



Investigation of foot-and-mouth disease outbreaks in Uttar Pradesh

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The annual economic loss due to foot-and-mouth disease (FMD), a highly contagious disease of cloven-hoofed animals, in India ranges from INR 12,000 crore to 14,000 crore (Singh *et al.* 2013).

Vaccination based FMD-control programme (FMD-CP) is in operation in India which involves biannual vaccinations of all cattle and buffaloes in selected areas, regular active surveillance and antibody monitoring in vaccinated population (Biswal *et al.* 2012). Among the present 7 types of FMD virus (FMDV) type O, A and Asia 1 are prevalent in South Asia. In recent past, about 80% of the outbreaks in India are attributed to serotype O (Subramaniam *et al.* 2012, Hegde *et al.* 2014). For effective control and prevention of the disease, timely investigation of field outbreaks aid rapid implementation of appropriate vaccination and zoosanitary measures for restricting the dissemination of the virus. In the present study, FMD outbreaks at various villages/farms in different districts of Uttar Pradesh (UP) were investigated. Majority of the outbreak fall under western plain and semi-arid zones.

Clinical tissue samples (18; vesicle/tongue epithelium) were collected in 50% phosphate buffered saline/glycerol medium (pH, 7.5) from cattle (3), buffalo (12), goat (2) and pig (1) from 10 FMD outbreaks attended during April 2013 to March 2014 in 8 districts of Uttar Pradesh. Serum samples (121) including cattle (65), buffalo (54) and goat (2) were also collected from FMD virus affected, convalescing and in-contact animals.

Supernatants of the homogenized clinical tissue materials were used in a serotype differentiating antigen detection ELISA as per Bhattacharya *et al.* (1996) for confirmation of serotype of the virus involved in the outbreaks.

To assess the protective antibody titre against FMDV structural protein (SP), liquid phase blocking enzyme linked immunosorbent assay (LPB ELISA) was performed as per

Ranabijuli *et al.* (2010) using the LPB ELISA kit [Project Directorate on Foot and Mouth Disease (PD-FMD), Mukteswar]. Two-fold dilution of serum samples were tested for serotype-specific FMDV SP-Ab titre. Antibody titres were expressed as the reciprocal log₁₀ dilution corresponding to 50% end-point titre, i.e. the dilution at which the reaction of the test sera results in an optical density equal to 50% inhibition of the median optical density of the antigen control wells. The serum samples with antibody titres >1.8 log₁₀ against a particular FMD virus serotype was taken as positive for the presence of antibodies against that particular FMD virus serotype in retrospective diagnosis.

3AB NSP ELISA for detection of anti-FMDV nonstructural protein (NSP) antibodies was performed as per Mohapatra *et al.* (2011) using the r3AB3 NSP ELISA kit supplied by PD-FMD, Mukteswar to assess antibodies against 3AB NSP of FMDV. Serum samples producing corrected optical density value ≥40% of that of the positive control were scored positive.

During the present investigation, 9 outbreaks occurred in FMD-CP districts where biannual vaccination was carried out in XIII (Jan 2013 to Mar 2013) and XIV (Sep 2013 to Nov 2013) phases of vaccination indicating some failure in the vaccination programme. The outbreaks were recorded in June, Sep, Oct, Nov and Dec. Clinical FMD in the form of vesicular/erosive lesions on tongue and feet was evident both in cattle and buffalo. In one outbreak at Bulandshahar district other animals including goat and pig were also affected. In pigs only foot vesicular lesions were observed while in goats characteristic mouth lesions as well as interdigital erosive lesion were observed.

Fourteen out of 18 clinical samples collected during the outbreaks, were typed as serotype O in serotype differentiating antigen detection ELISA, while in 4 samples no virus could be detected (Table 1). Species wise distribution of serotypes showed samples from cattle (2/3), buffalo (9/12), goat (2/2) and pig (1/1) as type 'O' (Table 2).

