive diagnosis of EIA relies on the detection of EIAV-specific antibodies in the serum of suspected animals, with a combination of serological tests recommended for accurate confirmation. Indirect ELISA (iELISA) is widely used as a sensitive screening test, while the Agar Gel Immunodiffusion (AGID) test remains the prescribed confirmatory gold standard. Advanced techniques such as PCR may further aid detection by targeting viral components. The present study was undertaken to determine the serosurveillance of Equine Infectious Anaemia Virus (EIAV) in horses of the Saurashtra region and to generate baseline epidemiological data with respect to district, age, sex, breed, and season. It also aimed to evaluate the diagnostic performance of indirect ELISA as a screening test in comparison with the Agar Gel Immunodiffusion test as the confirmatory gold standard, and to document the associated clinical, haematological, and serum biochemical alterations in suspected and seropositive horses.

Materials And Methods

A total of 514 horses were randomly selected from the Saurashtra region of Gujarat for blood and serum sample collection during January-June 2025 as part of Equine Infectious Anaemia Virus surveillance. The study was conducted at the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, in collaboration with the Veterinary Clinical Complex, Junagadh and the National Research Centre on Equines (NRCE), Hisar (Haryana), India, following approval of the Institutional Animal Ethics Committee (IAEC No. KU-JVC-IAEC-LA-147-24).

Epidemiological Surveillance

Epidemiological surveillance of Equine Infectious Anaemia Virus (EIAV) was conducted to record prevalence data in horses from the Saurashtra region. Information on district, age, sex, breed, and month of sampling (January to June 2025) was collected. A total of 514 horses (131 males and 383 females) from nine districts were included. Data were analysed for age, sex, breed, district, and month-wise distribution to assess overall prevalence and epidemiological patterns of EIAV infection.

Clinical Examination and Collection of Samples

A comprehensive clinical evaluation was performed on the horses included in the study. Parameters such as rectal temperature (°F), conjunctival mucous membrane status (normal, pale, congested, or icteric), presence of ectoparasites, feed intake, type of nasal discharge (unilateral or bilateral), and occurrence of nervous signs (if any) were recorded.

Under strict aseptic conditions, whole blood and serum samples were collected from horses for haematological, biochemical, and serological investigations. Approximately 2 mL of jugular blood was collected in K_3EDTA vacutainers and analyzed for haematological parameters using an automated haematology analyzer (Abacus ABJ Vet-5) following the manufacturer's guidelines. For serum biochemistry and serology, about 7 mL of blood was collected in gel-barrier clot activator vacutainers; the separated serum was stored at -4 °C for biochemical analysis using a DiatekDia-CHEM 240 Plus analyzer and at -20 °C for ELISA and AGID testing for Equine Infectious Anaemia. All samples were appropriately labelled and processed as per standard laboratory protocols to maintain quality and integrity.

Diagnosis by Enzyme-Linked Immunosorbent Assay (ELISA) and Agar Gel Immunodiffusion Test (AGID / Coggins Test)

Detection of antibodies against Equine Infectious Anaemia Virus (EIAV) was carried out using a recombinant p26 antigen-based indirect ELISA kits (Ochoa *et al*., 2000; Malik *et al*., 2013). Samples with PP% <20 were considered negative, 20-25 were classified as doubtful, and >25 were interpreted as positive for EIAV antibodies. All samples were run in duplicate to ensure reliability, with blank wells included to correct for background absorbance. ELISA-positive and doubtful samples were retested and further confirmed using AGID/Coggins test.

The Agar Gel Immunodiffusion (AGID) test was employed as the definitive (gold-standard) confirmatory assay for detecting antibodies against Equine Infectious Anaemia Virus (EIAV), using purified recombinant p26 antigen and reference positive control serum as per standard protocols followed at NRCE, Hisar (Malik *et al*., 2013). A distinct line of identity between the test serum and antigen wells was interpreted as positive, while absence of a line indicated a negative result; weak reactions were retested.

