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Research Article

SEROMONITORING OF FOOT-AND-MOUTH DISEASE VACCINATED ANIMALS IN EIGHT DISTRICTS OF HARYANA

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ABSTRACT

Foot-and-mouth disease (FMD) is a notoriously contagious and economically devastating viral disease of artiodactyles. In most Asian countries including India, FMD is still endemic and severely limits the region's ability to participate in international livestock trade. The IX phase vaccination campaign was executed and antibody titres monitored against foot-and-mouth disease virus (FMDV) serotypes O, A and Asia-1. FMD-Control Programme (FMD-CP) is being implemented in eight districts (Bhiwani, Fatehabad, Hisar, Jhajjar, Jind, Rohtak, Sirsa and Sonipat) of Haryana. As a part of the FMD-CP, a total of 1600 (800 pre- and 800 four weeks post-vaccination) serum samples collected from eight districts of Haryana under FMD-CP were seromonitored by Liquid Phase Blocking ELISA (LPBE) to determine vaccinal immune response. The overall percent of animals demonstrating protective LPBE antibody titres ($\geq$1.8 $\log_{10}$) against FMDV serotypes O, A and Asia-1 were 44.10, 40.60 and 26.40, respectively in pre-vaccinated and 83.10, 74.90 and 62.20, respectively in post-vaccinated cases from eight districts under FMD-CP. In cattle, the percent of animals demonstrating protective LPBE antibody titres against FMDV serotypes O, A and Asia-1 were 37.50, 33.20 and 21.80 in pre-vaccination; and 83.80, 76.00 and 63.00 in post-vaccination serum samples, respectively. On the other hand, 50.80, 48.00 and 31.00 percent buffaloes (pre-vaccination); and 82.50, 73.80 and 61.50 percent buffaloes (post-vaccination), respectively demonstrated protective antibody titres against FMDV serotypes O, A and Asia-1, respectively.

Key words: Foot-and-mouth disease, FMD-Control Programme, LPB ELISA, seromonitoring

Foot-and-mouth disease (FMD) is a highly contagious disease affecting both domestic and wild cloven-hoofed animals (OIE, 2012). FMD is economically the most devastating disease of farm animals due to loss in production and interference with international trade (Rufael *et al*., 2008). In most Asian countries including India, FMD is still endemic and one of the biggest impediments to growth of the livestock sector. The annual total economic loss due to this disease in India ranges from Rs. 12,000 crore to 14,000 crore (Singh *et al*., 2013). Foot-and-mouth disease virus (FMDV) is the type species of the genus *Aphthovirus* within the family *Picornaviridae*, order *Picornavirales* (Le Gall *et al*., 2008). The disease is characterized by fever, lameness and vesicular lesions on the tongue, feet, snout and teats. Although, FMD does not result in high mortality in adults, the disease has debilitating effects including weight loss, decrease in milk production and loss of draught power resulting in a loss in productivity for a considerable time. Mortality can be high in young ones. The clinical signs can appear within 2 to 3 days after exposure and can last for 7 to 10 days.

The hallmarks of the FMDV infection include rapid replication of the virus, ability to infect even in small doses, the multitude of transmission routes and the multiple virus antigenic types and subtypes (Rodriguez and Grubman, 2009), which make the FMD very difficult to control. Thus the serological studies assume far more significance in endemic country like India. Since, humoral immune response is mainly responsible for protection against FMDV (Robinson *et al*., 2011) thus the seromonitoring forms an important component of FMD control programme (FMD - CP). The present study describes the status of the vaccinal immune response in bovines of eight districts of Haryana under FMD - CP during the IXth phase of vaccination.

MATERIALS AND METHODS

One hundred (50 pre- and 50 post-vaccination) sera sample each from cattle and buffaloes vaccinated against FMDV from eight districts (Bhiwani, Fatehabad, Hisar, Jhajjar, Jind, Rohtak, Sirsa and Sonipat) under FMD-CP were received in the year 2009 at Regional Research Centre on FMD, Department of Veterinary Microbiology of the university. Pre-vaccination serum samples were those which were collected before starting of IX phase vaccination while post-vaccination serum samples were those that were collected after four weeks of IX phase vaccination.

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Thus, a total of 1600 (800 pre- and 800 post-vaccination) serum samples collected from the eight districts of Haryana were used in the present study.

Detection and Quantification of Serotype Specific Antibodies Against Structural Proteins of FMDV Using Liquid Phase Blocking ELISA (LPBE): All the reagents used were generous gift from Central FMD Laboratory, Project Directorate on FMD, IVRI Campus, Mukteswar. LPBE was used for the detection of antibodies against FMDV as described by Hamblin *et al*. (1986). Briefly, 96 well flat bottom polystyrene micro ELISA plates (Nunc Maxisorb, Roskilde, Denmark) were coated with 50 µl/well of anti-146S rabbit sera optimally diluted in carbonate-bicarbonate buffer, pH 9.6 and kept at 4^0C overnight. Two-fold dilutions of the test sera starting with 1:16 upto 1:128 dilutions was made in phosphate-buffer-saline containing 0.1 % (v/v) Tween-20 in the master plate and mixed with equal volume of virus along with positive and negative controls and incubated overnight at 4^0C in the carrier plates. Next day, the pre-coated plates were washed thrice with washing buffer. Then, 50 µl/well of each dilution of test sera/virus mixtures was transferred in the corresponding wells in pre-coated ELISA plates and incubated for 1 h at 37^0C. After washing thrice with washing buffer, 50 µl/well of guinea pig anti-FMDV type specific serum (tracing serum) optimally diluted in blocking buffer was added in all wells of the respective plates and incubated for 1 h at 37^0C. Following washing thrice with washing buffer, 50 µl of polyclonal rabbit anti guinea pig IgG horse radish peroxidase (HRPO) conjugate (Sigma-Aldrich) optimally diluted (1:4000) in blocking buffer was added in all the wells and incubated further for 1 h at 37^0C. Finally, after washing thrice, 50 µl of freshly prepared substrate solution of orthophenylene-diamine (OPD)/H_2O_2 (Sigma Chemicals Co.) was added to each well and plates were left in dark for the development of colour. The reaction was stopped by adding 50 µl/well 1 M sulphuric acid. The optical density (OD) of the wells was measured using ELISA reader at 492 nm.

