

COMPARATIVE PERFORMANCE OF FOOT-AND-MOUTH DISEASE VACCINE PREPARED USING DIFFERENT ADJUVANTS AND PAYLOADS

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ABSTRACT

Foot and mouth disease is a highly infectious and an economically important disease of livestock especially in a country like India, where agriculture and livestock sector contributes a major portion to the country's economy. Constant surveillance and regular mass vaccination is the key to control and eradicate the disease when extreme measure like slaughtering cannot be implemented. A potent vaccine forms the integral part of this control strategy and constant improvement in the immunogenicity of the vaccine is highly desirable. Apart from selecting a proper vaccine strain of virus and right payload of antigen, the importance of adjuvant cannot be overlooked and therefore oil adjuvant are been used as they provide a longer duration of immunity. Here in this study we have evaluated these two aspects of the vaccine. The higher payload of antigen provides with a higher and a longer duration of immunity which was tested serologically using VNT. The second study was done to compare two different oil adjuvant-Montanide ISA 50 V2 versus Polyvac 50, and was seen to have no significant differences in the antibody titres in the vaccinated animals amongst these two groups.

KEYWORDS: Foot-and-mouth disease; Vaccine; Virus neutralization test; Oil adjuvant; Antibody titre.

INTRODUCTION

Gaali Kuntu Khur Paka-Munh Paka as it's known in rural India or Foot-and-mouth disease (FMD) as it is known throughout the world is caused by the member of the genus *Aphthovirus*

belonging to the family *Picornaviridae*, which is divided into seven serotypes (O, A, C, SAT1, SAT2, SAT3 and Asia1) that infect the cloven footed ruminants and swine. The infection with one serotype does not confer immunity against another serotype. (OIE terrestrial manual 2012) The disease is contagious and fatal to cloven footed animal which is characterized by vesicle in the mouth, tongue, hoofs and nipples along with increased body temperature and loss of appetite. (Park 2012) The disease is perhaps one of the important animal diseases limiting the trade of animal products. (Rodriguez 2009). It is estimated that the direct loss contributed with this disease is more than Rs. 20,000 Crore (4.45 Billion dollars USD) per year (Patnaik et al 2012, PDFMD 2012). To control and limit the spread of the disease to the disease-free pockets of the countries, vaccination based control programs with trivalent oil adjuvant vaccines, are been implemented in the country involving regular bi-annual vaccinations of cattle and buffaloes of select area and regular active surveillance and monitoring of sero-conversion of antibody in vaccinated animals is been carried on (PDFMD 2012). Since the inactivated vaccine has a disadvantages of short duration of immunity, oil adjuvant vaccines have been shown to be more effective in conferring a longer duration of immunity than the aqueous adjuvant based FMD vaccines. (Patil et al 2002, Luborth et al 2007). This study is aimed at comparing the potency of FMD vaccine blend with international oil adjuvant to that of indigenously prepared oil adjuvant with similar payload of antigen on the sero-conversion of the animals been vaccinated.

Further this study also aims at studying the relation of antigenic payload in terms serum antibodies in vaccinated animals.

MATERIALS AND METHOD

Animals: Around 15 indigenous non vaccinated cattle calves of age group ranging between 8 to 10 months were housed in a open shed farm. Along with grazing and concentrates these calves were provided with dry hay and water ad lib. These calves were divided into three groups each containing 5 animals. The calves were de-wormed with anthelmintic suspension one week prior to immunization.

Vaccination: Each calf were injected with a single injection of 2 ml of the respective vaccine blend deep intra-muscularly in the neck region taking care of sterility of the site of injection. The calves were closely observed for 30 minutes after injecting the vaccine for immediate anaphylactic reaction and then were observed subsequently for 72 hrs for any local or systemic reactions to the vaccine. The calves were further monitored routinely till the end of trial.