Statistical Analysis

Prevalence data were expressed as percentages. Epidemiological data were analyzed using the chi-square test, and haemato-biochemical parameters using the unpaired *t*-test. Sensitivity, specificity, and accuracy of i-ELISA and AGID were calculated with MedCalc (v19.3.1) and compared using McNemar's chi-square test. A p-value <0.05 was considered significant, while <0.01, <0.005, and <0.001 were considered highly significant.

Results and Discussion

Overall Prevalence of Equine Infectious Anaemia

A total of 514 horses from different districts of the Saurashtra region were screened for Equine Infectious Anaemia Virus (EIAV) during January-June 2025. Based on clinical examination and haemato-biochemical alterations, 47 horses (9.14%) were categorized as clinically suspected. Among these, two samples (0.39%) were reactive by indirect ELISA; however, both samples were negative by the confirmatory AGID test. Thus, no AGID-confirmed

EIAV-positive case was detected in the study population, indicating an extremely low circulation of EIAV in the region during the study period.

The overall seropositivity rate (0.39%; 2/514) observed in the present investigation was comparable with several earlier reports that documented very low EIAV prevalence in regions with effective surveillance, controlled animal movement, and favourable ecological conditions. Conversely, substantially higher prevalence reported in some endemic regions has been attributed to dense equine populations, unrestricted movement, and higher vector abundance. Therefore, the findings of the present study suggest that the Saurashtra region presently represents a low-risk epidemiological zone for EIAV infection.

Epidemiological Surveillance of Equine Infectious Anemia

Epidemiological surveillance of EIAV in the studied equine population revealed no statistically significant associations across district-, age-, sex-, breed-, or month-wise categories. Both ELISA-reactive horses were detected exclusively in Bhavnagar district, resulting in a district-specific seropositivity of 1.71% (2/117), whereas all other districts recorded nil seropositivity; however, chi-square analysis ($\chi^2 = 6.81$; $p = 0.55$) demonstrated no significant geographical association, indicating that the observed clustering was incidental rather than reflective of true spatial predisposition.

Age-wise evaluation identified seropositivity in horses aged 1-5 years (0.57%, 1/185) and >5-20 years (0.34%, 1/293), with no positive cases in horses <1 year or >20 years, and no significant age-related association was observed ($\chi^2 = 0.26$; $p = 0.96$), although higher numerical values in adult horses may be attributed to prolonged cumulative exposure to hematophagous vectors and increased animal movement. Sex-wise analysis showed marginally higher seropositivity in females (0.52%, 2/383) compared to males (0%), yet this difference was statistically non-significant ($\chi^2 = 0.68$; $p = 0.40$), supporting the vector-mediated and sex-independent nature of EIAV transmission.

Breed-wise assessment revealed higher numerical seropositivity in Thoroughbred horses (2.27%, 1/44), followed by Kathiawari horses (0.43%, 1/231), while Marwari and non-descript horses remained seronegative; nevertheless, no significant breed association was evident ($\chi^2 = 4.97$; $p = 0.17$), suggesting that management practices and exposure risk outweigh genetic susceptibility. Month-wise surveillance recorded seropositivity in January (2.85%, 1/35) and May (1.82%, 1/55), with no positive cases in other months; however, seasonal variation was not statistically significant ($\chi^2 = 10.05$; $p = 0.07$), indicating that EIAV transmission in the study area is not strongly seasonal and is likely influenced by multifactorial environmental and management-related factors.

Diagnostic Evaluation

Out of 514 samples, two samples were reactive by iELISA but negative by AGID, classifying them as false positives. A total of 512 samples were negative by both tests, constituting true negatives.

