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Detection of antibodies against structural proteins of foot-and-mouth disease virus in bovines of western Uttar Pradesh, India

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Abstract

Foot-and-mouth disease (FMD) is considered as one of the most important contagious viral diseases affecting cloven-footed animals. For effective control of FMD, immunization along with herd immunity is essential in the field conditions. To assure and track the coverage and effectiveness of the vaccination program, the serological studies are very much required after the vaccination program. The present study was aimed to investigate the prevalence of antibodies against structural proteins of FMD virus (FMDV) serotypes of O, A and Asia-1 in seven districts of western Uttar Pradesh, India, and assure the efficacy of vaccination under National Animal Disease Control Program. A total of 308 sera samples were collected from apparent healthy vaccinated cattle and buffaloes from seven districts including Amroha, Baghpat, Bareilly, Bulandsahar, Gautam Budh Nagar, Meerut and Muzaffarnagar of western Uttar Pradesh, India. Determination of antibodies against structural proteins of FMDV was carried out using solid-phase blocking enzyme-linked immunosorbent assay. The protective level of the FMDV serotypes O, A and Asia-1 included in the inactivated trivalent vaccine was 66.55, 48.05 and 47.08% in bovines, respectively. To provide the higher level of protection against the circulating FMDV, the present study recommended the thorough investigation of the immunogenic interaction between the vaccine strains and the field strains. Further investigations should also be conducted with larger sample size and across diverse geographical regions to gain a more comprehensive understanding of herd immunity.

Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting a wide range of animals, including domesticated ruminants, pigs, camelids, and over 70 wild-life species, including elephants.[1,2] It has a transboundary nature, posing a significant threat to global food security and inflicting substantial economic losses on livestock farmers and the industry.[3] The causative agent behind FMD is the FMD virus (FMDV), a member of the Aphtho-virus genus within the Picornaviridae family.[4] The virus is classified into seven immunologically distinct serotypes including A, O, C, Southern African territories 1, 2, 3, Asia-1, and each serotype comprises numerous strains exhibiting varying degrees of genetic and antigenic diversities.[5]

Clinical manifestations of FMD include fever, lameness, and development of vesicular lesions on the mouth, tongue, feet, snout and teats of infected animals.[2,3] The morbidity of disease is high, while mortality is low except in young animals due to myocardial complications.[2] The FMD is endemic in India and for prevention of the disease, all the cattle and buffaloes are vaccinated with inactivated trivalent vaccine containing FMD serotypes of O, A and Asia-1 under National Animal Disease Control Program. The protection levels against FMDV in the animals are detected by estimating the antibodies level against structural proteins of the virus in sera samples.[2,4,5]

World Organization for Animal Health recommends three serological tests including virus neutralization test (VNT), liquid-phase blocking enzyme-linked immuno-sorbent assay (LPBE) and solid-phase competitive enzyme-linked immunosorbent assay (SPCE) for detecting antibodies against the structural proteins of FMDV. The VNT is accurate, but requires intensive labor, sensitive cell

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lines, live FMDV, and good laboratory facility. In India, an indigenously developed LPBE had been extensively used to monitor post-vaccination antibody responses.[6-9] However, LPBE has faced criticism primarily concerning the specificity and variable stability of the inactivated antigen utilized in the test.[10] The SPCE has been found to offer higher specificity than LPBE; therefore, LPBE has been replaced with SPCE as a more specific and reliable serological assay for detecting antibodies against FMDV in India.[10-12]

The present study aimed to assess the antibody levels against structural proteins of FMDV serotypes of O, A and Asia-1 in cattle and buffaloes of seven districts of western Uttar Pradesh, India, and evaluate the vaccine protection levels in the livestock population.

Materials and Methods

Study area and period of study. Seven districts including Amroha, Baghpat, Bareilly, Bulandsahar, Gautam Budh Nagar, Meerut and Muzaffarnagar of western Uttar Pradesh, India, were included in the study during the period of 2020 - 2022.

Study design and sample collection. A total of 308 blood samples without anti-coagulant were collected from apparently healthy vaccinated cattle and buffalo (Ethical approval No. IAEC/SVPUAT/2022/85 dated 16.03.2022). Briefly, the blood samples were collected from jugular vein in vacuum blood collection tubes with a clot activator. After centrifugation of blood collection tubes at 3,000 rpm for 10 min, clear sera were collected and stored at – 20.00 ˚C till further testing. Livestock (cattle and buffalo) were vaccinated by Animal Husbandry Department, Uttar Pradesh, India, using oil-adjuvant trivalent vaccine containing O, A and Asia-1serotypes under National Animal Disease Control Program.

