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Research Article

Antigenic Relationship of Field Isolates of Foot-and-Mouth Disease Virus Type "O" with in-Use Vaccine Strain in Assam, India

Bornali Borah[1], Abu Kausar Hazarika[1], Tamil Selvan[2], Krishna Sarma[3], Muhammad Moin Ansari[4*] and Isfaqul Hussain[4]

[1]Department of Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India; [2]Indian Veterinary Research Institute, Mukteswar, Uttarakhand, India; [3]All India Co-ordinated Project on FMD, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India; [4]Faculty of Veterinary Sciences and Animal Husbandry, Srinagar, Jammu and Kashmir, India
***Corresponding author:** drmoin7862003@gmail.com

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ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals; it affects cattle, buffaloes, pigs, sheep, goats and about 70 wildlife species, e.g. African buffaloes. The disease has been present in almost every part of the world where livestock are kept. It can cause enormous economic losses when incursions occur into countries which are normally disease free. In addition, it has long-term effects within countries where the disease is endemic due to reduced animal productivity and the restrictions on international trade in animal products. In this study twenty two tissue samples from naturally infected cattle with foot-and-mouth disease virus (FMDV) type 'O' from different parts of Assam was examined for isolation of the virus in baby hamster kidney-21 (BHK-21) clone 13 cell line. Antigenic relationship of the isolated strains of FMDV with vaccine strain (IND R_2/75) were carried out by calculation of the relative homology value ('r') from liquid phase blocking ELISA (LPBE) and two dimensional micro neutralization test (2D-MNT). Out of 22 samples 8 isolates of FMDV was obtained. Five isolates were closely related with the vaccine strain as determined by LPBE. The 2D-MNT reveled that all the field isolates were closely related with the vaccine strain. The results suggest that the vaccine strain might confer protection against the field strains of FMD type 'O' virus.

Key words: Antigenic relationship, Assam, FMD virus type O, Vaccine strain

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals; it affects cattle, buffaloes, pigs, sheep, goats and about 70 wildlife species, e.g. African buffaloes. The disease has been present in almost every part of the world where livestock are kept. It can cause enormous economic losses when incursions occur into countries which are normally disease free. In addition, it has long-term effects within countries where the disease is endemic due to reduced animal productivity and the restrictions on international trade in animal products. The disease is caused by a single stranded positive sense RNA virus, foot-and-mouth disease virus (FMDV), belonging to the genus *Aphthovirus* within the family *Picornaviridae*. Seven different serotypes (and numerous variants) of FMDV have been identified. FMD is endemic in India and in recent years outbreaks due to types O, A and Asia-1 have been encountered throughout the year. Serotype 'O' virus is responsible for majority of the outbreaks (Yuvaraj *et al*., 2013). So, continuous monitoring and vaccine matching exercise of the field outbreaks is necessary in order to implement FMD vaccination and control programme in the country more effectively. Antigenic variation gives rise to virus populations different from those that initiated the infection. In addition, antigenic variation in FMDV has also been observed in tissue culture in the absence of immunologic pressure (Bolwell *et al*., 1989; Diez *et al*., 1989; Domingo *et al.*, 1993; Fares *et al.*, 2001).

Liquid phase blocking ELISA (LPBE) and two dimensional-micro neutralization test (2D-MNT) have been employed to determine the antigenic relationship between the field isolates and reference vaccine virus strains of FMD virus (Kitching *et al*., 1988; Gleeson *et*

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al., 1994). Antigenic analysis of field isolates with the vaccine strain is significant for testing the appropriateness of the existing vaccine strain as well as for the selection of new vaccine strain. The study was aimed to compare type 'O' field isolates of FMD virus with the in-use vaccine strain antigenically by LPBE and 2D-MNT.

MATERIALS AND METHODS

Sample

Twenty-two tissue samples in the form of tongue and feet epithelium infected with FMDV serotype 'O' were used in the experiment. The samples were collected from natural outbreaks of FMD in cattle during 2007- 2010 from different parts of Assam, India and stored at -80° C.

