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Epidemiological studies on Foot-and-Mouth disease in western Uttar Pradesh, India

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ABSTRACT

Foot-and-mouth disease (FMD), a highly contagious viral infection of cloven-hoofed animals, has a significant financial impact on the dairy industry. Despite the disease being endemic in India, little information is available on the current status of FMD in Uttar Pradesh. The present cross-sectional study was conducted in selected districts of western Uttar Pradesh to determine the seroprevalence, associated risk factors, circulating virus serotypes, and molecular characterisation. Six hundred bovine serum samples were collected using a random sampling approach, while five epithelial tissues were purposively collected from affected animals during FMD outbreaks for the identification and molecular characterisation of FMDV serotypes. Serum samples were processed for antibody detection against non-structural proteins of FMDV using recombinant 3AB3 NSP-ELISA. FMDV serotypes were determined using sandwich ELISA and reverse transcription PCR (RT-PCR). Associated risk factors were determined using logistic regression. The overall prevalence of antibodies against FMDV was 14.00 percent (84/600). Animal species and FMD seroprevalence were significantly correlated. Only FMDV serotype O was detected in all epithelial tissue samples after serotyping and molecular analysis. Effective FMD control requires identification of circulating serotypes and associated risk factors, along with the inclusion of multiple animal species in surveillance. Mass vaccination tailored to serotypes, farmer awareness, stricter movement control, improved quarantine, and robust surveillance are vital. Future research should assess vaccine efficacy, outbreak dynamics, and economic impacts to support sustainable control in endemic regions.

Keywords: Buffalo, Cattle, FMD virus, Molecular characterization, Serotype O, Sero-prevalence

Livestock diseases are responsible for an annual decrease in productivity of the dairy industry (Singh *et al.* 2013, Kappes *et al.* 2023). Among these, Foot-and-mouth disease (FMD) is a highly transmissible viral infection of cloven-hoofed animals that leads to losses in livestock productivity and trade embargoes (Verma *et al.* 2012, 2014, Chakraborty *et al.* 2014, Afroz *et al.* 2024). The FMD virus (FMDV) belongs to genus *Aphthovirus*, a member of the *Picornaviridae* family, and has seven serotypes: O, A, C, and South African territories (SAT)-1, SAT-2, and SAT-3 (Verma *et al.* 2012, Jamal and Belsham 2013). Clinically, the disease is characterized by pyrexia, inappetence, salivation, lameness, and vesicular epithelial lesions in the buccal cavity, dental pad, tongue, foot, and udder (Chakraborty *et al.* 2014). Direct interaction with infected animals or indirect contact with the environment, including clothing, shoes, vehicles, and veterinary instruments contaminated with the secretions and excretions of affected animals, can spread the FMDV (Auty *et al.* 2019). Additionally, the unrestricted movement of livestock and their products across international borders is responsible for the propagation of the disease (Verma *et al.* 2012).

With the largest bovine population on the planet, India is regarded as both a producer and consumer of dairy products (Hemme *et al.* 2003, Kumar *et al.* 2012); however, FMD outbreaks lead to economic losses of up to 200 billion INR per year (Govindaraj *et al.* 2021). The disease is still widespread in Uttar Pradesh, causing outbreaks in bovine populations, even after the Government of India mass-immunised the bovine population against FMD through the FMD Control Program (FMDCP), Assistance to States for Control of Animal Disease (ASCAD), and National Animal Disease Control Program (NADCP) in 2019 (now called Livestock Health and Disease Control Program (LHDCP)) (Verma *et al.* 2008, Gunasekera *et al.* 2022, Subramaniam *et al.* 2022). The possible reasons for the difficulty in controlling FMDV are inadequate disease surveillance, inadequate veterinary staff and infrastructure, practical difficulty in implementing disease control programs, low levels of biosecurity, poor outbreak reporting systems, and limited knowledge of farmers (Verma *et al.* 2008, 2012). Several FMD outbreaks

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have been reported in various areas of the nation, and many studies on seroprevalence and associated risk factors have been conducted; however, the western parts of Uttar Pradesh lack knowledge on such aspects. Furthermore, identification of the circulating FMDV serotype in the study area will help formulate control strategies and thus reduce economic losses to the dairy industry in the region. Therefore, the present study was conducted to determine the seroprevalence of FMD, identify FMDV serotypes, and perform molecular characterization in selected districts of western Uttar Pradesh, India.