Laboratory analyses. The present study employed the SPCE to evaluate anti-FMDV structural antibodies in bovine sera samples collected from seven districts of western Uttar Pradesh, India, according the Project Directorate on Foot-and-Mouth disease, IVRI Campus Mukteshwar, Uttarakhand, Indial. Briefly, 96-well immunoplates (Nunc Maxisorb; Thermo Fisher scientific, Waltham, USA) were coated with 50.00 µL *per* well of an optimal dilution of rabbit anti-FMDV serum specific for each serotype of O, A and Asia-1 in coating buffer (0.06 Mcarbonate/bicarbonate buffer; pH: 9.60) overnight at 4.00 ˚C (Table 1). The immunoplates were then washed three times using 0.01 M phosphate buffered saline (PBS) with 0.10% Tween-20 detergent (PBST; pH: 7.20) followed by the addition of 50.00 µL *per* well of antigen for each FMDV serotype diluted in PBS incubated at 37.00 ˚C for 1 hr. After that, the immunoplates were again washed three times with PBST. Duplicate wells of 50.00 µL *per* well of each serum sample (diluted 1:5 in blocking buffer) or blocking buffer alone (diluent controls) were added to specific wells, immediately followed by the addition of 50.00 µL *per* well of guinea pig anti-FMDV serum diluted in blocking buffer to all wells. Immunoplates were incubated at 37.00 ˚C for 1 hr and washed three times with PBST as above, followed by the addition of 100 µL *per* well of rabbit anti-guinea pig immunoglobulin conjugated to horseradish peroxidase diluted (1:16,000) in blocking buffer. Immunoplates were again incubated at 37.00 ˚C for 1 hr. After washing three times with PBST, 50.00 µL of substrate solution, containing o-phenylenediamine hydrochloride and H_2O_2, was added into each well and incubated for 15 min at 37.00 ˚C. The reaction was stopped by adding 50.00 µL of 1.00M Sulfuric acid and the absorbance was measured at 492 nm using a spectrophotometer (Merilyer EIAQuant, Vapi, India), with reference measurements taken at 620 nm.

Table 1. Immunoplates diagram showing the positioning.

Well positions												Dilutions
Ag	Ag	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	1:32
Ag	Ag	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	1:64
Ag	Ag	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	1:128
BG	BG	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	1:256
S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	1:32
S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	1:64
S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	1:128
S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	1:256

Ag: Antigen; BG: Background; S: Sample.

Interpretation. The result for each sample was expressed as a percent inhibition. The mean optical density at 492 nm (OD492) values of all samples were converted into percentages of inhibition relative to the OD492 value of the antigen control. Antibody titers were expressed as a reciprocal of the final dilution at which the test serum sample's reaction yielded an OD value corresponding to 35.00% inhibition of the median absorbance recorded in the antigen control wells.

Percentage of inhibition. If the 35.00% inhibition value falls between two dilutions, the arithmetic mean of the $\log_{10}$ values of the two surrounding dilutions may be considered as a titer. For instance, if the 35.00% inhibition point is between $\log_{10}$ titers of 1.80 and 2.10, the mean of these two dilutions (1.95) can be accepted as an antibody titer. Although precise endpoint determination might require software like Sigma Plot for linear regression analysis and interpolation, the approximate endpoint titer calculation presented here wouldn't significantly impact the overall interpretation of results. This is because a protective titer threshold of 1.65 and above is recognized, and a herd-level serological monitoring approach is practiced.

Threshold for interpretation of protective antibody level. Protective antibody level is denoted as a $\log_{10}$ titer, with an antibody titer (> 35.00% inhibition) of ≥ 1.65 considered protective.

Results

An analysis was conducted on 308 serum samples from bovines across seven districts (Amroha, Baghpat, Bareilly, Bulandsahar, Gautam Budh Nagar, Meerut and Muzaffarnagar) in Uttar Pradesh, India. Solid-phase blocking ELISA was employed to assess the presence of antibodies against FMDV serotypes of O, A and Asia-1. Of the 308 samples, 205 (66.55%), 148 (48.05%) and 145 (47.08%) samples exhibited protective antibody titers (ELISA titers ≥ log 1.65) against FMDV serotypes of O, A and Asia-1, respectively (Table 2).

