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Immunological Evaluation of Influence of Dose and Route of Immunization with FMD Serotype 'A' Vaccine in Guinea Pigs

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Abstract

A study has been conducted to measure the immunization efficacy of route of inoculation between intra dermal (ID) and intra muscular (IM) in guinea pigs, using FMD vaccines. Three different vaccine formulations of varying concentrations containing full dose, 1/5th dose, 1/10th dose were prepared and injected either intra muscularly or intra dermal to six different groups of guinea pigs. At 30th day post vaccination, all the animals were challenged. Efficacy of route was defined in terms of serum neutralizing antibodies, IgG1 and IgG2 isotype response and challenge studies. When compared with guinea pigs that were intramuscularly vaccinated with a full dose vaccine, guinea pigs vaccinated intra dermal with 1/5th dose of the vaccine were 60% protected against clinical disease. Thus, we conclude that ID route is a good alternative for IM route, as ID route has shown to induce a very efficient immunological response against FMD. Moreover, the dose required by the ID route is lower compared to the IM route with an added benefit of reduced requirement of antigen and costs per dose of FMD vaccine.

Keywords: Foot and mouth disease, vaccination, guinea pigs.

Introduction

Foot-and-mouth disease virus (FMDV) causes a high impact disease of cattle and other cloven-hoofed animals, with severe agricultural and economic implications(1, 2). Globally, the only well accepted disease control strategy aims at obtaining a long-term elimination of the virus, especially when it is associated with sanitary measures such as quarantine and farm biosecurity along with culling the infected animal (3). Till date, vaccination through intra muscular route is the only licensed method to control the disease around the globe (4). Hence various strategies like increasing antigen content, increasing the number of immunizations, adding adjuvant and/or alternative immunization routes are gaining importance to improve vaccine immunogenicity. The trials of intra dermal (ID) immunizations against various infectious diseases were undertaken in many animal species *viz*. cattle, sheep, pigs, and also human beings (5). This is mainly to exploit dermal dendritic cells that are known for its unique capacity to initiate primary T cell responses and stimulate memory responses (6, 7). ID mode of immunization confers better immunological responses because of the presence of several antigen presenting cells such as dermal dendritic

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cells and Langerhans which enhance the efficacy of the administered vaccine (8). In addition, recent studies in several parts of the world have revealed that Intramuscular (IM) mode of immunization renders fibrosis or formation of granuloma. Especially in pigs, this poses greater economic loss because this is one of the most expensive parts, which has to be cut and discarded owing to the deformation (9).

Earlier studies by Eble and coworkers showed ID vaccination of pigs against FMD at 1/ 10^{th} dosage confers comparable vaccine efficacy as IM vaccination with a full dose (10). These results suggest that, the current vaccine stocks can be extended many folds using ID inoculation. This is supported by other studies with hepatitis B, rabies and influenza virus, tuberculosis, cholera, Aujeszky's disease vaccines suggesting that ID vaccination results in enhanced immunogenicity (11, 12, 13, 14, 15). In view of this, the present study was conducted to compare the efficacy of ID administration of FMD vaccination to guinea pigs with intramuscular FMD vaccination using full dose, $1/5^{th}$ dose, and $1/10^{th}$ dose.

Materials and methods

Healthy Albino guinea pigs of both the sex weighing 450-500 g were grouped into seven groups, each consisting of twelve and maintained at Animal Experimental Station, Indian Veterinary Research Institute, Yelahanka, Bengaluru. All the groups were negative for neutralizing antibodies against FMD when the animals were immunized on day 0, with different dose and routes of inoculation, as shown in the **Table 1.** Different formulations of vaccines were prepared by incorporating different concentration of the inactivated FMD Virus type A IND 17/82 antigen in double oil emulsion adjuvant in different formulations. Volume of the vaccine injected was kept constant for all the animals.

Vaccine was injected at multiple sites on dorsal thoracic and lumbar region. After vaccination, the animals were observed daily for systemic and local reactions at the vaccination site. Blood was collected on 0 and 28^{th} day post vaccination and 15th day post challenge, from all the groups using silica coated vacutainers (Becton Dickinson and Company, UK). After challenging, clinical signs like rectal temperature and vesicle score for the disease symptoms were recorded daily.

