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Research article

Seroprevalence and post-vaccination antibody monitoring of foot-and-mouth disease in cattle at the community level using ELISA

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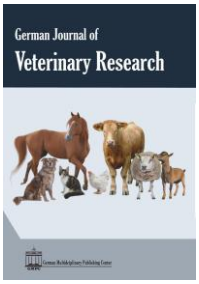

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Abstract

The livestock sector in Bangladesh is expanding rapidly and plays a vital role in meeting global protein demand. However, Foot-and-Mouth Disease (FMD) remains a significant threat to this sector. Vaccination is the primary strategy for controlling FMD, and evaluating vaccine effectiveness is essential. This study evaluated pre- and post-vaccination serum antibody titers in cattle using ELISA at the community level in 2023 across five districts: Bandarban, Jashore, Faridpur, Sirajganj, and Rajshahi. A total of 142 blood serum samples were randomly collected, with 92 samples obtained before vaccination and 50 samples collected 28 days after vaccination. The study utilized the locally available FMD vaccine. Pre-vaccination analysis showed that 35 of 92 sera (38.04%) were positive for non-structural protein (NSP) antibodies, indicating prior exposure to FMD virus. Serotype-specific antibodies were most frequently detected against serotype O (25.00%), followed by A (2.17%), while Asia-1 was not detected. Six sera (6.52%) were reactive to both O and A, and four sera (4.34%) were positive for O, A, and Asia-1, indicating the circulation of multiple FMDV serotypes in the region. Sirajganj exhibited the highest seropositivity, with 47.4% (9 samples) positive for NSP, and antibodies detected for serotype O (2 samples). Four sera reacted to both O and A, and seven sera tested positive for a combination of O, A, and Asia-1. In contrast, Jashore had the lowest seropositivity, with only one sample positive for NSP (7.1% for serotype O). Post-vaccination, seropositivity rates were 90.0% for serotype A, 92.0% for serotype O, and 76.0% for serotype Asia-1. These results indicate effective immune responses against serotypes A and O, suggesting satisfactory vaccine coverage. Bandarban demonstrated 100% seropositivity for all three serotypes. Conversely, lower antibody prevalence against Asia-1 was observed in Jashore (40%) and Sirajganj (70%), which may indicate limited immune stimulation for this serotype. The reduced response against Asia-1 underscores the need for ongoing surveillance, vaccine potency assessment, and potential booster immunization to maintain herd immunity against all circulating FMDV serotypes in Bangladesh.

Keywords: Bangladesh, Community, Effectiveness, FMD Vaccine, Seroprevalence

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Introduction

The livestock sector represents one of the fastest-growing economic sectors in developing countries, driven by increasing global demand for protein. On average, livestock contributes

approximately 40% to the agricultural Gross Domestic Product (GDP) in these nations, with country-specific contributions ranging from 15% to 80% (FAO, 2022). In Bangladesh, livestock, particularly ruminants, play a vital role in traditional agriculture, the supply of animal-derived foods, and employment generation. The country maintains 251.71 lakh cattle, 15.32 lakh buffalo, 39.81 lakh sheep, and 272.91 lakh goats (BBS, 2024-25). Furthermore, livestock accounts for 1.81% of Bangladesh's GDP, and the sector's GDP growth rate is 3.19% (DLS, 2024-25).

Despite livestock's substantial contribution to Bangladesh's economy, animal diseases remain a major constraint on economic growth, poverty alleviation, and food security (Khokon et al., 2017). Among these, Foot and Mouth Disease (FMD) is particularly concerning due to its widespread prevalence and significant impact on national economies and food security. Annual financial losses from FMD outbreaks in Bangladesh have been estimated at Tk. 188.57 billion (US$2.22 billion) (Giasuddin et al., 2020).

FMD is a transboundary animal disease characterized primarily by vesicle formation in the mouth, tongue, limbs, and interdigital spaces. Additional clinical signs include hyperthermia (105–106°F), anorexia, excessive salivation, stumping or kicking, reluctance to move, and abortion in pregnant animals (Azeem et al., 2020). FMD significantly reduces milk and meat production, decreases cattle value, and results in substantial treatment and vaccination expenses. The disease affects both adult and juvenile animals, with morbidity reaching 100% and mortality up to 20% in juveniles (Azeem et al., 2020). Given its considerable economic impact and endemic status, assessing FMD prevalence is essential for implementing effective control measures.