Discussion

India is endemic to FMDV serotypes such as O, A and Asia-1, resulting into significant burden to the farmers as direct and indirect losses due to outbreaks of FMD.[2] The disease is a serious threat to millions of bovine populations around the globe as well as trade of livestock and their products. Despite the vaccination, severe yearly outbreaks of FMD have been recorded in different areas of the country and there is evidence of sustained FMDV circulation in vaccinated cattle and buffalo populations.[2,3] Among dairy animals, cattle and buffaloes are considered at risk of FMD. The present work was carried out in seven districts of western Uttar Pradesh, India, during the period of 2020 - 2022 to assess the antibody titers against structural proteins of FMDV in cattle and buffaloes. The results indicated that 66.55%, 48.05%, and 47.08% of cattle and buffaloes showed protective antibody levels against FMDV serotypes of O, A and Asia-1, respectively. The FMD control program was initiated in 2004 across selected districts with the aim of establishing FMD-free zones. Presently, the National Animal Disease Control Program encompasses all districts in Uttar Pradesh, India, involving biannual vaccination against FMD for bovines. In the present study, a significant proportion of animals exhibited protective antibody titers (≥ 1.80 $\log_{10}$) against FMD serotype of O (66.55%), followed by serotype of A (48.05%) and serotype of Asia-1 (47.08%). This outcome could be attributed to the prevalent occurrence of FMD serotype of O in the country, as observed in prior studies.[9,13-16] Natural infections of O serotype within the susceptible bovine population likely contributed to the increased antibody titers against this specific serotype. This phenomenon has been noted in both the current study and earlier research highlighting the immunogenic nature of FMDV serotype of O compared to the A and Asia-1serotypes.[5,9] Another potential reason could be the higher immunogenicity of FMDV serotype of O or a greater quantity of O serotype antigen in the vaccine. The occurrence of outbreaks in districts like Meerut, Muzaffarnagar, and Bulandsahar in western Uttar Pradesh, India, could stem from factors such as sub-optimal vaccination coverage (< 60.00%) during various phases of FMD vaccination or movement of animals between these districts and neighboring states such as Punjab, Haryana and Rajasthan. Despite multiple phases of vaccination, the bovine population in the study area remained vulnerable to infection by any of the circulating FMDV serotypes in the country. Without implementing stringent biosecurity measures to control the animal's movement, there is a heightened risk of a large-scale outbreak occurring in the near future. Such an outbreak could have severe economic consequences for farmers.[16]

Effective control of FMD hinges on achieving vaccination coverage of at least 85.00%, if not 100%, within the susceptible livestock population. This approach is necessary to prevent the establishment and persistence of FMDV within a herd.[17-19] The feasibility of implementing a test-and-slaughter policy is limited in India, rendering mass immunization of susceptible livestock the most practical means of disease control.[2,3] The successful FMD vaccination campaign in Haryana, India, achieving over 80.00% herd immunity and a mere 2.00% anti-NSP antibody response, stands as an exemplary model for FMD-free status.[16,20] By implementing the FMD vaccine program thoroughly and inclusively, such as through the National Animal Disease Control Program, Uttar Pradesh, India, could work toward eradicating clinical cases of FMD and mirroring the successful story in Haryana, India.

The current study highlighted the improvement in the application of vaccination program in the study area. There is a requirement of further studies to ascertain the suitability of the existing O and A serotypes vaccines to protect from the currently circulating viruses, and encompass virus isolation, serotyping and molecular characterization of the FMDV across a broader scope.

Table 2. Animals showing protective antibody titers against different serotypes of foot-and-mouth disease virus.

Sample No.	District	Total samples tested	Samples showing antibody titer ≥ 1.80 against:		
			Type O	Type A	Type Asia-1
1	Amroha	3	02	01	00
2	Baghpat	64	38	23	26
3	Bareilly	7	03	01	02
4	Bulandsahar	6	01	02	01
5	Gautam Budh Nagar	49	30	20	21
6	Meerut	142	106	82	79
7	Muzaffarnagar	37	25	19	16
Total		**308**	**205 (66.55%)**	**148 (48.05%)**	**145 (47.08%)**

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Conflicts of interest

The authors declare no conflict of interest.

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