Isolation and serotyping of virus

The FMD virus from the field samples was isolated in baby hamster kidney-21 (BHK-21) clone 13 cell line as described by Longjam *et al.* (2010). The cell line was procured from the Project Directorate on Foot-and-Mouth Disease (PDFMD), Indian Veterinary Research Institute (IVRI), Mukteswar. Briefly, about two hundred milligram of infected epithelium was washed in sterile PBS and triturated in a pestle and mortar with 2 ml of PBS. Then the triturated material was treated with equal volume of chloroform and centrifuged at 3000×g for 15 min. The clear supernatant was again treated with penicillin (2000 IU/ml) and streptomycin (0.2 mg/ml) for 30 min at 37°C and stored at -20°C as inoculum for isolation of the virus. The cell line was grown in 25 cm^2 flasks with Glasssgow modified medium and infected with 350 µl of inoculum. The flasks were observed periodically during next 48 hours for any cytopathic effect (CPE). Two more passages were given till all the infected cell cultures showed CPE during 18-24 hours The cell culture fluid was harvested on attaining maximum CPE and centrifuged at 3000× g for 10 min. The supernatants containing the virus were stored at -80°C till further use. Isolates of the virus were reconfirmed as serotype 'O' of FMD virus by sandwich ELISA as described by Sarma and Sarma (2003).

Reference vaccine virus and serum

Foot and Mouth Disease virus strain type 'O' (IND R_2/75) was used as reference strain in the present study. The strain was also propagated in BHK-21 clone 13 cell line as mentioned above. Bovine antiserum against the reference strain and anti-146S FMDV rabbit and guinea pig sera against the vaccine strains O (IND R_2/75), A (IND 17/77, IND 489/97, IND 40/00), C (C/Bombay/64) and Asia-1 (IND 63/72) were obtained from PDFMD, IVRI, Mukteswar, India. These antisera were used as coating and tracing sera in sandwich-ELISA and LPBE.

Antigenic analysis of field isolates

Liquid phase blocking ELISA

Liquid phase blocking ELISA was carried as per the protocol of Sarma and Sarma (2003). The appropriate dilutions for LPBE of reference FMDV serotype 'O' strain (IND R_2/75) and the field isolates in the form of cell culture fluid were determined by sandwich ELISA, diluting the viruses two fold from neat to 1:10 in PBS-T (0.05% Tween-20). Reciprocal of the serum dilution corresponding to 50 per cent inhibition was considered as titre of the serum. The antigenic relationship expressed as 'r' value was obtained as per OIE (2006) guidelines. The r values were interpreted as per Ferris and Donaldson (1992). A 'r' value of 0.4 – 1.0 showed close relationship between field isolates and vaccine strains whereas the value of 0.2 - 0.39 indicated that the field isolates were antigenically related and a 'r' value of <0.2 indicated that they were distantly related.

Two dimensional-micro neutralization test

In order to assess the antigenic relationship of field isolates with the vaccine virus, two-dimensional micro-neutralization test (2D-MNT) was carried out in 96-well flat-bottomed tissue culture plates (Techno Plastic Products AG) using type-specific R_2/75 bovine vaccinal serum (BVS) as described by Rweyemamu *et al*. (1978) with little modifications. BHK-21 clone 13 cell line were used as the indicator system in the test. The plates were read microscopically after 48 hours of incubation for the presence or absence of CPE. Then they were stained with 0.1% crystal violet and final reading was taken. The reciprocal of the highest dilution of serum neutralizing exactly 100$TCID_{50}$ virus particles in 50% of the wells was considered as the titre of the serum. One-way antigenic relationships (r-value) of the field isolates relative to the reference strains was calculated, which is expressed as the ratio between heterologous/ homologous serum titre. The 'r' value was interpreted as per OIE (2006) guidelines as mentioned before. A 'r' value of >0.3 indicated that the field isolates were sufficiently similar to vaccine strains whereas the value of <0.3 indicated that the field isolates were different from the vaccine strains.

