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Sero-prevalence and Associated Risk Factors of Foot and Mouth Disease in Western Uttar Pradesh

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ABSTRACT: In India, foot-and-mouth disease (FMD) is considered as endemic and causes significant financial losses to the dairy sector. To effectively control the disease, there is requirement of sero-monitoring and risk factors associated with the occurrence of the disease. This cross-sectional study was conducted from January, 2021 to April, 2022 to estimate the seroprevalence of antibodies against r3AB3 non-structural protein of FMDV and the associated risk factors of the disease in western parts of Uttar Pradesh. A total of 130 sera samples from cattle and buffaloes from 06 districts of western Uttar Pradesh were collected and analyzed using indirect r3AB3 non-structural protein-ELISA kit, developed by Directorate on FMD, IVRI, Mukteshwar, India. For random bovine samples examined by the DIVA-ELISA assay, the apparent prevalence of anti-3AB3 antibodies was found to be 35.38%. Using logistic regression analysis, the effects of the many potential risk variables that were thought to influence the likelihood of FMD were calculated. In western Uttar Pradesh, sex was the risk factor that was most closely linked with FMD sero-positivity (OR = 3.4038; p = 0.041). The present study result confirms that FMD virus is circulating in the animals and the disease is highly prevalent in the study area. Hence, the regional concerned bodies should take attention on the implementation of National Animal Disease Control Programme.

Keywords: Bovines, ELISA, FMD, non structural protein, seropositivity, seroprevalence.

INTRODUCTION

Different species of cloven-footed domestic and wild animals are susceptible to the highly contagious, devastating, and economically significant Foot-and-Mouth disease, which causes direct losses to farmers in the form of reduced milk production and indirect losses in the form of trade restrictions on animals and their products (Perry and Rich 2007; Brito *et al*., 2017; Govindaraj *et al*., 2021). Foot-and-mouth disease virus, often known as FMDV, is the cause of FMD and is a member of the Picornaviridae family of viruses. Seven immunologically diverse serotypes of the virus, including O, A, C, Asia-1, SAT 1, SAT 2, and SAT 3, have varying levels of global spread (Wubshet *et al*., 2019). Due to the host and virus peculiarities of the Foot-and-Mouth disease, a variety of clinical manifestations are elicited. Clinically, the disease is typically marked by acute pyrexia, development of vesicular lesions in the mouth, tongue, buccal cavity, dental pad, nares, feet, udder, and teats, in appetence or anorexia, lameness, and occasionally mastitis (Meyer and Knudsen 2001). FMD is spreading among animals in India because of unfettered animal mobility, common watering sources at commercial livestock markets, and conventional livestock management (Subramaniam *et al*., 2013; Hegde *et al*., 2016). Numerous risk factors, including as the production system, age, season, and interaction with other animals, especially wildlife, have been discovered by epidemiological research on FMD (Bhattacharya *et al.,* 1996). The insufficient epidemiological research on FMD and the inadequate knowledge and application of preventative and control strategies may be to blame for the disease's persistence and ongoing evolution.

Despite the fact that numerous researches on the epidemiology of FMD have been carried out, there is a dearth of contemporary data regarding the sero-monitoring of the disease, and associated risk factors. The present study was conducted with the objective to study the prevalence of antibodies against r3AB3 non-structural protein of FMDV and to evaluate the potential risk factors for the disease prevalence.

MATERIALS AND METHODS

Place of study. The present study was conducted at Department of Veterinary Medicine, College of Veterinary and Animal Sciences, FMD Center of National Animal Disease Control Programme, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India and Directorate on Foot and Mouth Disease, ICAR-Indian Veterinary Research Institute, Mukteshwar campus, Nainital, Uttarakhand.