FMD is a highly contagious disease caused by the Foot and Mouth Disease Virus (FMDV), a single-stranded positive-sense RNA virus belonging to the genus Aphthovirus in the Picornaviridae family (Zell et al., 2017). The virus primarily infects artiodactyls, including cattle, buffalo, swine, sheep, goats, and various wild ungulate species (Longjam et al., 2011). Globally, seven serotypes of FMDV have been identified: O, A, C, South African Territories (SAT 1, SAT 2, SAT 3), and Asia-1 (Brito et al., 2017). These serotypes are immunogenically distinct, and vaccination against one serotype does not provide immunity to others or their subtypes (Paton et al., 2005).

The prevalence of FMD has fluctuated significantly over time, largely due to rapid genetic variation among serotypes (Aslam and Alkheraije, 2023). Assessing FMD prevalence is therefore essential for understanding affected populations and associated risk factors within the study area. Such data will inform stakeholders in developing effective prevention and control strategies, thereby safeguarding animal health, food security, and economic stability.

Reported morbidity and mortality rates for crossbred cattle were 55.43% and 5.15%, respectively, while native cattle exhibited rates of 77.83% and 12.39%, respectively (Giasuddin et al., 2020).

Materials and methods

Location and period of study

The study was carried out in different areas of five districts: Bandarban, Jashore, Faridpur, Sirajganj, and Rajshahi. The ELISA was done in the Central Disease Investigation Laboratory (CDIL), Dhaka. The study period was from July to December 2023.

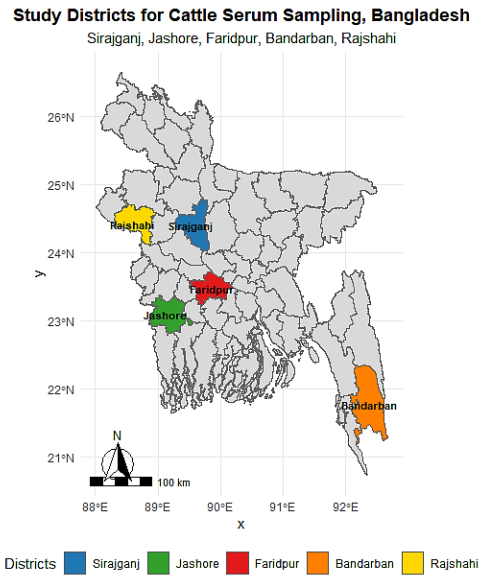

Figure 1: Geographical Presentation of the Study Districts in Bangladesh: Sirajganj (blue), Jashore (green), Faridpur (red), Bandarban (orange), and Rajshahi (yellow); the remaining administrative districts are shown in gray, with geographic coordinates, (latitude and longitude), a north arrow, and a 100-km scale bar are provided for spatial reference.

N=North, E=East, X-axis-wise Longitude, Y axis wise Lattitude, Km=Kilometer, 0= Degree

Sample collection procedures

A total of 142 blood serum samples were randomly collected from cattle in the study areas. Of these, 92 samples were obtained from unvaccinated cattle and categorized by age and sex. After 28 days post-vaccination, 50 samples were collected from vaccinated cattle. All samples originated from farms with comparable management practices and herd composition. Clinically sick animals were excluded from the study, and informed consent was obtained from the respective farm owners prior to sample collection. Blood was drawn from the jugular vein using the venipuncture method with a sterile 10 mL needle and syringe. Cattle were classified into age groups: young (≤1 year), adult (1–2 years), and old (>2 years). Animals younger than six months were excluded to reduce potential interference from maternally derived antibodies. Statistical analyses were performed using R software (version 2025). Associations between categorical variables were evaluated using the Chi-square test. Statistical significance was set at $P < 0.05$.

Serum preparation and ELISA testing

FMDV serotyping was conducted using antigen capture ELISA. Blood samples were kept at room temperature for 3–4 hours in a slightly inclined position to promote clotting and serum separation. Sera were stored at -20°C until analysis. The ELISA was performed using an Indirect ELISA kit (ID.Vet® Innovative Diagnostics, France) according to the manufacturer's protocol.