Virus neutralization test *:* A modified method of OIE (16) was employed to estimate the serum-neutralizing antibody titre. Briefly, a twofold dilution of serum was mixed with pre-titrated viral suspension containing 100 $TCID_{50}$ (Tissue Culture Infective Dose 50) of FMDV in flat bottomed 96-well microtitre plates (Nunc, Denmark) and allowed to stand for 1 hr at 37^0C in a humidified chamber with 5% CO_2. Each serum sample was tested in duplicate. BHK21 cell suspension was added and incubated for 48 h at 37^0C with 5% CO_2. Cells were observed for cytopathic effects (CPEs) and end-point titres were calculated as the reciprocal of the final serum dilution that neutralised 100 $TCID_{50}$ of FMDV in 50% of the wells.

Isotype-specific ELISA : On all serum samples ELISAs were performed for FMDV specific IgG1and IgG2 antibodies. Briefly, Polystyrene 96 wells micro titre plates (NUNC maxisorp) were coated overnight at 4°C with purified inactivated virus in carbonate buffer. Serum samples (1:200 dilution series) were added and after incubation for one hour at 37°C, peroxidase conjugated goat anti-guinea pig IgG1 and/or goat anti-guinea pig IgG2 (ICL) at 1:5000 in 1% BSA/PBS-T were added and incubated. Following which the samples were washed and substrate OPD-H_2O_2 added for the development of colour. Finally the reaction was stopped with the addition of 1M H_2SO_4 and absorbance was measured at 492nm.

Challenge test: Challenge test was carried out on 30th day after immunization to all the groups. Guinea pigs were challenged through foot pad injection of 100 $GPID_{50}$ of FMDV. All animals were monitored for major clinical signs of FMD, particularly characteristic vesicular lesion on the

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footpads for 7 consecutive days after challenge. The kinetics of the appearance of lesion was noted and severity of infection was scored. Animals showing primary lesions at the site of virus injection, without spreading to other feet throughout the period of observation were scored as 1+ and were considered as "protected". Animal showing delayed and mild secondary vesicle only on hind footpad without spreading to forefeet's were scored as 2+. Animals showing typical but delayed secondary lesions on both hind feet and at least on one forefoot were scored as 3+. Animals those reacted severely showing secondary lesions on all four feet within 48hr of challenge and high fever were scored as 4+. Any secondary lesions were considered as a sign of systemic viremia, and animals displaying such lesions (i.e. score 2+, 3+ and 4+) were considered as "unprotected."

Statistical analysis: ELISA and Log10SN_{50} data were analyzed with the aid of GraphPad Prism 5.0 software (San Diego, CA). Bonferroni post tests, two-way analysis of variance was used to compare the data between all the groups. P value of less than 0.005 was considered statistically significant and showed significantly high level of protection as compared to that of the control group.

Results

After vaccination, no systemic reactions were observed in any of the vaccinated guinea pigs. In the IM vaccinated guinea pigs no local reactions could be seen at the location of vaccination. In the ID vaccinated guinea pigs, a swelling at the location of vaccination could be observed with a diameter that differed per individual (ranged from 0.5 to 0.8 cm). Subsequently bleb got resolved within several minutes indicating the safety of ID inoculation.

Serology: Neutralizing antibody titers against FMDV type A antigen in all guinea pig serum samples were measured using the micro neutralization assay and end-point titers were calculated as the reciprocal of the final serum dilution that neutralized 100 $TCID_{50}$ of FMDV in 50% of the wells. The virus neutralizing antibody titer expressed in mean log10 SN_{50} per ml, were shown in Table1 and Fig. 1.