MATERIALS AND METHODS

Area of study, livestock and sampling: This study included 600 bovine serum samples from selected districts viz. Baghpat, Meerut, Bareilly, Bijnor, Hapur, Shamli, and Muzaffarnagar in Uttar Pradesh state from 2022 to 2023 to determine the FMDV seroprevalence and risk factors involved. The selection of these districts was based on transportation accessibility, rapid laboratory availability, a high number of livestock exchanges with adjoining states, and historical incidence of disease. The study included both (male and female) bovines (cattle and buffalo) of all breeds and ages viz. calves (<3 years), adults (3–7 years), and old (>7 years). Furthermore, bovines showing overt clinical signs of FMD, such as vesicle lesions on dental pads, tongue, and/or foot from the Meerut and Gautam Budh Nagar districts, were also included for serotype detection and its molecular characterization during outbreaks. Five cattle vesicular lesions during the epidemic in villages Bisola, Meerut, and village Kudi Khera, Gautham Budh Nagar, Uttar Pradesh from January to March 2024 were also collected for serotype identification and molecular characterization of FMDV.

Collection of blood samples: For the determination of antibodies prevalent against FMDV, approximately 03 mL of blood was collected aseptically from jugular vein puncture in blood collecting vials (BD, USA). The clotted blood was transported to the laboratory for serum separation and the sera were kept in sterilized cryovials at -20°C till further testing.

Detection of antibodies against the nonstructural protein FMDV by ELISA: The collected serum samples were processed for the detection of antibodies against the nonstructural protein (NSP) of FMDV using the indirect ELISA (r3AB3 DIVA kit) developed by the Project Directorate on Foot and Mouth Disease (PDFMD), ICAR-Indian Veterinary Research Institute (IVRI), Mukteswar campus, India (Mohapatra *et al.* 2011).

Serotype identification and molecular detection of FMDV: To identify the FMDV serotypes and their molecular characterization, vesicular lesions were collected from active clinical cases of FMD in bovines showing clinical signs, such as pyrexia, smacking of lips, salivation, lameness, and vesicular eruptions on the dental pad, tongue, and foot. Approximately 1–2 g of epithelial tissue was obtained from the tongue and dental pad of the affected animals and stored in 50% phosphate buffered saline and glycerin at -20°C for further analysis. Vesicular epithelial tissues were tested for FMDV serotyping using sandwich ELISA (Bhattacharya *et al.* 1996).

Molecular detection of FMDV: The presence of FMD viral genetic material was tested in all vesicular epithelial tissue samples (Giridharan *et al.* 2005). Total RNA from vesicular epithelial tissues was isolated by the use of the NucleoSpin RNA Plus kit (MN, Germany) based on the manufacturer's guidelines. OligoDT primers and the PrimeScriptTM 1st strand cDNA Synthesis Kit were used to create cDNA in a thermal cycler (Takara, Japan) at 42°C for 45 min. Thereafter, an additional incubation to inactivate the enzyme was performed at 95°C for 5 min. The cDNA derived from FMDV RNA reverse transcription was amplified by PCR with the use of Emerald Amp GT PCR Master Mix (Takara, Japan) and 10 pmol each of forward primer Serotype O (DHP13-GTGACTGAACTGCTTTACCGCAT), Serotype A (DHP15- CAACGGGACGARCAAGTACTC) and Asia-1 (DHP9-GACCTGGAGGTYGCGCTTGT) and reverse primer NK61 (GACATGTCCTCCTGCATCTG) (Giridharan *et al.* 2005). The 1.5% agarose gel electrophoresis was used to visualize the amplified PCR products.

Data management and statistical analysis: A Microsoft Excel spreadsheet was used to enter the raw data. The seroprevalence was calculated by dividing the number of seropositive samples by the total number of samples examined, then multiplying the result by 100. Both univariate and multivariate logistic regression analyses were conducted using SPSS® statistical software. The threshold for statistical significance was $p<0.05$.

RESULTS AND DISCUSSION

Overall seroprevalence of FMD: Antibodies against the non-structural protein of the Foot-and-Mouth Disease Virus (FMDV) were found in 14.00% (84 out of 600) of the serum samples. Notably, seroprevalence was highest in Shamli district compared to other districts (Table 1).