Study Areas and Study Animal Population. The goal of the current study was to determine the risk factors for FMD and estimate its sero-prevalence. The study was conducted in the 06 districts of Western Uttar Pradesh between 2021 and 2022. These districts included Baghpat, Bareilly, Bulandsahar, Gautambudh Nagar, Meerut and Muzaffarnagar. In rural areas, agriculture and animal husbandry account for the majority of the economy. Sahiwal, Haryana, and crossbreeds of Holstein Friesian and Jersey were the most prevalent cattle breeds, and Murrah was the most prominent buffalo breed. The humid subtropical climate of this region is monsoon-influenced, with hot summers and milder winters.

Data and sample collection. A structured questionnaire was used to gather the epidemiological data. The questionnaire was prepared for details such as the animal's species, sex, age, location (district), method of animal rearing, past health issues, and other clinical signs, if any. In the current investigation, three ml of blood from 130 animals of the study area was collected under aseptic conditions using labelled sterile disposable syringes (Dispovan) or vacutainers (BD, USA). After the blood had clotted, the serum was separated and brought to the lab on ice. All serum samples were kept in storage at -20°C until testing.

Laboratory Diagnosis:

DIVA-ELISA (Recombinant 3AB3 Nonstructural Protein (NSP)-Based INDIRECT ELISA) (r3AB3 DIVA Kit). All the 130 bovine (cattle and buffalo) sera samples were screened for the presence of antibodies against FMD virus non-structural proteins using the DIVA-ELISA test-kit developed by PDFMD, IVRI Campus Mukteshwar, Uttarakhand. On the sera samples, the DIVA-ELISA was carried out in accordance with the manufacturer's procedure. If the mean adjusted absorbance of the positive control wells is more than or equal to 0.8, the test is judged to be valid. The plate must also be rejected if the supplied negative control serum's mean adjusted absorbance is greater than 20 PP. Background control wells' O.D. should be below 10 PP. Additionally, the positive control's duplicate wells' OD values shouldn't deviate from the mean OD of the duplicate wells by more than 20%.

Final results for each test serum must be presented as the PP value, which is derived by dividing the test serum's reaction by the positive control serum's reaction and multiplying the result by 100, i.e., the percent positivity value or PP value > 40%. The following cut-off zones should determine how the results are interpreted: (Table 1)

1. PP value > 40% indicates a positive 3AB3 NSP reactivity.
2. PP value < 40% indicates a negative 3AB3 NSP reactivity.

RESULTS AND DISCUSSION

The current investigation reported the FMD seropositivity in all study locations, with a total sero-prevalence of 35.38 percent in bovines indicating the endemicity of FMD virus in the districts of Western Uttar Pradesh. The presence of antibodies against the FMD virus Non-Structural proteins in the sera samples of bovine indicates that the animals had exposure to the FMD virus (OIE 2019). The high prevalence of FMD in the present study could be attributed to geographical location of the area, no vaccination of the animals, poor management practices, movement of animals from neighbourhood districts or states, contact of animals to the stray animals roaming in the area, where they can get infections from disease affected animals. Additionally, clinical cases of FMD are unreported or underreported (Verma *et al.,* 2010).

Tables 2 to 7 display various epidemiological data addressing the relationship between risk factors such as place, species, breed, sex, age, and management practices. Because there were extremely few animals in some areas, not all of them were included in the district wise analysis of prevalence. Gautam Budh Nagar was found to be having the highest sero-prevalence of FMD (46.67%), followed by Muzaffarnagar (40.98%), Meerut (35.13%), Baghpat (33.33%), and Bulandsahar (16.67%) districts. These differences in prevalence among districts could be explained by the fact that the placement of farms in each district differs, as do the places where outbreaks are prevalent as well as the agroecological conditions of the various areas. All the five districts of western Uttar Pradesh were at higher risk of FMD indicating the impact of biotic and abiotic factors on the disease prevalence. Temperature and relative humidity are considered as crucial environmental factors for the development and transmission of disease as the virus can travel up to 250 km (Constable *et al.,* 2017). The primary source of the spread of transboundary disease is the movement of animals for their trade (Fevre *et al.,* 2006). In terms of species, cattle had a higher prevalence (39.53%) than buffalo (27.27%) (Table 3). This echoes with the study conducted by Hegde *et al.* (2014) which highlights cattle as the main indicator species in the epidemiology of FMD in Karnataka, India. Similarly, Rout *et al.,* 2016 reported the total positivity of 53 (51.46%) cattle bulls and 14 (37.84%) buffalo bulls for NSP-Ab indicating an exposure to FMDV using 3AB3 NSP ELISA. Our findings are also supported by the recent studies conducted by Krishnamoorthy *et al.* (2022) in which they found higher prevalence in cattle (45%) as compared to buffaloes (30%).This higher seroprevalence in cattle may be attributed to the fact that buffaloes tend to more resistant to the various diseases and more or less they are indigenous in origin while cattles are mostly crossbreeds.