Owing to the absence of AGID-confirmed positives, diagnostic sensitivity could not be estimated. Nevertheless, iELISA demonstrated high specificity and overall diagnostic accuracy (99.61%), indicating excellent performance in correctly identifying non-infected animals. The McNemar test showed no significant difference between iELISA and AGID ($\chi^2 = 0.501$; $p = 0.48$), while the kappa value ($\kappa = 0.705$) indicated good agreement, supporting the reliability of iELISA as a screening assay in conjunction with AGID for confirmation. Minor discrepancies between ELISA and AGID may be attributed to low antibody titres, early or inapparent infections, or non-specific ELISA reactivity, underscoring the need for confirmatory testing in EIAV surveillance. Comparable low seroprevalence has been documented in several earlier studies (Turan*et al*., 2002; Issel *et al*., 2013; Scicluna *et al*., 2013; Malik *et al*., 2013; Alvarez *et al*., 2015; Alneem *et al*., 2019; Kasem*et al*., 2022; Vieira *et al*., 2023; Carvelli *et al*., 2024), further corroborating the findings of the present investigation. In contrast, higher seroprevalence reported elsewhere has been linked to increased vector density, unrestricted animal movement, suboptimal biosecurity, and endemic viral circulation. Collectively, these results indicate limited EIAV circulation in the study area, supporting the classification of the Saurashtra region as a low-risk zone for equine infectious anaemia at the time of sampling.

Haemato-Biochemical Alterations

Seropositive horses exhibited haematological alterations characteristic of EIAV infection (Table 1). Significant reductions in haemoglobin ($p<0.05$) and packed cell volume ($p<0.01$) were observed, confirming anaemia as a consistent pathological feature of EIAV. Although total erythrocyte count was comparatively lower, the difference was not statistically significant, likely due to the limited number of seropositive animals and compensatory erythropoietic responses during chronic or inapparent infection. Differential leukocyte analysis revealed significant neutropenia ($p<0.05$) with marked lymphocytosis ($p<0.001$), accompanied by increased monocyte and eosinophil proportions, reflecting persistent immune activation and involvement of the monocyte–macrophage system in viral persistence. Platelet counts showed a mild, non-significant decline, consistent with intermittent thrombocytopenia reported in chronically infected horses. These findings are in agreement with previous reports describing similar haematological alterations in EIAV-seropositive horses, including reductions in haemoglobin and packed cell volume and changes in leukocyte profiles (Craigo and Montelaro, 2011; Agina *et al*., 2017).

Serum biochemical evaluation did not reveal statistically significant differences between seropositive and seronegative horses with respect to total protein, albumin,

creatinine, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total bilirubin, and creatine kinase (Table 1). The absence of marked biochemical alterations suggests minimal hepatic, renal, or muscular involvement at the time of sampling and is indicative of inapparent or chronic EIAV infection. These findings emphasize that EIAV-infected horses may remain biochemically and clinically stable for extended periods, thereby highlighting the limited diagnostic value of routine biochemical parameters alone. Consequently, the results reinforce the importance of serological surveillance and haematological evaluation as essential tools for effective detection and epidemiological monitoring of EIAV under field conditions.

Table 1: Haemato-biochemical alterations in EIAV positive and negative horses

Parameter	EIAV negative group (n=512)	EIAV positive horses (n=2)
Haemaoblobin (g/dL)	10.65±0.18	9.75±0.15*
PCV (%)	42.18±0.57	39.79±0.02**
TEC ($\times 10^6$/µL)	8.67±0.39	7.75±0.34
TLC ($\times 10^3$/ µL)	7.61±0.51	8.66±0.18
Platelets count (10^5/ µL)	1.65±0.01	1.34±0.16
Neutrophils (%)	58.73±0.89	42.50±1.41*
Lymphocytes (%)	33.49±0.72	46.50±0.70**
Monocytes (%)	5.31±0.33	6.40±0.20
Eosinophils (%)	2.03±0.48	3.85±0.35
Total Protein (g/dL)	6.94 ± 0.26	5.07±0.73
Serum Albumin (g/dL)	3.63 ± 0.25	3.28±0.40
Serum Creatinine (mg/dL)	1.23 ± 0.17	1.18±0.16
AST (IU/L)	230.88 ± 13.59	198.55±12.21
GGT (IU/L)	20.76 ± 1.81	22.50±7.50
Total bilirubin (mg/dL)	1.11 ± 0.19	1.13±0.30
Creatinekinase (IU/L)	20.7 ± 1.44	24.00±2.00

