

Sero-surveillance of Foot and Mouth Disease (FMD) Virus Non-Structural Protein (NSP) Antibodies in the Bovines of Western Uttar Pradesh

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Abstract

Foot and mouth disease (FMD), caused by aphthovirus of picornaviridae family, is a highly contagious disease leading to severe economic losses to dairy industries in the affected countries. A cross sectional study was conducted to estimate the prevalence of circulating antibodies against non-structural proteins of FMD virus in the livestock in Uttar Pradesh. A total of 400 sera samples from the animals (200 cattle and 200 buffaloes) were tested for the presence of antibodies against r3AB3 non-structural protein (NSP) of FMDV using ELISA. The overall prevalence of anti-r3AB3 antibodies in bovine samples tested by the r3AB3-ELISA was 27.75%. No significant difference in prevalence of anti-r3AB3 antibodies was found in species and sex. At age level seroprevalence varied significantly among animals of <6 months (10%), 6 Months to 1 Year (13.33%), 1-3 Years of age (28.00%) and > 3 years of age (34.71%). At region-level, the significantly higher prevalence was recorded in Bareilly (47.50%) followed by Moradabad (40.00%), Bijnor (37.50%), Amroha (7.50%) and Rampur (6.25%). From the study, it can be concluded that natural infection of FMDV is widespread in the area and further studies using large number of samples including the molecular typing of FMD virus isolated from outbreaks and serum neutralization studies should be employed to develop an effective control program in India.

Keywords: Buffalo, Cattle, FMDV, NSP, r3AB3- ELISA

Introduction

Foot and mouth disease (FMD) is a highly contagious viral disease of cloven footed animals is caused by aphthovirus of picornaviridae family and clinically characterized by formation of vesicles in foot, mouth, buccal cavity, tongue, and udder (Verma *et al.*, 2012; Hussain *et al.*, 2019). The disease causes significant economic losses to dairy industry particularly in the developing countries like India (Knight Jones and Rushton, 2013; Singh *et al.*, 2019). The FMD virus is icosahedral, non-enveloped with a positive-sense single-stranded RNA genome of approximately 8,500 bases encoding for structural and non-structural proteins (Mason *et al.*, 2003). The virus is having seven distinct serotypes (O, A, C, Asia1, SAT1, SAT2 and SAT3) and more than 60 subtypes (OIE, 2017). FMD is endemic in India and the circulation of these serotypes was reported in country (Pattnaik *et al.*, 2012; Verma *et al.*, 2012; Chakraborty *et al.*, 2014). In recent years, there is considerable interest in the techniques that can differentiate between the vaccinated and infected animals. Several diagnostic tests have been developed to distinguish infected from vaccinated animals, all were based on detection of antibodies to the non-structural proteins (NSPs) of FMD virus (Gelkop *et al.*, 2018; Fukai *et al.*, 2018; Wang *et al.*, 2019; Hussain *et al.*, 2019).

In Uttar Pradesh, despite the endemicity of the disease, few studies have been carried out on sero-prevalence of antibodies against non-structural proteins of FMD virus (Verma *et al.*, 2010). So, the present cross-sectional study was conducted to estimate the prevalence of circulating antibodies against non-structural proteins of FMD virus in the livestock in Uttar Pradesh.

Materials and Methods

Study Area and Sampling

The study area includes five districts (Amroha, Bijnor, Bareilly, Rampur and Moradabad) of western parts of Uttar Pradesh, India (Figure 1). This cross-sectional study for sample collection was conducted in the months of August to March. A total of 400 blood samples from 200 cattle and 200 buffaloes were collected with epidemiological details, viz. species, sex, age groups and places (districts). The blood samples were collected by jugular venepuncture under aseptic condition and sera were separated and stored at -200C till further use.