First, all reagents were equilibrated to room temperature and homogenized by vortexing. The assay was conducted in 96-well microplates. Each well received 50 µL of dilution buffer 18. Positive control (30 µL) was added to wells A1 and B1, and negative control (30 µL) to wells C1 and D1. The remaining wells were filled with 30 µL of test sera. The microtiter plate was covered and incubated for 2 hours ±10 minutes at 37°C (±3°C). After incubation, sera were removed, and each well was washed five times with approximately 300 µL of washing solution. Subsequently, 100 µL of conjugate 1× was added to each well, followed by incubation for 30 ± 3 minutes at 21°C (±5°C). Wells were then washed five times with 300 µL of washing solution. Next, 100 µL of substrate solution (TMB) was added to each well and incubated for 15 minutes at 21°C (±5°C) in the dark. The reaction was stopped by adding 100 µL of stop solution to each well. Optical density (OD) readings were recorded using a spectrophotometer at 450 nm. The validity of the FMD NSP Competitive ELISA results was confirmed by a validity test. For a valid FMD NSP

c-ELISA result, the mean OD value of the negative control serum was greater than 0.7 (ODNC > 0.700), and the mean value of the positive control optical density (ODPC) was less than 30% of the ODNC (ODPC/ODNC < 0.3). To interpret the results, test sera were expressed as an index, calculated by dividing the absorbance of the tested serum by the OD of the reference negative control. For each sample, the competition percentage (S/N %) was determined. The formula used to calculate seroreactivity is = OD sample / ODNC × 100.

Results

This test distinguishes between samples from infected animals, which possess antibodies against the NSP of the FMD virus, and vaccinated animals, which lack these antibodies.

Serological monitoring of FMDV serotypes

Among the 92 sera tested, 35 (38.04%) were positive for NSP antibodies, indicating prior exposure to the FMD virus through natural infection. Serotype-specific antibodies were most frequently detected against serotype O (25.00%), followed by serotype A (2.17%), and serotype Asia-1 (0%). Co-positivity was observed in 6 sera (6.52%) reactive to both O and A, and in 4 sera (4.34%) positive for O, A, and Asia-1. These results indicate the circulation of multiple FMDV serotypes in the study areas (Table 1).

A district-wise analysis revealed that Sirajganj exhibited the highest seropositivity, with 9 sera (47.4%) testing positive for non-structural protein (NSP) antibodies and 2 samples positive for serotype O-specific antibodies. Four sera demonstrated reactivity to both serotypes O and A, while seven sera tested positive for a combination of serotypes O, A, and Asia-1, suggesting exposure to multiple serotypes. In Bandarban, 19 out of 31 sera (61.29%) were positive for NSP antibodies; 18 (58.06%) were positive for serotype O, 1 (3.2%) for serotype A, and none for Asia-1. Despite limited serotype diversity, the high NSP positivity indicates ongoing viral circulation in this district. Faridpur showed a moderate seropositivity level, with 3 of 16 samples (18.75%) positive for NSP antibodies. Serotype-specific analysis identified one sample positive only for serotype A, and two samples positive for both serotypes O and A, indicating co-infection or exposure to multiple serotypes. In Rajshahi, 3 sera (25%) were positive for NSP antibodies. Of these, two were positive solely for serotype O, while one exhibited reactivity to serotypes O, A, and Asia-1, indicating mixed infections. Jashore district had the lowest seropositivity, with only one sample (7.1%) positive for NSP antibodies, specifically for serotype O, and no reactivity to serotypes A or Asia-1. In summary, these findings indicate that FMDV serotype O is the predominant circulating strain, followed by serotypes A and Asia-1. The serological data support the co-circulation of multiple FMDV serotypes across different regions of Bangladesh.

Table 1: Seroprevalence of foot and mouth disease in different regions (serotypes and antibodies against non-structural proteins)

District	Number of sera tested	Number of sera positive by serotype					
		Asia-1	O	A	O+A	A+O+Asia-1	NSP
Sirajganj	19	0	2	0	4	3	9
Jashore	14	0	1	0	0	0	1
Faridpur	16	0	0	1	2	0	3
Bandarban	31	0	18	1	0	0	19
Rajshahi	12	0	2	0	0	1	3
Total	92	0	23	2	6	4	35
Percentage		0	25	2.17	6.52	4.34	38.04
Chi-square value		NA	28.35	2.41	11.49	9.21	16.87
P-value		NA	0.000	0.660	0.021	0.056	0.002

Abbreviation: NSP, nonstructural protein

Among the age groups, animals older than 24 months exhibited the highest antibody rates, with 33.33% for serotype O and 6.66% for serotype A. The 13-to-24-month group demonstrated moderate positivity rates, whereas the youngest group (6 to 12 months) had the lowest seroprevalence, with 17.3% for serotype O only (see Table 2).