Collection of Serum: 0 day blood samples were collected at the start of the trial and each calf was immunized with their respective vaccine blend. Subsequent blood collections were done on day 28th, 90th, 180th and 270th days post immunization. 10 ml of blood samples were collected in a sterile 15 ml tubes from each calf from each respective group and were allowed to clot. The serum separated from the clot was collected and transferred into a sterile 15 ml tube and then were heat inactivated at 50°C for 30 mins in water bath and then stored at -20°C for further testing.

Vaccine: The FMD trivalent vaccine was blend using three FMD serotype- O, A and Asia 1. Three blends of trivalent Foot and Mouth Disease virus vaccine were prepared using two adjuvant oils from two different sources and two different

antigen payloads. Blend 1 had normal antigen payload along with Montanide ISA 50 V2 as an adjuvant, Blend 2 & 3 consists of 25% less payload than Blend 1, with Montanide ISA 50 V2 and Polyvac 50 as an adjuvant.

Serum neutralizing antibodies: The heat inactivated serum samples were subjected to virus neutralization test (VNT) and the test was performed following the OIE Terrestrial manual. In brief, the serum samples were diluted two fold and then incubated with a virus dose of 100 TCID₅₀ for each respective FMD virus serotype (O, A & Asia 1) and incubated for 1 hour at 37°C. These samples were then transferred to BHK21 monolayer and incubated for 72 hours at 37°C. The CPE was used to determine the end point titres that were calculated as the reciprocal of the last serum dilution to neutralize virus in 50% of the well.

Statistics: All data are expressed as the mean \pm standard deviation (SD) unless otherwise specified. Analyses were performed with SPSS version 16.0 software (SPSS, Chicago, IL, USA). A non-parametric Mann-Whitney U test was used to analyze significant differences between the three vaccine blends at all the time point, amongst all the three serotypes. Differences were considered to be statistically significant when the P-values were \leq 0.05 or 0.01.

RESULTS

The vaccine Blend 1 and Blend 2 are having same adjuvant oil- Montanide ISA 50 V2 (SEPPIC, France) but the antigen payload for Blend 1 (FV/04/13) is 25% more than that of Blend 2 (FV/05/13). Whereas the antigen payload in Blend 2 (FV/05/13) and 3 (FV/06/13) are similar, but the adjuvant oil used is from different sources. The adjuvant oil used in Blend 2 is Montanide ISA 50 V2, (Seppic France) whereas the adjuvant oil used in Blend 3 is Polyvac 50 (Mukta, India).

The calves of all the groups did not show any acute or transcend untoward reactions to any of the vaccine blend after been injected. There was no local reaction at the site of injection in any of the calves of any of the vaccine blend group. Although some of the calves did show slight elevated in body temperature after 24 hrs, which subsided after 48-72 hrs. None of the calves showed any signs of depression or off feed. The data is been provided in Table 1.

The calves used in this trial were unvaccinated; however the 0 day serum samples from some of the calves showed detectable level of FMD specific antibodies but considered as sero negative. These could be attributed to the maternal antibodies transferred from the cow to its calf through colostrums. (Shankar 1982; Rajkumar 2008).

FMD vaccine Blend 1, 2 and 3 were tested in three groups of cattle calves each having five calves. These calves were vaccinated with a single 2 ml injection of respective blend of vaccine at 0 day and then the serum samples was collected at 0, 28th, 90th, 180th and 270th day post vaccinations.

The average VNT titres for 15 animals vaccinated with their respective vaccine blends at each time point is shown for serotype O, A and Asia1 (Fig. 1 & Fig. 2).

The Sero-conversion amongst each serotype of FMD virus-O, A & Asia1, is seen higher in the group vaccinated with vaccine Blend 1 in comparison to the group vaccinated with either of vaccine Blends 2 or 3 when observed till the end of the trial. The vaccine Blend 1 had shown a significant difference in serotype O to that of blend 2 at 270th day post vaccination and continued to remain high and above the protective titre of >48 throughout the trial. Although in serotype A there was a sudden drop in serum antibody titre at day 90th post vaccination, but

still it maintained above the protective titre of >32; and in serotype Asia1 it had maintained serum antibody titre of above >32, which is recommended for this type and showing significant difference with Blend 2 at 28th day post vaccination. The Blend 2 which had low antigen payload was seen to have not achieved the protective titre of >48 in serotype O, but could reflect the sero-conversion in serotype A and Asia1 above the protective titre of >32 and was seen to be maintained till the end of trail. The vaccines Blend 3 which had similar antigenic payload but different adjuvant, was seen to have similar pattern of sero-conversion in all the three serotypes of foot and mouth disease virus. Even though it had shown slightly higher titre in serotype Asia1 throughout the trial, but there was no statistical significance between them.