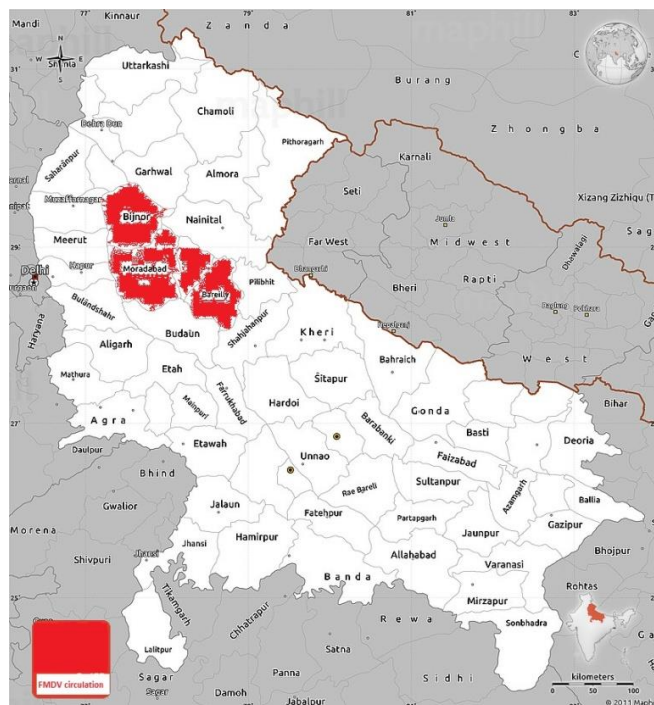


Figure 1: Map showing the study area in Uttar Pradesh state

Laboratory Analyses

An indirect ELISA was performed using r3AB3 DIVA ELISA kit procured from Project Directorate on Foot-and-

mouth disease (PDFMD, Mukteswar) to assess antibodies against 3AB NSP of FMDV as per the kit's recommendations. Briefly, 96-well micro-titer plates were coated with recombinant 3AB protein, and the plates were incubated at 4°C overnight. Test serum samples, negative and positive control serum (supplied in the kit) were diluted (1:50) in the diluent buffer and were transferred to the coated plates. Then, the plates were incubated for 1 h at 37°C followed by washing. Subsequently, anti-bovine-horse radish peroxidase (HRP) conjugate was dispensed and continued with incubation as mentioned earlier. After the substrate reaction had developed, the optical density (OD) values were measured at 492 nm. Results were expressed as a ratio of mean OD of the test sample to the mean OD of negative control (T/N ratio). Serum producing a T/N ratio more than 2 was scored as positive.

Statistical Analysis

The results were analyzed using Chi-square test as described by Snedecor and Cochran (1994).

Results and Discussion

Out of 400 sera samples collected from five districts of Western Uttar Pradesh, 111 (27.75%) of the samples scored positive in NSP ELISA (Table 1).

Table 1: District-wise distribution of sera samples found positive by NSP-ELISA

Districts	Total Samples Tested	Negative	Suspected	Positive
Rampur	80	67	8	5 (6.25)
Moradabad	80	38	10	32 (40.00)
J.P. Nagar	80	65	90	6 (7.50)
Bareilly	80	26	16	38 (47.50)
Bijnor	80	39	11	30 (37.50)
Total	400	235 (58.75)	54 (13.50)	111 (27.75)

*Values written in parentheses are in percentage

This signifies the FMDV circulation in the bovine population of Western Uttar Pradesh. The percentage of seropositive (NSP reactors) animals varied widely from 6.25% in Rampur districts to 47.5% in Bareilly district. This variation in seropositivity of FMDV observed in different districts was very much expected and also in concurrence of previous report in an earlier study from Uttar Pradesh (Verma *et al.*, 2010) and was almost similar to the pattern of different districts within the state, which reported the variation of 5.0% to 52.0% in seropositivity of animals (Verma *et al.*, 2010). The reasons for the differences in seropositivity of FMDV among the districts of the Uttar Pradesh state could not be fully explained based on the available data, but may be related to differences in animal management practices like animal markets, purchase of infected animals for replacement or upgrading, demographic factors, climatic conditions, rearing of different species together and wild life interaction. High circulation of anti NSP antibodies in animals of districts Moradabad, Bareilly and Bijnor might be due to sharing of their boundaries with other state. In districts Amroha and Rampur, the percent positivity against NSP ELISA were low, which might be because of the better availability of the vaccination services and the awareness among the livestock owners about FMD vaccination. Such a large variation of antibody prevalence values among the villages surveyed most likely reflects variation in the extent of FMDV infection rather than any difference in the agro-climatic environment or in husbandry practices. These figures were obtained from a small sampling area and should not be considered as representative for the entire district. There was no significant difference in percent positivity of FMDV in cattle (29.00%) and buffaloes (26.50%) (Table 2).

