Kinetics of humoral immune response in pigs vaccinated against foot and mouth disease

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The present investigation was conducted to study the foot and mouth disease virus (FMDV)-specific humoral immune response (HIR) in pigs, following vaccination with oil adjuvanted foot and mouth disease (FMD) vaccine, up to 90 days post vaccination (dpv). For this, 40 Large White Yorkshire (LWY) pigs (20; one-year old female (gilts) and 20; three-month old piglets) were vaccinated @ 2 ml/animal, subcutaneously. Sera samples were collected at fortnight interval from all the animals. The log₁₀ SN₅₀ antibody titres against all the serotypes (Type O, A and Asia-I) were detected in both gilts and piglets from day 7 to 90 dpv indicating the persistence of HIR up to the last day of sampling. The maximum antibody titres were observed on 28 dpv, thereafter, titres started declining, but were present till 90 dpv against all the three FMDV serotypes. HIR was more pronounced in piglets in comparison to gilts, as group mean SN antibody titres against all the three FMDV serotypes were found to be more maintained and significantly higher in piglets.

Keywords: Foot and mouth disease virus, FMD vaccine, Gilt, Piglet, SN

Foot and mouth disease (FMD), a highly contagious disease, characterized by vesicular lesions is responsible for heavy economic losses in meat and milk producing countries.¹² Pig, cattle, buffalo, sheep and goat are the main domesticated species susceptible to this disease. It has been seen that pigs are quite resistant to infection (>800 TCID₅₀) as compared to cattle (10 TCID₅₀), but once disease occurred, the mortality rate is higher in pigs (mainly suckling piglets). Also the pigs are potent amplifier of FMD through aerosol transmission to other susceptible species mainly cattle.³

In India, there are reports of several FMD outbreaks in organized pig farms including that of Haryana. In developing countries like India, where disease is endemic, vaccination can play an important role in prevention and control of disease.⁴⁻⁶ Vaccination with inactivated FMD vaccine protect pigs against clinical disease⁷ as protective immunization has been shown to reduce greatly the excretion of FMD virus (FMDV) following exposure of pigs to infection.⁸⁻¹³ FMDV represents a good example of antibody mediated protective immunity to virus. A correlation has been established between high titres of circulating neutralizing antibodies and the capacity of vaccinated animals to resist experimental FMDV infection⁹⁻¹³.

In India, no effective FMD vaccine is available so far which can protect swine against FMD. However, some of the commercial vaccine manufacturers have launched oil adjuvanted FMD vaccine for pigs. There is paucity of literature on the systematic study of humoral immune responses in pigs following FMD-vaccination in India. The present study was planned to analyze the kinetics of humoral immune responses up to 90 days of post vaccination in gilts and piglets with an objective to assess the antibody response against various FMDV serotypes (O, A, Asia-1).

Materials and Methods

Cell culture and medium supplements — Baby hamster kidney (BHK-21) clones 13-cell line was used for preparation of viral antigens and for performing SNT. Cells were maintained in minimum essential medium (MEM; GIBCO BRL) supplemented with 5% fetal calf serum (FCS), lactalbumin hydrolysate, tryptose soya broth, sodium bicarbonate, strepto-penicillin and nystatin.

Virus — FMD virus reference serotypes O, A and Asia-1 were procured from Central Laboratory of Project Directorate on FMD, IVRI, Mukteshwar.
Kumaon, Uttrakhand, India. Antigens of FMD virus serotypes were grown in BHK-21 cell monolayer. The cells were harvested when they were showing 90% virus-specific cytopathic effect (CPE). The virus was harvested from the cells by freezing and thawing. The cell suspension was used as antigen of a particular serotype in SNT.

Experimental animals — A total of forty LWY pigs [20; one-year-old adult females (gilts), and 20; three months old piglets], were selected at Govt. Pig Breeding Farm, Hisar, India for this study. Identification of animals was made through ear tattooing. The animals were kept at different sheds [20; having 10 animals per shed. Gilts were divided into two groups A and B each of 10 animals, while piglets constituted group C and group D. Before transferring animals to these sheds, these were thoroughly cleaned and disinfected. The feed obtained from HAFED, Rohtak, India was fed (2.0 kg/day) to gilts and (1.25 kg/day) to piglets throughout the experiment. The water was provided ad libitum.