This study reported a significant relationship (P<0.05) between the seroprevalence of FMD and the sex of the animals, with more females (40.38%) exposed than males (15.38%). The high seroprevalence FMD in female animals during the present study was consistent with the findings of previous studies (Mazengia *et al.,* 2010; Olabode *et al.,* 2013; Wungak *et al.,* 2016; Mesfine *et al.,* 2019; Chowdhury *et al.,* 2019; Atuman *et al.,* 2020). The unequally distributed sample size, in which the sample size of female animals was higher than male animals, might be responsible for the observed variance in FMD seroprevalence between the sexes of animals. In most parts of the world including Uttar Pradesh, the female animals are retained for milk production and breeding, so they have lower offtake rates than males (Mazengia *et al.,* 2010; Olabode *et al.,* 2013; Wungak *et al.,* 2016; Atuman *et al.,* 2020). Due to their propensity to remain in the herd for longer periods of time, the female animals are presumably more likely to be exposed to FMDV or its serotypes during their life span (Mesfine *et al.,* 2019). This higher seroprevalence in female animals may also be attributed to the fact that females are in much more stress due to production, heat and breeding stress. Contrary to the present findings Chowdhury *et al.* (2019) reported that male cattle (35.88%) were more commonly infected with FMD than female cattle (15.80%). They further reported that the risk of getting FMD cases in male cattle was 2.98 times higher than female cattle, which might be due to use of male cattle for draught purposes that may expose the skin for damages and increase the chances of infection. However, these findings were solely based on the clinical signs, no laboratory studies were conducted in determining the results. Singh *et al.* (2020) found no significant difference in prevalence of anti- r3AB3 antibodies in species and sex.

All 130 animals were split into two categories: young (under 3 years old) and adults (three years old or over). Amongst age categories, adult animals (>3 yrs of age) were marked with non-significant (p>0.05) high level of sero-positivity (36.44%) in comparison to that of young animals (30.43%) (Table 5). This is in agreement with the previous reports (Gelaye *et al.,* 2009; Esayas *et al.,* 2009; Wungak *et al.,* 2016) who documented no significant association between seropositivity of FMD between groups of different ages of cattle. On the other hand, Thrusfield, 2018, states that Young cattle who had less prior exposure to FMD did not exhibit a discernible reaction. In contrast various studies (Rufael *et al*., 2008; Megersa *et al.,* 2009; Ahmed *et al.,* 2020) revealed significantly higher FMD seroprevalence in young as compared to adult cattle, while other studies (Megersa *et al.,* 2009; Bayissa *et al.,* 2011; Habtamu *et al.,* 2011; Beyene *et al.,* 2015; Arzt *et al.,* 2018; Navid *et al.,* 2018; Mesfine *et al.,* 2019; Chowdhury *et al.,* 2019; Singh *et al.,* 2020; Shurbe *et al*., 2022) found that the seroprevalence increased with age in the sampled cattle. The high prevalence of FMD infection in animals may be due to cumulative infection over time, where older animals have a higher risk of contracting the disease due to their longer retention in the community. This variation might be attributable to the sample sizes being distributed unevenly among age groups and by differences in how young animals are handled, such as sheltering them separately or separating them from adult animals when they are around the farm and camps (Murphy *et al.,* 1999; Alexandersen and Mowat 2005; Maclachlan and Dubovi 2010; Awel *et al.,* 2021). Shurbe *et al*. (2022) stated passive maternal immunity to be the possible reason of lesser seroprevalence in young animals. Regarding the breed of animals, the prevalence was slightly higher but non-significant (p>0.05) in crossbreds (38.23%) than indigenous breeds of animals (32.25%) (Table 6). This is in agreement with the previous reports (Misgana *et al.,* 2013; Awel *et al.,* 2021) who reported non-significance difference in seroprevalence of FMD between indigenous and crossbreeds. However, Chowdhury *et al.* (2019) revealed that the indigenous cattle were found to be significantly associated with the FMD infection compared to crossbreed cattle. Shurbe *et al*. (2022) also reported significantly higher seroprevalence in local breeds. His findings are in accordance with the study of Awel and Dilba (2021), however their findings were non significant. Contrary to this, Sulayeman *et al.* (2018) reported the higher FMD seroprevalence in crossbred than local cattle, which might be attributed to the genetic variation among the breed of animals (Sahle *et al.,* 2007; OIE, 2019; Ahmed *et al.,* 2020).