Table 2: Species-wise distribution of sera samples found positive by NSP-ELISA

S. No.	Species	Total sample tested	Negative	Suspected	Positive
1	Cattle	200	112	30	58 (29.00)
2	Buffaloes	200	123	24	53 (26.50)
3	Total	400	235	54	111 (27.75)

*Values written in parentheses are in percentage

Such comparable level of seropositivity demonstrated in these animals could be attributable to active infection and it would indicate the presence of FMDV in the herd. There was no significant difference in percent positivity of FMDV in male (25.88%) and female animals (28.25%) (Table 3).

Table 3: Sex-wise results of sera samples positive by NSP-ELISA

S. No.	Species	Total sample tested	Negative	Suspected	Positive
1	Male	85	48	15	22 (25.88)
2	Female	315	187	39	89 (28.25)
3	Total	400	235 (58.75)	54 (13.50)	111 (27.75)

*Values written in parentheses are in percentage

Animals were grouped into four age groups, viz. <6 Months, 6 Months-<1 year, 1-<3 year and ≥ 3 year. The seroprevalence of FMD virus was the significantly ($P < 0.05$) higher in age group ≥ 3 years (34.71%), while it was lowest in age group <6 Months (10.00%) (Table 4).

Table 4: Age-wise results of sera samples positive by NSP-ELISA

S. No.	Age Groups	Total sample tested	Negative (% negativity)	Suspected (% of suspected)	Positive (% positivity)
1	<6 Month	20	14	4	2 (10.00)
2	6 Month-<1 yr	60	42	10	8 (13.33)
3	1-<3 yr	150	89	19	42 (28.00)
4	Above 3 yr	170	90	21	59 (34.71)
Grand Total		400	235	54	111 (27.75)

*Values written in parentheses are in percentage

Age wise seroprevalence results revealed that among all, the seroprevalence in the age group ≥ 3 years was highest and increased steadily with advancement of age. The age of the animals is always a crucial risk factor associated with most of the diseases. The possible reason for higher seropositivity of FMDV in aged animals might be due to continuous exposure to infectious organism as previously reported in other diseases like brucellosis (Bandyopadhyay *et al.*, 2009) and salmonellosis (Verma *et al.*, 2010).

NSP expression of FMDV and their applications as antigen in ELISA were shown to be promising diagnostic methods that can differentiate the vaccinated animal from FMDV infected animals in field (Rodriguez *et al.*, 1994; Bergmann *et al.*, 2000; Wang *et al.*, 2019; Hussain *et al.*, 2019). As the bovine (cattle and buffalo) population in these districts are regularly vaccinated against FMD since the commencement of FMD control programme, any evidence for NSP-Ab could be considered infection-related seroconversion (Berger *et al.*, 1990; Verma *et al.*, 2010); hence, these animals can be the source of FMDV infection. In previous studies in-house indirect ELISA utilizing baculovirus-expressed FMDV non-structural protein (NSP) 3A was also used to find evidence for virus circulation (prevalence of anti-NSP 3A-specific antibodies) by examining serum samples in Haryana (Kumar *et al.*, 2007). NSP ELISA was easy to perform, quicker with reproducible results and with high specificity (De Diego *et al.*, 1997; Georgiev *et al.*, 2003; Verma *et al.*, 2010; Wang *et al.*, 2019; Hussain *et al.*, 2019). The test for the detection of antibodies against NSP has a significant advantage in detection of carrier animals.

Conclusion

The presence of anti NSP antibodies in animal population is suggestive of infection. The results of the present study provided serological evidence of widespread endemic circulation of FMD virus in bovine of western Uttar Pradesh, India. Various risk factors are associated to FMD and play crucial role in its spread. age wise significant difference can be mentioned here. The study was conducted in very small number of samples. Thus, authors recommend further detailed epidemiological investigation with a greater number of samples districted in a greater number of districts to find out the exact status of FMDV circulation in the state.

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Conflict of Interests

There is no conflict of interest.

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