Vaccine — FMD vaccine containing quadrivalent O, A, C and Asia-1 strains of FMDV, BEL inactivated, adjuvanted with mineral oil and thiomersal (0.01% w/v) added as preservative, was used in the present study. All the animals were vaccinated by inoculating 2 ml of vaccine per animal, subcutaneously (sc). The animals were dewormed 15 days prior to vaccination.

Collection and processing of samples — For serum collection, blood samples (=5ml) without anticoagulant were collected from each animal of all the groups at fortnight intervals. Blood samples from group A and D were collected at odd week intervals [0, 7, 21, 35, 49, 63, 77 and 90 days post vaccination (dpv)], while from group B and C were collected at even weeks post vaccination (0, 14, 28, 42, 56, 70 and 84 dpv). The serum was separated, filtered and heat inactivated at 56°C for 30 min and stored at -20 °C till used.

Serum neutralization test (SNT) — The test was performed by constant virus and varying serum technique as per recommendation of OIE14. Serum samples were tested using two fold dilutions in triplicates in flat bottom tissue culture plates having 96 wells. Dilution of serum samples were made in a volume of 160 μl in complete MEM. From each (dilution) well, 50 μl was transferred to three different tissue culture plates meant to be used for three different serotypes (O, A and Asia-1). To these plates, 50 μl of a particular virus serotype diluted to 100 TCID₅₀ in MEM maintenance medium was added. The cell controls were kept without virus in each plate. Appropriate virus controls were also maintained. The plates were sealed with pressure tolerant cellophane tape and incubated at 37 °C for 1 hr. After 1 hr, 100 μl of BHK-21 cells suspension was added to each well having cells (1x10⁶cells/ml). The plates were again sealed and incubated at 37 °C for 2-3 days. The plates were examined daily for appearance of CPE. When virus control wells showed 90% CPE, the plates were unsealed, well’s medium was discarded with a jerk and the cells were stained with crystal violet dye (0.5%) for 1/2 hr. After washing and drying the plates, results were noted macroscopically. Positive wells, where the virus had been neutralized and the cells remained intact were seen to contain blue-stained cell sheet. The negative wells, where virus had not been neutralized, remained unstained. The SN antibody titres expressed as log₁₀ SN₅₀ were calculated by Karber method15.

\[
\text{Log}_{10} \ SN_{50} = \text{L-d (s-0.5)}
\]

where, L-log₁₀ of the most concentrated serum dilution tested; d-log dilution factor; and s-sum of proportions of culture protected.

Results and Discussion

Induction of humoral immune response (HIR) was demonstrated by determining SN antibody titers in the post vaccinal sera samples against various FMDV serotypes (Type O, Asia-1 and A). SN antibody titer has already been demonstrated to be a good correlate of HIR against FMD3,10,12,16.

The mean log₁₀ SN₅₀ antibody (Ab) titres against type O, type A and type Asia-1 on various days post vaccination for groups A, D, B and C are shown in Table 1. No statistically significant difference in SN Ab titres among various FMDV serotypes on various dpv was observed with in all the groups except in group C which showed significantly higher Ab titres (Table 1) against FMDV type Asia-1 on 14 dpv. These findings are in agreement with earlier studies5,13,17,18 where it has been reported that neutralization response against oil emulsion FMD vaccine can be first demonstrated only after 3-8 dpv.

The peak SN Ab titres in group A were obtained on 21 dpv against type O and Asia-1, while on 35 dpv against type A. In group B, the peak SN Ab titres against all the three FMDV serotypes were obtained on 28 dpv. In group C (piglets), the neutralizing antibody titres got the peak on 42 dpv against type O.
Table 1 — Serum neutralizing antibody titres of group A, B, C and D against different FMDV serotypes (O, A and Asia-1) as determined by SNT  

[Values are mean ± SE of 10 animals]  

<table>
<thead>
<tr>
<th>SN Ab titres (log$<em>{10}$SN$</em>{SO}$);</th>
<th>dpv</th>
<th>Group A</th>
<th>Group D</th>
<th>dpv</th>
<th>Group B</th>
<th>Group C</th>
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<td></td>
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<td>Type A</td>
<td>Asia-1</td>
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<td>Type A</td>
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<td>1.25±0.06</td>
<td>1.26±0.1</td>
<td>1.41±0.07</td>
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|                                 |     | Group A | Group D | Group B | Group C |
| mean                            |     | 1.36±0.03 | 1.37±0.02 | 1.34±0.03 | 1.38±0.02 | 1.36±0.02 |
| Group mean                      |     | 1.28±0.03 | 1.30±0.02 | 1.30±0.02 | 1.42±0.03 | 1.48±0.03 | 1.47±0.03 |