DISCUSSION

The commercial Foot-and-Mouth Disease vaccine is an inactivated trivalent vaccine against the three serotypes- O, A & Asia1, which are prevalent in here in India. The vaccine is supplemented with an oil adjuvant to enhance the immunogenicity of the vaccine. The FMD is a prevalent disease in India and so a constant surveillance of the disease along with mass vaccination practices with a potent trivalent vaccine is being undertaken by the government for individual states under the FMD control program- FMDCP (Pattnaiket al 2012; PDFMD 2012) As the disease is of an important economic importance, efforts are been made by the government to cover maximum cattle under this vaccination program. Similarly, efforts are been made by the vaccine manufacturer to improve and maintain the immunogenicity of the vaccine. The integral part of this process is the use of right adjuvant. It has been recognised worldwide that the oil adjuvant vaccine produces a longer immunity than the alum adjuvant and this is

desirable in case of FMD in an endemic country like India (Sivakumar et al 2011; Cloete et al 2008; Li et al 2013; Mohan et al 2013; Patil et al 2002, Luborth et al 2007)

In this study, we have attempted to evaluate the sero-conversion of antibodies against the serotypes-O, A & Asia1 of Foot and Mouth disease virus vaccine using three different vaccine blends, Blend 1(FV/04/13) having a normal antigenic payload and Montanide ISA 50 V2 oil adjuvant, Blend 2 (FV/05/13) having a lower payload of antigen and Montanide ISA 50 V2 oil adjuvant and Blend 3 (FV/06/13) having a lower payload of antigen and Polyvac 50 oil adjuvant.

The Blend 1 has shown a higher sero-conversion in the vaccinated animals than the Blend 2 as determined by the VNT test done on day 28th, 90th, 180th and day 270th. These observations correlate with antigenic payload used in blending these two formulations.

The quality and the duration of immune response shown by the vaccinated animals is correlated to the vaccine used to immunize; which partly is attributed to the antigen quantity used and the supporting agent like the adjuvant. Thus it can be seen from the above data that, when the adjuvant along with the blending agents and conditions are kept common, the antigenic payload would determine the amount of immune response elicited by the vaccinated animals which has also been observed by other researchers in the past (Barnett et al 2004; Cox et al 2006; Cox et al 2010; Madanmohan et al 2010). The duration of antibody titres in animals vaccinated with the Blend 1 were observed to be beyond day 270th and which could be attributed to the higher payload of antigen in comparison to the other blends and also to the oil adjuvant used (Aucouturier et al 2001; Barnett and Caraben 2002)

The second important aspect of a potent FMD vaccine is the adjuvant used to blend the vaccine. This fact is well accepted and therefore there has been a constant improvisation on the adjuvant technology which is evident by work done by several researchers (Zhou et al 2014; Cox and Bernett 2008; Arousa et al 2013; Selim et al 2010) and more emphasis has been made in the oil adjuvant field (Cloete et al 2008; Aucouturier et al 2001; Li et al 2013; Muhammad et al 2013).

The Blend 2 and Blend 3 were having a similar payload of antigen of all three serotypes of FMD virus, but had two different oil adjuvants. The comparison of the sero-conversion in the vaccinated animals was observed till 270th day post vaccination. Throughout the trial there were no significant differences seen in the VNT titre in between these groups in any of the serotypes in any of the animals. Although the Blend 3 had shown a higher mean antibody titre value in serotype A and Asia1 at 28th day, but the difference was not found significant when analysed using Mann-Whitney U test.