In cattle, the % of animals showing log₁₀ antibody titre of >1.8 for O, A and Asia 1 was 64.61%, 56.92% and 50.76% (Table 3). Similarly, for buffalo it was 46.29%, 29.63% and

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Table 1. District wise distribution of FMD outbreaks and serotypes involved between Apr 2013 and Mar 2014 in Uttar Pradesh.

Month/year	District	No. of outbreaks	No. of clinical samples	FMD serotype involved			No virus detected (NVD*)
				O	A	Asia 1	
Jun/2013	Agra	1	4	4	-	-	-
Sep/2013	Bulandshahar	2	8	6	-	-	2
Oct/2013	Fatehpur	1	-	-	-	-	-
Oct/2013	Ghaziabad	1	-	-	-	-	-
Oct/2013	Meerut	1	-	-	-	-	-
Nov/2013	Mainpuri	1	1	1	-	-	-
Nov/2013	Mujaffarnagar	1	2	2	-	-	-
Dec/2013	Saharanpur	2	3	1	-	-	2
	Total	10	18	14	-	-	4

*NVD, Conclusive FMD virus serotype could not be established.

Table 2. Species wise distribution of clinical samples from FMD outbreaks and serotype involved between April 2013 and March 2014 in Uttar Pradesh

Species	No. of outbreaks	No. of samples	FMD serotype involved			NVD*
			O	A	Asia 1	
Cattle	10	3	2	-	-	1
Buffalo	10	12	9	-	-	3
Goat	1	2	2	-	-	-
Pig	1	1	1	-	-	-

*NVD, conclusive FMD virus serotype could not be established.

27.78%, respectively. In goat, none of the sample showed protective titre. The antibody titres showed maximum number of animals possessing protective titre for type O. As serum samples are from FMD virus affected/convalescing animals, high percentage of type O antibodies may be because of the outbreaks that were typed as O in the present study. The proportion of animals having protective immunity against all 3 FMDV types was 38.46% and 24.07% in cattle and buffalo, respectively. These values indicated poor herd immunity that might be one reason for outbreaks as suggested earlier (Parida 2009, Rout *et al.* 2014).

In 3AB NSP ELISA, 86.15% cattle and 62.90% buffalo were positive for anti-FMDV NSP antibodies (Table 3). The high seropositivity in 3AB NSP ELISA correlates with FMDV infection and exposure of these animals to FMD virus as the serum samples were from FMD affected/convalescent animals. Rout *et al.* (2014) obtained a total of 42.85% cattle, 59.93% sheep and 73.9% goats as positive

reactors in 3AB NSP ELISA in serum samples (357) collected from clinically sick, recovered and in-contact animals during investigation of FMD outbreaks in various villages of Chikballapur district in Karnataka. In random sero-monitoring studies at Regional Research Centre on FMD, Mathura a 56.40% positive reactors were found in 3AB NSP ELISA in 1023 random bovine serum samples collected during the same period from western region of Uttar Pradesh, indicating a high level of FMDV circulation in the area (Annual Report, AICRP for epidemiological studies on FMD, Mathura, 2013–14); whereas, according to Pattnaik *et al.* (2012), average prevalence of FMD infection in bovine population is approximately 27.5% based on anti-3AB NSP ELISA. In another study, overall 6.67% buffaloes were found infected and 10.67% were in doubtful condition in screening of 300 random serum samples from the border area between Uttarakhand and Uttar Pradesh (Mohan *et al.* 2014). The present finding strengthens further the usefulness of 3AB NSP ELISA test for FMD outbreak prediction.