Briefly, all vaccinated groups of guinea pigs developed neutralizing antibodies against FMDV after vaccination but the individual group titers varied. The titers of the ID vaccinated guinea pigs were in general higher than those of the IM vaccinated guinea pigs. For comparison of results, group A (IM vac 0.6ìg) was taken as standard. All guinea pigs which were sero negative on day 0 showed sero positive at 28th day and on 45th day post vaccination. After challenge, i.e. on 45 days post vaccination, irrespective of vaccination all groups showed increased levels of antibody

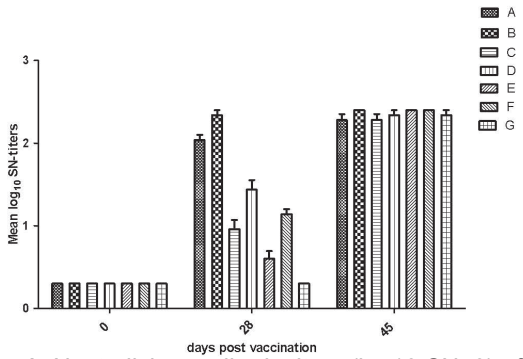

Fig. 1: Neutralizing antibody titers (log10 SN50) of FMD type A 17/82 vaccine in different groups of guinea pigs at different time interval.

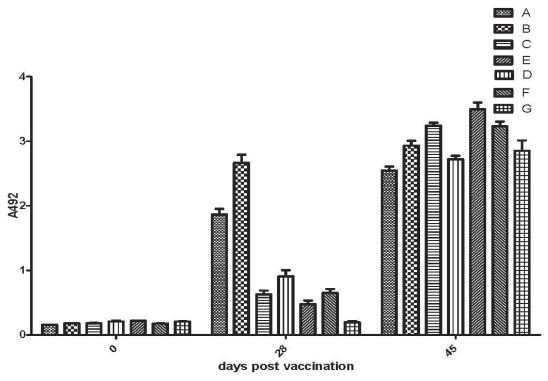

Fig. 2: Mean OD values of FMD Type A specific IgG1 antibody response of various vaccinated groups of guinea pigs at different time intervals

response. Statistically, at 28 days post-vaccination the SN-titers of the guinea pigs of all vaccinated groups differed significantly ($p<0.05$) from the non-vaccinated ones. The mean titers were found to be highest for group B (ID vac 0.6ìg) followed by group A (IM vac 0.6 ìg) but no significant difference ($p<0.005$) was observed among these groups. Among low dose vaccinated groups, Group D (ID vac 0.12ìg) differed significantly ($p<0.05$) from F (ID 0.06ìg) and other low dose C (IM vac 0.12 ìg) and E (IM vac 0,06 ìg) IM vaccination groups with regard to SN titers. Antibody response on 45 DPV was found to be higher (>2) in all groups and no significant difference was observed among the groups.

Isotype-specific ELISAs response: On all serum samples, ELISAs were performed for FMDV specific IgG1 and IgG2 antibodies. Generally IgG2 response was found to be higher in all the vaccinated animals. Mean response was found to be higher in all the ID vaccinated groups comparable to the IM vaccinated animals. The mean titers of all groups were shown in Table. 2 and 3, Fig 2 and 3.

The IgG1 and IgG2 response against FMDV 'A IND 17/82' were found to be significantly higher ($P<0.05$) at day 28 in all the vaccinated groups compare to the non-vaccinated controls. In addition, the mean titers were higher in group B

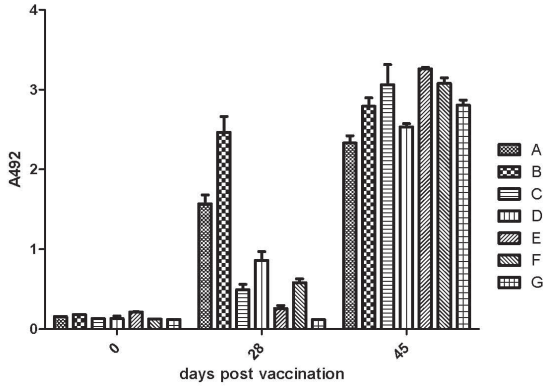

Fig. 3: Mean OD values of FMD Type A specific IgG2 antibody response of various vaccinated groups of guinea pigs at different time intervals

followed by group A, D, F. C and E. At day 28, mean titers of group B (ID vac 0.6 ìg) was found to be significantly higher ($p<0.05$) than group A (IM vac 0.6ìg) but no significance was found with group B (ID vac 0.6 ìg group). Among low dose immunized groups, group D (ID vac 0.12ìg) was found to be highly significant ($p<0.05$) than other low dose groups except for group F (ID vac 0.06ìg). At 45th day post vaccination, both IgG1 and IgG2 response was found to be higher in all the groups. Specifically, C, E and F groups showed higher response compared to their response on day 28.