There was a sudden drop in the antibody titre for serotype A at 90th day post vaccination in all the three vaccine blends, this observation could not be explained and was thought to be due to environmental stress or due to a mild infection of FMD A serotype as there is a subsequent rise in the titre at 180th day post vaccination.

CONCLUSION

The above study concludes that a higher FMD antigen payload accompanied by an oil adjuvant is important to elicit a higher and a longer immune response. Further this study also concludes that the oil adjuvant Polyvac 50 is as efficient as Montanide ISA 50 V2, in eliciting an immune response against the FMD trivalent vaccine when blended with similar antigenic payload.

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Table no. 1: Health record of calves after vaccination

Sr. No	Animal No & Details	Vaccine Blend	Dose & Route of Inj.	Body temp (°F)					Local reaction (If any) Size: L X B (cm)				
				0 H	4 H	24 H	48 H	72 H	0 H	4 H	24 H	48 H	72 H
1	1061, (10 mth, Male)	1	2 ml (I/M)	100.6	100.8	102.0	101	101.4	Nil	Nil	Nil	Nil	Nil
2	1063 (8 mth, Female)	1	2 ml (I/M)	100.4	100.8	100.4	100.4	101.6	Nil	Nil	Nil	Nil	Nil
3	1064 (10 mth, Female)	1	2 ml (I/M)	100.2	100.4	100.2	101.2	101.4	Nil	Nil	Nil	Nil	Nil
4	1068 (10 mth, female)	1	2 ml (I/M)	101.2	101.0	101.4	102.6	102.8	Nil	Nil	Nil	Nil	Nil
5	1070 (8 mth, Female)	1	2 ml (I/M)	101.0	101.6	101.4	101.6	102.2	Nil	Nil	Nil	Nil	Nil
6	1071 (8 mths, Female)	2	2 ml (I/M)	100.0	101.4	103.6	103.2	102.6	Nil	Nil	Nil	Nil	Nil
7	1073 (10 mth, Female)	2	2 ml (I/M)	101.2	101.2	102.2	102.4	102.2	Nil	Nil	Nil	Nil	Nil
8	1074 (10 mth, Female)	2	2 ml (I/M)	101.4	101.4	101.2	101.2	100.8	Nil	Nil	Nil	Nil	Nil
9	1077 (8 mth, Female)	2	2 ml (I/M)	100.8	100.6	100.8	101.4	101.2	Nil	Nil	Nil	Nil	Nil
10	1078 (8 mth, Female)	2	2 ml (I/M)	101.2	101.2	101.4	101.0	101.6	Nil	Nil	Nil	Nil	Nil
11	1079 (8 mth, Male)	3	2 ml (I/M)	100.6	101.0	101.2	100.8	100.8	Nil	Nil	Nil	Nil	Nil
12	1080 (10 mth, Female)	3	2 ml (I/M)	101.2	101.2	101.8	102.2	102.2	Nil	Nil	Nil	Nil	Nil
13	1081 (10 mth, Female)	3	2 ml (I/M)	100.4	100.2	100.8	101.4	101.2	Nil	Nil	Nil	Nil	Nil
14	1082 (8 mth, Male)	3	2 ml (I/M)	100.2	100.2	101.2	101.0	101.4	Nil	Nil	Nil	Nil	Nil
15	1083 (10 mth, Male)	3	2 ml (I/M)	101.6	101.6	102.4	102.6	102.4	Nil	Nil	Nil	Nil	Nil