Serotype O was the most prevalent across all age groups, consistent with its predominant circulation in endemic regions. No antibodies specific to serotype A were detected in animals aged 6 to 12 months or 13 to 24 months.

Mixed serotype infections were identified. In the youngest age group (6 to 12 months), neither dual serotype reactivity (O + A) nor triple serotype reactivity (A + O + Asia-1) was observed. In contrast, within the 13 to 24 month group, 6.25% of samples exhibited dual serotype reactivity (O + A), and 3.12% demonstrated triple serotype reactivity. The highest proportion of mixed infections occurred in animals older than 24 months, with 13.33% showing dual reactivity (O + A) and 10.00% exhibiting triple-serotype reactivity. These findings suggest that exposure and cumulative infection risk increase with age.

The detection of antibodies against the non-structural proteins (NSPs) of FMDV indicates ongoing viral circulation among vaccinated cattle. Seropositivity increased with age: NSP-ELISA positivity was 13.33% (4/30) in animals aged 6–12 months, 37.50% (12/32) in the 13–24 months group, and 63.33% (19/30) in animals older than 24 months. The observed increase in NSP antibody detection across age categories likely reflects cumulative exposure to FMDV or repeated infection cycles. NSP-ELISA positivity was marginally higher among females (43.48%; 20/46) than males (32.61%; 15/46) (Table 3). This difference may result from the longer retention of breeding females in herds, which increases their opportunities for exposure during multiple FMD outbreaks.

A total of 50 bovine serum samples were collected to assess the post-vaccination antibody response against Foot-and-Mouth Disease Virus (FMDV). Serotype-specific ELISA tests were conducted to determine antibody levels against FMDV serotypes A, O, and Asia-1. Overall seropositivity rates were 90.0% for serotype A, 92.0% for serotype O, and 76.0% for serotype Asia-1 (Table 4). In Bandarban, 100% seropositivity was observed for all three serotypes, indicating robust vaccine-induced protection. Faridpur also demonstrated strong immune responses, with 100% positivity for serotypes A and O and 80% for Asia-1. In contrast, lower antibody prevalence against Asia-1 was detected in Jashore (40%) and Sirajgonj (70%), which may reflect antigenic variation or limited immune stimulation for this serotype (Table 4). Regional differences in seropositivity were not significant for serotype A (P = 0.340) or serotype O (P = 0.433). However, significant regional heterogeneity was observed for serotype Asia-1 (P = 0.020), with lower seropositivity in Jashore (40%) compared to other districts.

Table 2: Seroprevalence of foot and mouth disease (serotype) according to the age of animals

Age group	No. of samples tested	A positive (n)	A (%)	O positive (n)	O (%)	O + A (n)	O + A (%)	A + O + Asia-1 (n)	A + O + Asia-1 (%)
6–12 months	30	0	0	4	17.39	0	0	0	0
13–24 months	32	0	0	9	28.12	2	6.25	1	3.12
>24 months	30	2	6.66	10	33.33	4	13.33	3	10.00
Overall	**92**	**2**	**2.17**	**23**	**25.00**	**6**	**6.52**	**4**	**4.34**

Table 3: Seroprevalence of foot and mouth disease (antibodies against non-structural proteins), according to age and sex of animals

Variables	Category level	No. of samples tested	NSP-ELISA-Positive	Proportion NSP-ELISA-positive	Chi-square value	P-value
Age	6–12 months	30	4	13.33	8.77	0.012
	13–24 months	32	12	37.50		
	>24 months	30	19	63.33		

Sex	Male	46	15	32.61	0.737	0.390
	Female	46	20	43.48		

Abbreviation: NSP, nonstructural protein.

Table 4: Post-vaccination antibody response to different serotypes against the foot-and-mouth disease virus

Region	Number of sera tested	Number of sera positive by serotype			NSP-ELISA Positive
		A	O	Asia-1	
Bandarban	10	10	10	10	0
Jashore	10	08	09	04	0
Faridpur	10	10	10	08	0
Rajshahi	10	08	08	09	0
Sirajgonj	10	09	09	07	0
Total	50	45	46	38	0
Percentage	100	90.00	92.00	76.00	0
Chi-square value	-	4.44	3.80	11.62	-

These findings indicate satisfactory immune responses against FMDV serotypes A and O, suggesting effective vaccine coverage. However, the comparatively lower antibody response against serotype Asia-1 underscores the need for ongoing surveillance, vaccine potency testing, and consideration of booster immunization or antigenic matching to maintain herd immunity across all circulating FMDV serotypes in Bangladesh.