Analysis of FMD risk factors: Risk factor analysis revealed that male, adult, crossbred cattle exhibited higher FMD seroprevalence compared to other animals (Table 1). The relationship between FMD seropositivity and intrinsic risk factors such as species, breed, and age was explored using univariate logistic regressions. FMD seroprevalence was found to be significantly associated with species, with cattle exhibiting a higher seroprevalence (17.02%) than buffaloes (15.49%). Specifically, cattle had an almost threefold higher risk of FMDV infection (95% CI, 1.540–5.775; $p= 0.001$) (Table 2).

FMDV serotype identification: During the FMD outbreaks in village Bisola, district Meerut and village Kudi khera, district Gautam Budh Nagar, Uttar Pradesh, antigen detection sandwich ELISA was performed on five vesicular epithelial tissue samples to determine the serotypes of FMDV. All 05 vesicular epithelial tissues were identified as FMDV serotype O.

Table 1. Univariate logistic regression analysis for identification of associated risk factors with bovine seropositivity for Foot-and-Mouth Disease (FMD)

Risk factor	Category	Number of animals positive/ Total animals (%)	ß	SE	Odd ratio	95% CI		*p*-value
						Lower	Upper	
District#	Baghpat	9/63 (14.29)	-0.84	0.077	0.0920	0.790	1.070	0.279
	Meerut	59/381 (15.48)						
	Bareilly	0/8 (0.00)						
	Hapur	2/37 (5.40)						
	Shamli	3/11 (27.27)						
	Muzaffarnagar	11/88 (12.50)						
	Bijnor	0/12 (0.00)						
Species#	Cattle	73/429 (17.02)	1.012	0.407	2.750	1.239	6.103	0.013
	Buffalo	11/171 (15.49)						
Breed	Murrah	11/159 (6.92)	0.49	0.185	1.050	0.730	1.510	0.792
	Crossbred	59/359 (22.78)						
	ND	4/29 (13.79)						
	HF	10/53 (18.87)						
Sex#	Male	05/35 (14.29)	0.085	0.517	1.088	0.395	3.000	0.870
	Female	79/565 (13.98)						
Age	Calf (< 2 years)	02/31 (6.45)	0.171	0.261	1.186	0.711	1.978	0.514
	Adult (3 – 7 years)	72/493 (14.60)						
	Old (> 7 years)	10/76 (13.16)						
	Total	84/600 (14.00)						

Table 2. Multivariate logistic regression analysis for identification of associated risk factors with bovine seropositivity for Foot-and-Mouth Disease (FMD)

Risk factor	ß	SE	Odd ratio	95% CI		*p*-value
				Lower	Upper	
Constant	-3.770	0.637				
Species	1.093	0.337	2.983	1.540	5.775	0.001

Molecular detection and characterization of FMDV: In the current study, all five tissue samples from cattle were subjected to molecular confirmation with the use of reverse transcription polymerase chain reaction (RT-PCR) assay. All samples yielded amplicons of 249 bp, confirming the presence of FMDV serotype O (Fig. 1).

The present study revealed that five of the seven districts surveyed showed FMD seropositivity. In bovines, the overall sero-prevalence of FMD was 14.0 percent, indicating that the FMDV was endemic in the study area. Antibodies against FMDV's non-structural proteins were found in the serum samples of bovines, indicating that the animals had been previously exposed to the virus (OIE 2019). The findings of this study are consistent with previous reports: 27.75% in some districts of Uttar Pradesh using the r3AB3 non-structural protein (NSP) ELISA (Singh *et al.* 2020), 23.7% in Haryana using the 2c NSP ELISA (Mahajan *et al.* 2013), and 27% in India (Anon 2015). In contrast, previous studies conducted in various regions of India have reported a lower seroprevalence of FMD, specifically 7-9% in the Andaman and Nicobar Islands (Sunder *et al.* 2015). The geographic location of the region and variations in the distribution of livestock, management techniques, and unrestricted animal movement may be the possible reasons for discrepancies in the frequency of FMD observed in the current study (Verma *et al.* 2010, Ali *et al.* 2022).