Management system had also shown no significant association with FMD seroprevalence, however, the seroprevalence of FMD was higher in animals reared in organized farm or under intensive system (46.67%) than individually reared or semi-intensive (33.91%) farming systems (Table 7). The observed differences in FMD seroprevalence between the management system of animals might be due to the unproportionate sampling, in which the sample size of individually reared animals was much higher than organized farms. In line with the results of the present study, previous studies (Vosloo *et al.,* 2002; Mesfine *et al.,* 2019; Awel *et al.,* 2021; Ali *et al.,* 2022) reported that intensive livestock production and large herd size are highly vulnerable to FMD infection. Hegde *et al,* (2014) observed a significant correlation between livestock density and the number of outbreaks reported and number of cases for all the agro-climatic zones in Karnataka. This may be related to animal crowding, which increases the likelihood of FMD transmission by facilitating frequent direct contact (Mesfine *et al.,* 2019). Additionally, this may be explained by the fact that the FMD virus can survive for several months in shady areas that aren't exposed to sunlight (Constable *et* *al.,* 2017). The effects of the various risk factors that supposed to affect the occurrence of FMD were computed using logistic regression analysis (Table 8 and 9). One potential risk factors *i.e.* sex was identified by univariate logistic regression analysis (p<0.5) with the strongest association with disease presence. A multivariate logistic regression model was also built with the risk factors (Table 8). Hosmer-Lemeshow test indicates a good fit of the model to the data (*P*=0.4024) and the area under the ROC curve (AUC) is 0.601 (95% CI: 0.512–0.686).Analysis of sero-prevalence of FMD in bovines with respect to place, species, breed, sex, age and management practices of animals revealed that the sex to significantly influence the occurrence of FMD infection in bovines, while age, species, breed, place and management practices had no significant influence (P<0.05) on the prevalence of FMD in animals though they also serve as potentially influencing risk factors to be analyzed.

Table 1: Interpretation of ELISA plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	TS1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	PC	PC	NC	NC	BG	BG

Table 2: District wise sero-prevalence of Foot-and-Mouth disease in bovines.

Sr. No.	District*	Number of animals tested	Number of Positive animals (%)
1.	Baghpat	03	01(33.33)
2.	Bareilly	01	0 (0.00)
3.	Bulandsahar	06	01 (16.67)
4.	Gautambudh Nagar	15	7 (46.67)
5.	Meerut	74	26 (35.13)
6.	Muzaffarnagar	31	11 (40.98)
	Total	**130**	**46 (35.38)**

Value in parentheses indicates prevalence in percentage

Table 3: Species wise sero-prevalence of Foot-and-Mouth disease in bovines.

Sr. No.	Species	Number of animals tested	Number of Positive animals (%)
1.	Cattle	86	34 (39.53)
2.	Buffalo	44	12 (27.27)
3.	**Total**	**130**	**46 (35.38)**

Value in parentheses indicates prevalence in percentage

Table 4: Sex wise sero-prevalence of Foot-and-Mouth disease in bovines.