High seropositivity against foot-and-mouth disease virus (FMDV) serotypes A, O, and Asia-1 was observed across all age groups of vaccinated cattle. Among animals aged 6 to 12 months, seropositivity rates were 93.75% for both serotypes A and O, and 81.25% for Asia-1. In the 13 to 24 months age group, seropositivity was 87.50% for serotype A, 93.75% for serotype O, and 75.00% for Asia-1. For cattle older than 24 months, antibodies were detected in 88.88% of samples for serotypes A and O, while serotype Asia-1 exhibited a lower seropositivity rate of 72.22% (Table 5). Serotype O consistently demonstrated high antibody levels across all age groups. Comparable patterns were observed between sexes. In male cattle, seropositivity was 88.88% for serotypes A and O, and 72.00% for Asia-1. Female cattle exhibited slightly higher seropositivity for serotype A at 92.00% and for Asia-1 at 80.00%, while serotype O remained at 88.88%. These results indicate robust post-vaccination immune responses against serotypes A and O across age and sex, whereas serotype Asia-1 elicited comparatively lower antibody responses.

Table 5: Post-vaccination antibody response against foot-and-mouth disease according to age and sex of animals

group		No. of samples tested	A Pos	Proportion A pos	O Pos	Proportion O pos	Asia-1 Pos	Proportion Asia-1 pos
Age	6–12 months	16	15	93.75	15	93.75	13	81.25
	13–24 months	16	14	87.5	15	93.75	12	75
	>24 months	18	16	88.88	16	88.88	13	72.22
Sex	Male	25	22	88.88	23	88.88	18	72
	Female	25	23	92.00	23	88.88	20	80

Discussion

Foot-and-mouth disease virus (FMDV) comprises three serotypes that are distributed globally and frequently cause epidemics in Asia, complicating disease control efforts (Li et al., 2023; Rodriguez et al., 2017). Serotype O is the predominant strain in Asia (Knowles et al., 2005; Zhu et al., 2019), leading to substantial and

persistent economic losses in export markets (Yadav et al., 2022). Despite exhibiting limited antigenic variability, the widespread distribution of FMDV type O presents significant challenges for eradication (Yang et al., 2020; Kitching, 2005). Multiple epidemiological studies have documented a high prevalence of FMD in Bangladesh.

The overall non-structural protein (NSP) seroprevalence identified in this study was 38.04%, indicating prior natural exposure of cattle to FMDV in the surveyed regions. This prevalence is higher than those reported in Bhutan (17.6%) (Dukpa et al., 2011), Western Uttar Pradesh, India (29.0%) (Singh et al., 2020), Ethiopia (25.3%) (Batu et al., 2025), Eritrea (26.0%) (Tekleghiorghis et al., 2017), the Haor areas of Bangladesh (24.71%) (Chowdhury et al., 2020), and Meghna Upazila (24.51%) (Mannan et al., 2009), but lower than the 54.7% reported by (Hossain et al., 2023) and 56.68% reported by (Jahan et al., 2024). These differences are likely attributable to variations in geographical location, management practices, vaccination coverage, and disease epidemiology.

Variation in NSP seroprevalence was evident at the district level. In Rajshahi, the observed seroprevalence (25.0%) was nearly identical to the 25.07% reported by (Mannan et al., 2009). Similarly, the prevalence in Sirajganj (47.4%) was comparable to the 48.0% reported by (Giasuddin et al., 2017). This study confirmed the circulation of FMD virus serotypes A, O, and Asia-1 in Bangladesh. These findings align with previous reports identifying these three serotypes as the predominant circulating strains in the country (Nandi et al., 2015; Siddique et al., 2018; Hossain et al., 2023). Prior research has consistently shown that serotype O is the dominant serotype in Bangladesh, followed by serotypes A and Asia-1. Specifically, serotype O accounted for 62.2% of circulating strains (Nandi et al., 2015), 82% of outbreaks (Siddique et al., 2018), and 85% of outbreaks reported during 2012–2021 (Hossain et al., 2023). (Giasuddin et al., 2016) also reported that serotypes O and Asia-1 predominated over serotype A. The detection of co-infections in this study is consistent with previous reports by (Islam et al., 2017) and (Islam et al., 2021), which documented co-infections involving FMDV serotypes O, A, and Asia-1. These results indicate the continued co-circulation of multiple serotypes in Bangladesh.