AST= Aspartate Aminotransferase, GGT= Gamma-glutamyltransferase; *p<0.05, **p<0.021, between groups

Conclusion

The present investigation demonstrates an extremely low seroprevalence of EIAV (0.39%, 2/514) in horses of the Saurashtra region, with no AGID-confirmed positive cases. Epidemiological variables including district, age, sex, breed, and month showed no statistically significant association with EIAV occurrence. Diagnostic evaluation confirmed high specificity, excellent accuracy, and good kappa agreement between iELISA and AGID. Haematological findings in seroreactive horses were consistent with EIAV-associated anaemia and immune modulation, while serum biochemical parameters remained largely unaltered. Overall, the findings indicate low EIAV circulation, effective disease control, and justify the continued use of iELISA as a screening tool with AGID confirmation for routine EIAV surveillance in the region.

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ANNOUNCEMENT - III
FELLOWS AND ASSOCIATE FELLOWS AWARDS: SVSBT-2026

(For details please visit society's website: https://www.svsbt.com)

Applications in the prescribed formats are invited from the **Life Members** of the Society for the **Fellows and Associate Fellows Awards of SVSBT-2026.** The awards will be honoured to the deserving candidates during the inaugural ceremony of ***XIII Annual Convention*** and ***National Conference*** to be held **during October 6-7, 2026** at College of Veterinary Science, **BASU, Patna-800014,** Bihar, India.

The application format can be had on request from the President, SVSBT by e-mail at ajdhami59@gmail.com or WhatsApp No. 9898262498. The **application must be submitted only as soft copy in 2 PDFs** to the President, SVSBT, Anand, Gujarat at ajdhami59@gmail.com. **Part-1:** Application in detail along with self-assessed scorecard, and **Part-2:** All other supporting documents for each claim, including only the first page of published articles/books/manuals etc. **on or before 31st July, 2026**. **It is mandatory that the applicant must be LM of the Society**, or else he/she should become life member by due date of application through paying prescribed fees online in the UCO Bank A/C as detailed below. Incomplete applications and those received after the due date will not be entertained. SVSBT reserves the rights for the final selection of the awardees.

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- The applicant must be a life member of the SVSBT, If not, must register before due date of application.
- Applicant must have a minimum experience of 25 years in the field of teaching research and extension, and should be above 50 years of age, as on the last date of application.
- He/She should be Indian national, who is working in the field of Veterinary Science & Animal Biotechnology & its Allied/Corporate sector in India.
- Awardees may also be nominated by the executive committee from the corporate/ entrepreneurs, on the basis of distinguished contribution in scientific arena of Veterinary Science & Animal Biotechnology towards development of animal sciences.
- Application must include Full details as per the format that can be had from the President SVSBT on request and submitted online in PDF format with all necessary documents/evidences of each claim made to: ajdhami59@gmail.com **on or before 31st July, 2026**.
- Processing fee Rs. 2000/- is to be paid online in SVSBT A/C by the applicant before submission of application.
- Selected candidate shall pay Rs. 7000/- online within 15 days of intimation of results

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- The applicant must be having MVSc/PhD degree in Veterinary Science or Animal Biotech, and life membership of the SVSBT
- Applicant should be between 40 and 50 years of age on the last date of application.
- He/She should be Indian national, who is working in the field of Veterinary Science & Animal Biotechnology in India
- Application must include Full details as per the format that can be had from the President SVSBT on request, and submitted online in PDF format with all necessary documents/evidences of each claim made to: ajdhami59@gmail.com **on or before 31st July, 2026**.
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