Sr. No.	Sex*	Number of animals tested	Number of Positive animals (%)
1.	Male	26	04 (15.38)
2.	Female	104	42 (40.38)
3.	**Total**	**130**	**46 (35.38)**

Table 5: Age wise sero-prevalence of Foot-and-Mouth disease in bovines.

S. No.	Age*	Number of animals tested	Number of Positive animals (%)
1.	Young (≤3 yrs)	23	07 (30.43)
2.	Adult (>3 yrs)	107	39 (36.44)
3.	**Total**	**130**	**46 (35.38)**

Table 6: Breed wise sero-prevalence of Foot-and-Mouth disease in bovines.

Sr. No.	Breed	Number of animals Tested	Number of Positive animals (%)
1.	Crossbred	68	26 (38.23)
2.	Indigenous	62	20 (32.25)
3.	**Total**	**130**	**46 (35.38)**

Table 7: Rearing practices wise sero-prevalence of Foot-and-Mouth disease in bovines.

Sr. No.	Management Practice	Number of animals tested	Number of Positive animals (%)
1.	Organized farm	15	07 (46.67)
2.	Individually reared animals	115	39 (33.91)
3.	**Total**	**130**	**46 (35.38)**

Table 8: Univariable logistic regression for risk factors for Foot-and-Mouth disease in bovines.

Risk Factor	Category	No. +ve/total (%)	OR	95% CI		*P*-value
				Lower	Upper	
Species	Cattle	34/86 (39.53)	0.5735	0.2598	1.2661	0.1648
	Buffalo	12/44 (27.27)				
District	Baghpat	01303(33.33)	1.0692	0.7366	1.5520	0.7248
	Bareilly	0/01 (0.00)				
	Bulandsahar	01/06 (16.67)				
	Gautambudh Nagar	7/15 (46.67)				
	Meerut	26/74 (35.13)				
	Muzaffarnagar	11/31 (40.98)				
Sex#	Male	04/26 (15.38)	3.7258	1.1974	11.5928	0.0231
	Female	42/104 (40.38)				
Breed	Crossbred	26/68 (38.23)	0.7692	0.3733	1.5851	0.4769
	Indigenous	20/62 (32.25)				
Age	Young (≤ 3 years)	07/23 (30.43)	1.3109	0.4962	3.4635	0.5849
	Adult (> 3 years)	39/107 (36.44)				
Management	Organized Farm	07/15 (46.67)	0.5865	0.1981	1.7363	0.3352
	Individual rearing	39/115 (33.91)				

Table 9: Multivariable logistic regression analysis on the occurrence of FMD in bovines as a function of various risk factors.

Risk Factor	ß	SE	OR	95% CI		P value
				Lower	Upper	
Age	0.3162	0.5248	1.3720	0.4905	3.8375	0.5468
Breed	-0.0276	1.2913	0.9727	0.0774	12.2249	0.9829
District	0.0905	0.2157	1.0947	0.7172	1.6710	0.6749
Management	-0.4185	1.3908	0.6580	0.0431	10.0494	0.7643
Sex	1.2249	0.5995	3.4038	1.0511	11.0230	0.0410
Species	-0.1988	1.3182	0.8197	0.0619	10.8579	0.8801
Constant	-2.79079	3.4026				

CONCLUSIONS

In the present study, using 3AB3 DIVA-ELISA, out of 130 sera samples, 46 (35.38%) was found positive indicating the circulation of the FMDV in the study area. On analysis of various risk factors, sex wise seroprevalence of FMD was significantly higher ($P<0.05$) in females (40.38 percent) than in males. The endemicity of FMD in western Uttar Pradesh has been confirmed and established by current study.

FUTURE SCOPE

Since the current investigation was having several limitations including sample size and uneven distribution of sample therefore further studies must be carried out in order to access current status of FMD in various regions of India. Moreover this study is quite helpful in sero-monitoring and sero-surveillance as well as strengthening the ongoing NADCP programme for control of FMD in India.

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Conflict of Interest. None.

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