Mean of all the log Ab titres of one serotype in a group. Means having same superscripts do not differ significantly at \( P<0.05 \)

Means with superscripts are compared row wise using DMRT in a group. ND: Not done, dpv – Days post vaccination.
and type A and on 14 dpv against type Asia-1. In this group, blood samples could not be collected on 28 dpv, otherwise it was possible that peak Ab titres in group C might have been on 28 dpv against all the three serotypes. In group D, highest antibody titres against all the three FMDV serotypes were obtained on 21 dpv. Overall, we have obtained peak serum neutralizing Ab titres between 21-28 dpv following vaccination of pigs and piglets. This is in consistent with earlier studies\textsuperscript{16,19} which also reported peak neutralizing Ab titres on 21 dpv. SN Ab titres between 8-14 dpv and on 14 dpv\textsuperscript{20} in pigs vaccinated with oil adjuvanted FMD vaccine. In buffalo calves vaccinated with oil-adjuvanted FMD vaccine, peak SN Ab titres against different FMDV serotypes were recorded between 14-21 dpv. After getting peak titres, the Ab titres started declining on various days up to 90 dpv, but the Ab titres against all the three FMDV serotypes were still present on 90dpv. These findings are consistent with the earlier reports\textsuperscript{18,21}. All of them have reported the persistence of SN Ab titres at 90 dpv. One study\textsuperscript{12} had reported Ab titres persistence even at 112 dpv in pig after oil emulsion FMD vaccination. On the other hand, one report\textsuperscript{9} could not demonstrate the Ab titres even at 67 dpv in vaccinated pigs vaccinated against FMD.

Group A and D — Against FMDV type O, no significant difference in SN Ab titres between two groups on any day of sampling was found (Fig. 1). Against type A, significantly higher SN Ab titres were found in group D piglets on 49 and 90 dpv as compared to gilts. Against type Asia-1, significantly higher neutralizing antibody titres were found in group A on 7 and 35 dpv, while group D exhibited higher titres on 90 dpv. No significant difference in group mean antibody titre was found for Type O and Type Asia-1 between group A gilts and group D piglets while for type A, the group mean was significantly higher in piglets.

Group B and C — Against FMDV type O and A, significantly higher antibody titres were detected on 42, 70 and 84 dpv in group C piglets as compared to gilts (Fig. 2). Also against type Asia-1, significantly higher Ab titres were observed on 14, 70 and 84 dpv in piglets as compared to gilts. Group mean titres against all the three FMDV serotypes (O, A and Asia-1) were significantly higher in group C piglets as compared to gilts of group B.

Overall, the group mean serum neutralizing Ab titres in piglets (group D and C) against all the three serotypes viz., 1.41±0.02 for type O, 1.42±0.02 for type A and 1.40±0.02 for type Asia-1 were significantly higher as compared to gilts (group A and B) i.e. 1.32±0.02 for type O, 1.30±0.01 for type A and 1.31±0.01 for type Asia-1 and also the antibody titres were more maintained in piglets. This observation was in agreement with the earlier reports\textsuperscript{16} that reveal higher SN Ab titres against FMDV serotypes in piglets as compared to gilts. These workers have also emphasized that ideal age for vaccinating piglets, first time for FMD, should be 10-12 weeks of age and then the animals should be revaccinated just before breeding. In our study, the piglets were 3 month old at the time of vaccination and also gilts were vaccinated before breeding.
In the present study, low serum SN Ab titres against various serotypes were observed. Similar low serum antibody titers in pigs have also been reported earlier by many workers. It has been claimed that even low levels of Ab titers are able to protect the animal against challenge. Because of some restrictions imposed by Animals Ethical Act, it was not possible to study the protection against challenge at the end of experiment. Several workers have reported that protection cannot be entirely correlated to SN Ab titers as other mechanisms e.g. mucosal immunity, cellular immune responses and innate immunity do play an important role in process of protection in pigs. Francis and Black have found protection against various serotypes were observed. Similar low immunity do play an important role in process of protection in pigs.

References