NSP seropositivity was significantly higher in female cattle (43.48%) than in male cattle (32.61%). This observation is consistent with findings by (Akter et al., 2024) and (Jahan et al., 2024), who also reported increased FMD prevalence among females. The higher prevalence in female cattle may be due to physiological stress associated with pregnancy, lactation, and reproductive activity, which can increase susceptibility to infection.

NSP seropositivity increased markedly with age, rising from 13.33% in young cattle to 63.33% in animals older than two years. This trend is consistent with studies indicating that older cattle experience greater cumulative exposure to FMDV in endemic regions (Fakhrul-Islam et al., 2016; Chowdhury et al., 2020; Singh et al., 2020; Chepkwony et al., 2021). (Akter et al., 2024) reported similar age-related patterns, while (Jahan et al., 2024) observed a higher incidence in younger cattle, suggesting that age-related susceptibility may vary depending on local epidemiological and management factors. Antibodies against NSPs have been shown to persist in hosts for extended periods, sometimes up to three years (Elnekave et al., 2015). Seropositivity was observed following trivalent vaccination against FMDV serotypes A and O across all study regions, with consistently high seropositivity and no significant regional differences. This outcome suggests effective vaccine performance in community settings. In contrast, serotype Asia-1 exhibited lower seropositivity (76.0%) and significant regional variation ($p = 0.020$), potentially reflecting uneven immune responses due to differences in viral characteristics, vaccine quality, or field-level administration practices. No NSP-positive sera were detected among vaccinated animals, supporting the DIVA (Differentiating Infected from Vaccinated Animals) principle and confirming that the antibodies were a result of vaccination. Overall, the findings indicate robust protective immunity against serotypes A and O, while immunity against Asia-1 remains weaker and less consistent. Comparisons by age and sex revealed generally high seropositivity across all groups, with no significant sex-based differences, suggesting minimal influence of sex on vaccine-induced antibody development in field conditions.

Study limitations

Several limitations should be considered when interpreting these findings. Serum samples were collected at the community level without longitudinal follow-up of individual animals, precluding repeated measurements over time. As a result, the study offers a cross-sectional view of post-vaccination antibody status rather than insights into long-term immune dynamics. The relatively small number of post-vaccination sera (n = 50) compared with pre-vaccination sera (n = 92) may have reduced the statistical power of subgroup analyses. Additionally, the absence of paired pre- and post-vaccination samples from the same animals prevented assessment of individual-level changes in antibody responses. Only serological responses were evaluated, with no virological or molecular characterization to confirm circulating FMDV strains or assess vaccine–field strain compatibility. Furthermore, information on potential confounding factors, such as vaccine handling, cold-chain maintenance, and farm management practices, was not systematically collected, although these factors may influence immune response.

Conclusions

Foot-and-mouth disease continues to impede livestock development in Bangladesh, causing significant economic losses due to calf mortality, reduced milk yield, and poor weight gain in beef cattle. In the absence of specific antiviral treatments, vaccination remains the primary strategy for disease prevention and control. Serological evidence confirmed the predominance and recent circulation of FMDV serotype O in the study area, with lower detection rates for serotype A and occasional presence of serotype Asia-1. The locally available trivalent vaccine achieved over 90% seropositivity against serotypes A and O, highlighting its potential utility in regional control programs. However, definitive evaluation of vaccine efficacy was constrained by the absence of clinical sample collection and vaccine-matching analyses.

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Conflict of interest.There is no conflict of Interest regarding this research and manuscript.

Author's contributions

This work was completed in collaboration among all authors. Razia Khatun and Md. Ashraful Islam conceptualized and designed the study. Md. Ashraful Islam and Syidul Islam implemented the experiment in the community of cattle farmers. Md. Ashraful Islam wrote the research methodology, study protocol, and the manuscript. Syidul Islam, Bijoy Barua, Md. Abu Haris Miah, Sukumar Roy, Md. Tarikul Islam assisted in the data collection and formal analysis. Md. Ashraful Islam and Syidul Islam reviewed and edited the manuscript for final submission. Razia Khatun provided guidelines for writing the manuscript and financial support for the research. All Authors read and agreed to the final version of the manuscript.

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Availability of data and materials. The data are available upon request from the corresponding author.

Ethical considerations

All authors have reviewed and agreed on ethical issues, including fabrication of data, double publication and submission, redundancy, plagiarism, consent to publication, and misconduct, for this article to be published with a high scientific quality in the present journal.

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