LPB-ELISA is being used for sero-monitoring of antibody response to FMD virus serotypes specially, when it becomes difficult to collect suitable clinical material due to either late receipt of information about FMD outbreak or mild/ sub-clinical form of the disease in animals. Serotype-specific spike in animals should be suggestive enough of the circulating serotype of the virus. It has been observed that following infection, animal may show sero reactivity for structural proteins of FMDV for several years. In the present study, in 3 outbreaks no clinical material was obtained. In retrospective diagnosis of these outbreaks (Table 4), 33.33 and 40% samples were showing log₁₀ titre of ≥ 1.8 for serotype O in Fatehpur and Ghaziabad outbreak,

Table 3. Seropositivity for FMD virus SP and NSP antibodies in serum samples collected from FMD outbreaks

Species	Total serum samples	No. of animals showing log ₁₀ titre of >1.8				3AB NSP-Ab positive
		O	A	Asia 1	All three	
Cattle	65	42 (64.61%)	37 (56.92%)	33 (50.76%)	25 (38.46%)	56(86.15%)
Buffalo	54	25 (46.29%)	16 (29.63%)	15 (27.78%)	13 (24.07%)	34(62.96%)

Table 4. Retrospective diagnosis of FMD outbreaks between April 2013 and March 2014 in Uttar Pradesh

District	Total serum samples	No. of animals showing log ₁₀ titre of >1.8			3AB NSP-Ab positive	SP-Ab 4 fold spike		
		O	A	Asia 1		O	A	Asia 1
Fatehpur	18	6 (33.33%)	0(0%)	2 (11.11%)	14 (77.77%)	5 (27.77%)	0(0%)	1 (5.55%)
Ghaziabad	10	4(40%)	0(0%)	0(0%)	10(100%)	4(40%)	0(0%)	0(0%)
Meerut	30	23 (76.66%)	24 (80%)	23 (76.66%)	29 (96.66%)	2(6.66%)	4 (13.33%)	3(10%)

respectively. A clear spike in SP-Ab response against serotype O was evident i.e. ≥ 4 fold increase in the titre (0.6 log₁₀ titre increase) compared to each of the other 2 serotypes in samples from Fatehpur (27.77%) and Ghaziabad (40%) outbreaks indicating an extensive circulation of serotype O virus in the area. A few samples (5.55%) from Fatehpur also showed clear spike in SP-Ab response against serotype Asia 1. In outbreak at Meerut, 76.66, 80.0 and 76.66% samples were showing log₁₀ titre of ≥ 1.8 for serotypes O, A and Asia 1, respectively. Although cattle from this organized farm revealed clinical symptoms, clear 4 fold spike in SP-Ab was not apparent. History of this organized farm revealed vaccination of animals biannually. Absence of clear ≥ 4 fold spike in SP-Ab may be because of antibody response against all the vaccinal types that may have masking effect. In mass vaccinated population, the antibody titres detected in LPB-ELISA might interfere with the retrospective diagnosis in convalescent/recovering animals (Kakker and Sharma 2008). In 3AB NSP ELISA on serum samples from FMD affected/convalescent animals 77.77, 100 and 96.66% were detected to be positive reactors in 3 outbreaks, respectively, suggesting an exposure of these animals to FMD virus.

From the present investigation of FMD outbreaks throughout the year, serotype O was involved as the major serotype. Serotype O has been reported to cause maximum number of outbreaks (79.8%) in the year 2012–13 in India (PD-FMD Annual Report, 2012–13). In UP, both serotype O and A was responsible for outbreaks in 2012–13 (PD-FMD Annual Report, 2012–13). Further work is required to draw the phylogenetic relationship and lineages of FMDV isolates in present investigation. Thus, information on each and every outbreak in endemic region is important in understanding the epidemiology of FMD virus and vaccine efficacy.

SUMMARY

FMD outbreaks (10) in Uttar Pradesh during 2013–2014, were investigated. Fourteen clinical samples were typed as serotype O in antigen detection ELISA. In 3AB NSP ELISA on serum samples from FMD affected/convalescent animals 86.15% cattle and 62.90% buffalo were tested positive for anti-FMDV NSP antibodies indicating an extensive FMDV activity. The protective immunity against all 3 FMDV types showed poor herd immunity. For individual O, A and Asia 1 protective titre was 64.61, 56.92 and 50.76% in cattle and 46.29, 29.63 and 27.78% in buffalo, respectively. High

percentage of Type O antibodies in convalescing serum samples corroborated with serotype O in antigen typing. In retrospective diagnosis of 3 outbreaks, a clear spike in SP-Ab response against serotype O was evident in 2 outbreaks. The study revealed serotype O as cause of outbreaks throughout the year.