RESULTS

Out of 22 numbers of samples positive for FMDV type 'O' inoculated into BHK-21 cell line, only 8 isolates FMDV type 'O' could be isolated (Table 1). The presence of virus and its type in cell culture was confirmed by CPE and S-ELISA. At first passage, CPE could be observed in seven samples 24-48 hours post inoculation. The CPE was observed by 18- 24 hours post infection at sixth passages in all the samples. The 8 positive samples showed corrected optical density (OD) of more than 1.00 in the 'O' type specific S-ELISA (Table 1) while the vaccine starins gave an OD of 2.096.

The infectivity titer ($log_{10}TCID_{50}$) of all the eight field isolates and the vaccine virus (IND R_2/75**)** which was determined in BHK-21 cell line by 2D-MNT ranged between $10^{3.34}$ and $10^{5.27}$ $Log_{10}TCID_{50}$/50 µl and it was highest for G-54/07. Out of eight isolates, three samples (G-30/09, G-52/10, G-53/10) showed same infectivity titre ($10^{3.83}$). G-19/09 showed a lowest titre of $10^{3.34}$ $Log_{10}TCID_{50}$/50 µl (Table 2).

The antigenic relationship ('r'-values) of field isolates of FMD virus with vaccine strain determined by one-way (unidirectional) LPBE are shown in Table 3. The 'r' values ranged from 0.32 to 1.00. Five isolates had an 'r' value of >0.40 indicating close antigenic relationship with the vaccine strain. Only one isolate (G-31/07) showed 100% relationship ('r' value 1.00) with that of the reference vaccine strain, whereas three isolates had lowest

Table 1: Details of samples of FMDV type 'O' isolates in BHK-21 clone 13 cell line confirmed by S-ELISA

Sl. No.	Sample No	Place of outbreak (District)	Type of samples	Vaccination status	S-ELISA OD at 492 nm
1	G-31/07	Kamrup	FE	NA	1.030
2	G-54/07	Nagaon	TE	UV	1.007
3	G-11/09	Kokrajhar	FE	UV	1.114
4	G-19/09	Darrang	FE	UV	1.055
5	G-25/09	Kamrup	TE	V	1.114
6	G-30/09	Golaghat	TE	NA	1.016
7	G-52/10	Kamrup	FE	UV	1.008
8	G-53/10	Goalpara	FE	UV	1.006

NA -Not Available, UV -Unvaccinated, V- vaccinated;, TE -tongue epithelium, FE -feet epithelium.

Table 2: Infectivity titre /50 µl ($log_{10}TCID_{50}$) of field isolates and reference strain of FMDV type 'O' determined by 2D-MNT

Sl. No.	Sample/isolate No	$Log_{10}TCID_{50}$/50 µl
1	G-31/07	3.99
2	G-54/07	4.94
3	G-11/09	3.61
4	G-19/09	3.34
5	G-25/09	3.66
6	G-30/09	3.83
7	G-52/10	3.83
8	G-53/10	3.83
9	IND R_2/75*	5.27

*Reference vaccine strain

Table 3: Comparison of antigenic relationship (r value) of field isolates of FMDV type 'O' reference vaccine strain virus by LPBE and 2D-MNT

SL. No	Sample/isolate No	'r' value	
		LPBE	2D-MNT
1	IND R_2/75*	1.00	1.00
2	G-31/07	1.00	0.52
3	G-54/07	0.75	1.00
4	G-11/09	0.32	0.44
5	G-19/09	0.37	0.39
6	G-25/09	0.33	0.48
7	G-30/09	0.69	0.95
8	G-52/10	0.50	0.50
9	G-53/10	0.71	0.68

*Reference vaccine strain

'r' value (0.32 - 0.37). The 'r'-values (Table 3) of field isolates as determined by 2D-MNT revealed that all the isolates have antigenic similarity with the vaccine strain. The isolates showed an 'r' value > 0.30 with IND R_2/75. The isolate G-54/07 showed very close relationship (100%).