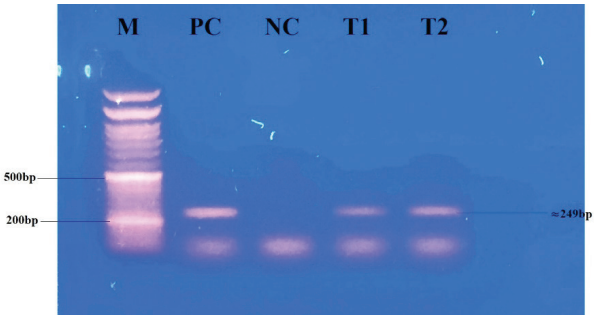

Fig. 1. Reverse transcription polymerase chain reaction test to detect of serotype 'O' of FMDV

Regions with dense livestock populations or mixed farming systems often provide favourable conditions for viral transmission, whereas differences in husbandry practices, such as communal grazing, inadequate biosecurity, and lack of routine vaccination, can further amplify the spread of infection (Batu *et al.* 2025). Similarly, the unrestricted movement of animals across districts or even international borders facilitates the introduction and reintroduction of new viral strains, thereby sustaining endemicity. Furthermore, the presence of various serotypes

and FMDV subtypes complicates disease control, as immunity developed against one serotype often does not confer protection against another, making vaccinated herds susceptible to outbreaks despite prior immunization efforts (Paton *et al.* 2005). This antigenic diversity poses significant challenges for cross-protection and underscores the need for region-specific vaccination strategies. The findings of the current study suggest that cattle are nearly three times more (95% CI 1.540–5.775; p= 0.001) likely to be infected with FMDV than buffaloes. This aligns with a previous study by Hegde *et al*. (2014), who found that the main indicator species for FMD epidemiology in Karnataka, India were cattle. Additionally, Rout *et al.* (2016) found that 51.46% of cattle bulls and 37.84% of buffalo bulls tested positive for NSP-Ab using 3AB3 NSP ELISA, indicating exposure to FMDV. These results underscore the importance of cattle in the transmission and prevalence of FMDV and highlight the need for targeted preventive and control measures in livestock populations.

FMD is widespread in India, with three main FMDV serotypes, O, A, and Asia-1, circulating throughout the country (Verma *et al.* 2014). Previous studies have used molecular and serological techniques to identify FMDV serotypes O, A, C, and Asia-1 during FMD epidemics in India (Pattnaik *et al.* 2012, Verma *et al.* 2008, 2012, Dahiya *et al.* 2021). Furthermore, molecular characterization of FMDV and sandwich ELISA revealed that FMDV serotype O was responsible for the disease epidemics in Meerut and Gautam Budh Nagar districts of Uttar Pradesh. These results are consistent with those of earlier studies that demonstrated FMDV serotype O to be the most common FMDV serotype (Verma *et al.* 2012, 2017). Serotype O of FMDV has been found to be the most common serotype causing outbreaks in India's surrounding nations, including Pakistan (Jamal *et al.* 2021, Ijaz *et al.* 2022), Afghanistan (Wajid *et al.* 2020), Bhutan (Dukpa *et al.* 2011), Nepal (Adhikari *et al.* 2018), Bangladesh (Rahman *et al.* 2020, Hossain *et al.* 2023), and China (Ren *et al.* 2021).

The present study demonstrated that Foot-and-Mouth Disease Virus (FMDV) is endemic in the bovine population of western Uttar Pradesh. Clinical signs, serological analyses, and molecular characterization confirmed that serotype O was the predominant circulating strain responsible for the outbreaks in the region. Multivariate logistic regression further revealed a strong association between FMD seropositivity and risk variables, such as animal species, highlighting that cattle are at a greater risk than buffaloes. In light of these findings, it is imperative for government authorities to prioritize comprehensive mass vaccination programs tailored to circulating serotypes, coupled with awareness campaigns to educate farmers about preventive strategies. In addition, enforcing livestock movement restrictions, strengthening quarantine facilities, and improving regional surveillance systems are essential for curbing the spread of the disease. Future research should focus on assessing vaccine efficacy against prevalent serotypes in the region, conducting longitudinal studies to better understand the dynamics of outbreaks, and evaluating the economic impact of FMD on livestock productivity and farmers' livelihoods. These steps will not only strengthen disease control policies but also contribute to the development of sustainable strategies for FMD management in endemic conditions.

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