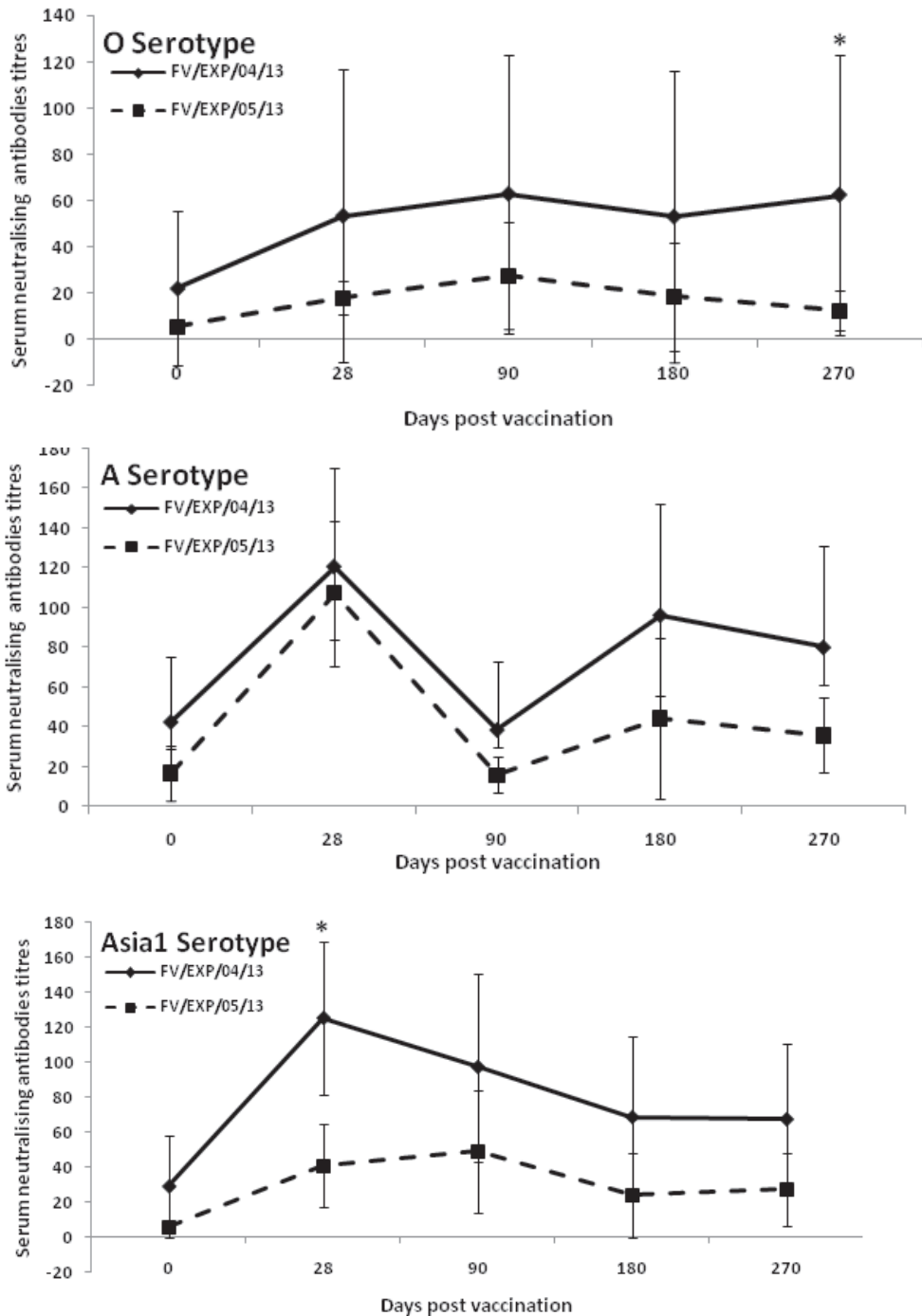


Figure 1: Serum neutralising antibody titres was detected using Virus Neutralising Test (VNT) of the serum samples collected on the day 0, 28, 90, 180 and 270 post vaccination from calves of vaccine group Blend 1 (FV/EXP/04/13) and Blend 2 (FV/EXP/05/13). Values are shown as Mean \pm S.D of 5 calves per group and were considered statistically significant. The values were considered statistically significant, if p values were ≤ 0.05 (*p ≤ 0.05)