Viral challenge test in guinea pigs: Guinea pigs were challenged with FMD Type A IND17/82 on day 30 post immunization along with unimmunized control group. Animals were observed for one week after challenge and scored at the end of observation period (Table. 4). All control group animals developed the major clinical sign of FMD, such as appearance of vesicles on the feet and fever within 48 hr of challenge. None of the guinea pigs from group B (ID vac 0.6ìg) developed generalized FMD after challenge, although vesicles at the inoculated foot were observed. Except one animal, all guinea pigs from group A (IM vac 0.6ìg) showed 90% of clinical protection. Among the low dose vaccinated animal group, most of the guinea pigs developed lesions at the right (opposite to inoculated) legs, some showed within 48 hrs and some after a week. Only in group D (ID vac 0.12 ìg) about 60% of animals did not develop vesicle on the right leg and were found to be clinically protected.

Discussion

The effectiveness of ID route was demonstrated by the number of guinea pigs that were protected against generalization of FMD after challenge. Vaccination with Type A FMDV irrespective of antigen pay load and route of immunization, has induced antibody production in all the animals. ID vaccination has shown superior response over IM at all varying doses of immunization. Protective humoral response at 28 day post vaccination was observed through neutralization test in the groups where full dose

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Table 1: Experimental design and vaccine trial

Group	Route of administration	Antigen Administered	Vaccine dose	Formulations
A	IM	0.6 µg	0.2ml	A
B	ID	0.6 µg	0.2ml	A
C	IM	0.12 µg	0.2ml	B
D	ID	0.12 µg	0.2ml	B
E	IM	0.06 µg	0.2ml	C
F	ID	0.06 µg	0.2ml	C
G	IM		0.2ml	PBS

Table.2: Neutralizing antibody titers (log_{10} SN_{50}) of FMD type A 17/82 Vaccine in different groups of guinea pigs at different time interval.

Days post vaccination	**GROUPS**						
	A	**B**	**C**	**D**	**E**	**F**	**G**
0 DPV	0.30±0.0	0.30±0.07	0.30±0.0	0.30±07	0.3±0.06	0.3±0.06	0.3±0.0
28 DPV	2.04±0.6	2.3±0.0	0.9±0.12	1.4±0.12	0.6±0.1	1.1±0.1	0.3±0.0
45 DPV	2.29±0.73	2.4±0.0	2.2±0.073	2.3±0.06	2.40±0.0	2.40±0.0	2.3±0.06

***Note**: A= IM vac 0.6µg, B=ID vac 0.6 µg, C=IM vac 0.12 µg. D= ID vac 0.12 µg, E= IM vac 0,06 µg, F=ID vac 0.06 µg, G= Non vac. Each value is mean of ten values ± SE (each sample assayed in duplicates). DPV: Days post vaccination.*

vaccine was injected irrespective of route. Among low dose immunization groups, only some of the animals from 1/5th dose intra dermally vaccinated group showed protective titers. Upon challenge with the virus, 30th post vaccination all the groups showed high titers.

Antigen specific IgG1 and IgG2 response pattern followed the SNT titers. Increase in neutralizing antibody response on 45 days post vaccination in group C, E and F indicates the boosting effect of infection after vaccination. Factors like type of vaccine, strain or species used in study, boosting the vaccine dose, specificity of the anti-isotypic antibody (17) used for vaccination place a role in directing the level of isotype response to FMD. Aluminium hydroxide/ saponin adjuvanted vaccines generally produces response more towards IgG1 and peptide vaccines produces more towards IgG2 (18). The booster dose of the same antigen evokes an important increase in IgG2a subclass levels in the ID route for malaria synthetic peptides loaded microparticle vaccination (19).Serological and mucosal immune response study conducted by Eble et al (20) with FMDV O TAW 3/97 antigen elicited more IgG2 response than IgG1. Whether or not the IgG1 and IgG2 isotypes against FMDV have different protective capacities is not clearly understood, but a balanced Th1 and Th2 response is required for the immune response to be effective in any disease (21, 22). In the present study, Guinea

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Table.3: Serum IgG 1 response assayed by indirect ELISA against FMD type A 17/82 in different immunized groups of guinea pigs at different time intervals.