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REFERENCES

- Bhattacharya S, Pattnaik B and Venkataramanan R. 1996. Development and application of sandwich enzyme-linked immunosorbent assay (ELISA) for type identification of foot and- mouth disease (FMD) virus in direct field materials. *Indian Journal of Animal Sciences* **66**: 1–9.
- Biswal J K, Sanyal A, Rodriguez L, Subramaniam S, Arzt J, Sharma G K, Hammond J M, Parida S, Mohapatra J K, Mathapati B S, Dash B B, Ranjan R, Rout M, Venketaramanan R, Misri J, Krishna L, Prasad G, Pathak K M L and Pattnaik B. 2012. Foot-and-mouth disease: Global status and Indian perspective. *Indian Journal of Animal Sciences* **82** (2): 109–31.
- Hegde R, Gomes A R, Girdhar P, Kowalli S, Shivshankar B P, Sudharshana K J, Nagaraj K, Sesharao R, Mallinath K C, Shankar B P, Nagaraj D, Seema C M, Khan T A, Nagaraj G V, Srikala K, Dharanesh N K, Venkatesha M D and Renukprasada C. 2014. Epidemiology of foot and mouth disease in Karnataka state, India: a retrospective study. *Virus Disease* **25** (4): 504–09.
- Kakker N K and Sharma R. 2008. Retrospective diagnosis of foot and mouth disease outbreaks by liquid phase blocking enzyme linked immunosorbent assay. *Haryana Veterinarian* **47**: 28–31.
- Mohan A, Upadhyay A K, Kumar M, Kushwaha N, Singh R and Rastogi S K. 2014. Seroprevalence of foot-and-mouth disease and protective antibody titre against it in buffaloes. *Buffalo Bulletin* **33** (4): 284–92.
- Mohapatra J K, Pandey L K, Sanyal A and Pattnaik B. 2011. Recombinant non-structural polyprotein 3AB-based serodiagnostic strategy for FMD surveillance in bovines irrespective of vaccination. *Journal of Virological Methods*

- 177: 184–92.
- Parida S. 2009. Vaccination against foot-and-mouth disease virus: strategies and effectiveness. *Expert Review Vaccines* **8**: 347–65.
- Pattnaik B, Subramaniam S, Sanyal A, Mohapatra J K, Dash B B, Ranjan R and Rout M. 2012. Foot-and-mouth disease: Global status and future road map for control and prevention in India. *Agricultural Research* **1**: 132–47.
- Ranabijuli S., Mohapatra, J K, Pandey L K, Rout M, Sanyal A, Dash B B, Sarangi L N, Panda H K and Pattnaik B. 2010. Serological evidence of foot and mouth disease infection in randomly surveyed goat population of Orissa, India. *Transboundary and Emerging Diseases* **57**: 448–54.
- Rout M, Biswal J K, Dash B B, Hegde R, Subramaniam S and Mohapatra J K. 2014. Investigation of foot-and-mouth disease outbreaks in Chikkaballapur district of Karnataka. *Indian Journal of Animal Sciences* **84** (3): 231–35.
- Singh B, Prasad S, Sinha D K and Verma M R. 2013. Estimation of economic losses due to foot and mouth disease in India. *Indian Journal of Animal Sciences* **83** (9): 964–70.
- Subramaniam S, Pattnaik B, Sanyal A, Mohapatra J K, Pawar S S, Sharma G K, Das B and Dash B B. 2012. Status of foot and- mouth disease in India. *Transboundary and Emerging Diseases* **60**: 197–203.