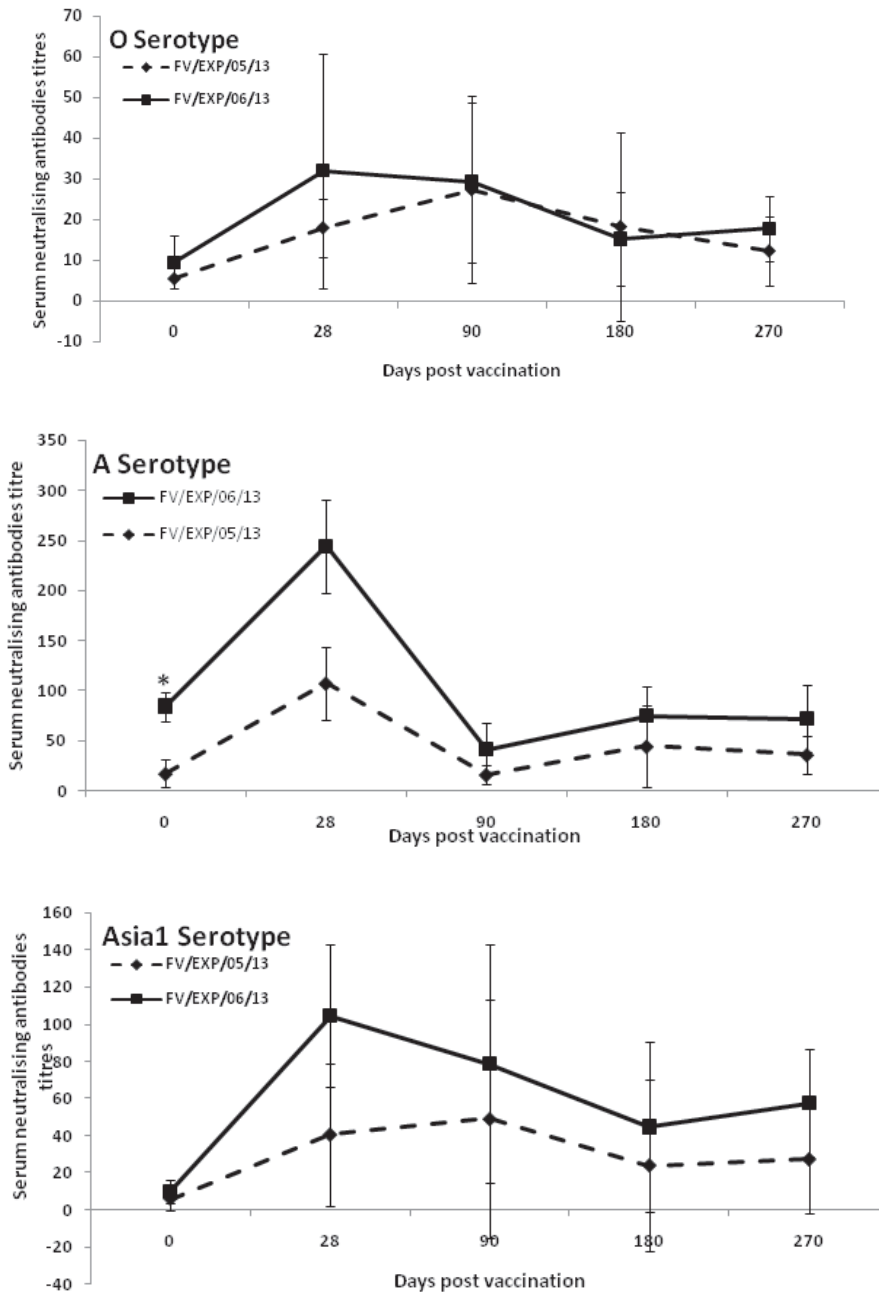


Figure 2: Serum neutralising antibody titres was detected using Virus Neutralising Test (VNT) of the serum samples collected on the day 0, 28, 90, 180 and 270 post vaccination from calves of vaccine group Blend 2 (FV/EXP/05/13) and Blend 3 (FV/EXP/06/13). Values are shown as Mean ± S.D of 5 calves per group and were considered statistically not significant. The values were considered statistically in significant, if p values were ≤ 0.05 (*p ≤ 0.05)