Days post vaccination	GROUPS						
	A	**B**	**C**	**D**	**E**	**F**	**G**
0 DPV	0.15±0.0	0.18±0.0	0.13± 0.03	0.16±0.0	0.21±0.01	0.12±0.03	0.12±0.0
28DPV	1.57±0.1	2.4±0.2	0.49±0.06	0.86±1.09	0.26±0.03	0.58±0.04	0.12±0.04
45 DPV	2.3±0.9	2.7±0.1	3.26±0.01	2.53±0.04	3.06±0.25	3.08±0.0	2.8±0.04

Each value is Mean OD±S.E of ten animals in each group (each sample assayed in duplicates.

Table.4: Serum IgG 2 response assayed by indirect ELISA against FMD type A 17/82 in different immunized groups of guinea pigs at different time intervals.

Days post vaccination	GROUPS						
	A	**B**	**C**	**D**	**E**	**F**	**G**
0 DPV	0.15±0.03	0.16±0.06	0.17 ±0.03	0.23± 0.06	0.23±0.3	0.17 ±0.02	0.20±0.03
28 DPV	1.86±0.08	2.66±12	0.62±0.06	0.90±0.09	0.47±0.06	0.64±0.06	0.19±0.01
45 DPV	2.54±0.05	2.93±0.07	3.23±0.05	2.72±0.05	3.49±0.10	3.23±0.07	2.85±0.16

Each value is Mean OD±S.E of ten animals in each group (each sample assayed in duplicates).

pigs vaccinated with Montanide ISA-206 adjuvanted vaccines showed more IgG 2 response than IgG1 response. The possible reason to this could be due to adjuvant influence on the class switching of antibody. But both IgG1 and IgG2 responses has given protection to animals after challenge.

Challenge experiment in guinea pig confirmed the protection of antibody neutralization response. All animals injected with full dose vaccine intra dermally (group B) showed 100% protection and antibody titers were highest at 28 dpv compare to rest of the groups and reduced at 45 dpv suggesting the low level of viral replication. Similarly full dose vaccine injected intramuscularly showed protective levels of antibody response with 90% of protection after challenge. Among the 1/ 5^{th} and $1/10^{th}$ dose injected groups (group C, E and F) 0% protection was observed irrespective of route of immunization. Only in $1/5^{th}$ dose intradermally injected group 60% of animals exhibited protection. However the ID route of inoculation proved to be effective in our study did not correlated with the ID and IM comparison study against FMD in pigs where 1/ 10^{th} of the dose by ID inoculation has provided an effective immune response against FMD (10). The possible reason for this could be change in the Type of virus or quantity of full dose virus used for vaccination or host difference or the day of challenge. Our study is also is in agreement with a recent study that demonstrated the efficacy of ID route of vaccination using ISA 201 as an adjuvant which proved to more potent in preventing the formation of granuloma or fibrosis that is likely to be formed due to the IM delivery system (23).

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Table.5: Protective response of immunized Guinea Pigs after challenge with Type A FMDV

Scoring one week after challenge (No. of Guinea pigs in score/total no guinea pigs in group)					
Group	4+	3+	2+	1+	%of animals protected
A	0/10	0/10	1/10	9/10	90
B	0/10	0/10	0/10	10/10	100
C	0/10	1/10	9/10	0/10	0
D	0/10	0/10	4/10	6/10	60
E	0/10	6/10	4/10	0/10	0
F	0/10	2/10	8/10	0/10	0
G	10/10	0/10	0/10	0/10	0

Score 4+: *Guinea pig showing severs secondary lesions on all four feet within 48 hrs of challenge and high fever.* ***Score 3+****: Guinea pig showing delayed secondary lesions on both hind feet and at least on one forefoot.* ***Score 2+****: Guinea pig showing delayed and mild secondary vesicle only on hind feet without spreading to forefeet.* ***Score 1+:*** *Guinea pig showing primary lesions at the site of virus injection, without spreading to other feet throughout the period of observation and were considered as protected.*

Conclusion

In conclusion, the study indicated that ID route is more economical and better way of injecting the FMD vaccine as compared to intra muscular route with efficacy at even lower doses. Therefore, the same concentration of the vaccine can facilitate preparation of several doses higher for ID than for IM routes.

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