Estimation of Antibody Titres: The percent inhibition in each well was calculated in relation to antigen control using the formula:

$$\text{Percent inhibition} = 100 - \frac{\text{OD of the test well - Background OD}}{\text{OD of antigen control - Background OD}} \times 100$$

Antibody titres were expressed as 50 % end-point titre i.e. the reciprocal $\log_{10}$ dilution at which the wells with test sera showed 50 % inhibition with respect to antigen control wells was considered to be the titre of the serum. The serum samples which had antibody titres of ≥ 1.8 $\log_{10}$ were considered to have protective immune response against FMD virus infection (Anon, 2008).

RESULTS AND DISCUSSION

In cattle, percent animals demonstrating protective LPBE antibody titres (≥ 1.8 $\log_{10}$ antibody titres) against FMDV serotypes O, A and Asia-1 were 37.50, 33.20 and 21.80, respectively, in pre-vaccination and 83.80, 76.00 and 63.00 in post-vaccination serum samples, respectively. On the other hand, percent buffaloes demonstrating protective antibody titres were 50.80, 48.00 and 31.00 in pre-vaccination and 82.50, 73.80 and 61.50 in post-vaccination serum samples, respectively. Overall (cattle and buffaloes) percent animals demonstrating protective antibody titres against FMDV serotypes O, A and Asia-1 were 44.10, 40.60 and 26.40, respectively, in pre-vaccinated and 83.10, 74.90 and 62.20, respectively, in post-vaccinated serum samples (Table 1).

The FMD-CP was launched in 2004 covering 54 districts across the country [including eight districts of Haryana during X Five year plan (2002-07)] to create FMD free zones. The livestock population in eight districts was administered inactivated oil adjuvanted trivalent FMD vaccine bi-annually. Sero-monitoring of vaccinal immune response against FMDV in Haryana, the backbone of FMD-CP is regularly being done by this centre using the LPBE. Besides, a continuous surveillance for the FMDV is also undertaken by this centre. After the start of FMD-CP, the outbreaks have been reduced to a greater extent as only one outbreak occurred in the year 2008 (Anon, 2009). An inactivated (mineral oil adjuvanted) vaccine that contains a mixture of FMD serotypes O, A and Asia-1 is being currently used in India (Nagarajan *et al*., 2008). Higher percent of animals demonstrating protective antibody titres during post-vaccination indicated that the vaccination resulted in boosting up the immune response of both cattle and buffaloes against the three FMDV serotypes i.e., O, A and Asia-1. These findings are in agreement with the observations recorded by Moonen *et al*. (2004) and Cox *et al*. (2005). But the target level of herd immunity was achieved only against FMDV serotype O and not against serotype A and Asia-1. When proportions of protected animals reach a critical level (approx. 80 %) then herd immunity is achieved and new introduction of virus will not result in an outbreak of disease (Anderson and May, 1985).

In the present study, the buffaloes demonstrated

Table 1

Vaccinal protective immune response of animals in eight districts of Haryana following IX phase of vaccination

Species	No. (%) of animals showing titers ≥1.8 $\log_{10}$ against FMDV					
	Serotype O		Serotype A		Serotype Asia-1	
	Pre-Vac	Post-Vac	Pre-Vac	Post-Vac	Pre-Vac	Post-Vac
Cattle	150 (37.50%)	335 (83.80%)	133 (33.20%)	304 (76.00%)	87 (21.80%)	252 (63.00%)
Buffalo	203 (50.80%)	330 (82.50%)	192 (48.00%)	295 (73.80%)	124 (31.00%)	246 (61.50%)
Cattle and buffaloes	353 (44.10%)	665 (83.10%)	325 (40.60%)	599 (74.90%)	211 (26.40%)	498 (62.20%)

better protective immune response as compared to cattle. It is difficult to explain the possible reason and further studies are needed on this aspect. Furthermore, since the start of FMD-CP in Haryana most of the outbreaks were recorded in cattle despite the fact that both cattle and buffaloes were housed together. Percent animals demonstrating ≥1.8 $\log_{10}$ antibody titres were highest against FMDV serotype O followed by serotype A and Asia-1 in both pre- and post–vaccination samples. Similar type of results were also observed during the VIII phase of FMD vaccination as overall (cattle and buffaloes) percent of animals demonstrating protective antibody titres against FMDV serotypes O, A and Asia-1 were 61.34, 56.69 and 49.45, respectively, in pre-vaccinated and 83.70, 74.41 and 71.49, respectively, in post-vaccinated serum samples of VIII phase. This observation is in agreement with the findings of Patil *et al*. (2002). The relevant explanation for this observation can be that FMDV serotype O is the most predominant type in India (Subramaniam *et al*., 2013). If the animals were having natural infection with FMDV serotype O, the antibody titres would be higher against the same. Further, it might be possible that FMDV serotype O is more immunogenic than serotype A and Asia-1 or the vaccine was having more amount of FMDV serotype O antigen.

Although, no significant incidence of the disease have been reported after launching FMD-CP in the state, yet the vaccination campaign should be carried out stringently, meticulously and honestly for longer period to create and maintain protective herd immunity. This will lead to reduction in the effective virus transmission and subsequent decline in the FMD carrier animals in Haryana.

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