The comparison of antigenic relationship by LPBE and 2D-MNT revealed that the 'r' values obtained by 2D-MNT were marginally higher than the values obtained by LPBE. Majority of the isolates were found to be closely related with vaccine strain in both the assays. In case of G-52/10, the 'r' values obtained by LPBE and 2D-MNT were similar to each other. However, the values obtained by the two tests did not differ significantly.

DISCUSSION

As neutralizing antibody titre correlates well with protection in the animal, two dimensional-micro neutralization test (2D-MNT)) is used as gold standard and reference test system for vaccine strain selection and serological differentiation of FMD virus strains. This test is not influenced by antigen-antibody reactions involving non-immunogenic antigen (Pay, 1985) and involves exclusively the antigenic determinant on the virus capsid. The results of neutralization tests are expressed as 'r'-values and calculated as the ratio between heterologous and homologous serum titre as per the criteria of Rweyemamu (1984). Relationship values ('r') of more than 0.30 indicate a close relationship between the vaccine strain and the field isolate. Conversely, values less than 0.30 suggest that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect. Liquid phase blocking ELISA has also been used for comparison of the field isolates of FMD viruses with vaccine strain which is comparable to the virus neutralization test (VNT) (Hamblin *et al*., 1986; Arauzo *et al.*, 1996).

All the eight isolates used in the present study had antigenic relationship r >0.3. This indicated close antigenic relationship with the vaccine strains. Similar observation was made by Sarma and Sarma. (2003), who reported close antigenic relationship of field isolates of FMDV type 'O' with reference vaccine strain in Assam. The annual report (2008-09) of Project Directorate on FMD also revealed that about 94% of field isolates of type 'O' were closely related to the reference vaccine strain. The present observation also corroborates with the findings of Samuel *et al.* (1990), in Thailand and Rai (1980) in India. They reported close antigenic relationship of field isolates of type 'O' with vaccine strain. Jangra *et al*., 2005, also reported similar result in case of type 'A' virus. Sanyal *et al.* (2003), who used LPBE to determine the polyclonal relationship of the field isolates of type 'Asia-1' with vaccine strain virus concluded that the current vaccine was expected to give a good antigenic coverage. Likewise, Linchongsubongkoch *et al.* (2000) who reported that field isolates of type 'Asia-1' had close antigenic relationship with the vaccine strain in Thailand. On the other hand, three of the isolates had a weak antigenic relationship (r < 0.4) with the vaccine strain in LPBE.

A difference between 2D-MNT and LPB ELISA were observed in case of the isolates like in case of G-54/07 showed 75% homology in case of LPB ELISA where as it showed an 100% homology in case of 2D–MNT in relation with the vaccine virus. Similar kind of discrepancy was seen in case of G-31/07, which showed 100% homology in LPBE, where as it showed an 52% homology in case of 2D–MNT. Similarly, G-30/09 showed 69% homology in LPBE and 95% homology in 2D-MNT with the vaccine virus. The difference may be due to the antibody population measured by the two assays. The ELISA is more discriminating than 2D-MNT test and field observation tend to bear out results obtained with the ELISA (Samuel *et al.*, 1990). The change in the

antigenic relationship of the Middle East type 'O' isolates away from European to the Middle East reference strain (01/BFS, 1860) in 1988 was identified using ELISA technique before it was seen using serum neutralization test (SNT). In contrast, Gleeson *et al*., (1994) studied the relationship between type 'O' FMD virus isolates and vaccine strain in Thailand. He observed slightly less close relationship as compared to that shown by SNT.

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