



Serological response against foot-and-mouth disease virus to FMD-haemorrhagic septicaemia-black quarter combined vaccine and FMD vaccine alone in sheep and goat

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ABSTRACT

Foot-and-mouth disease is subclinical in small ruminants and they may play role in spread of disease. Presently, they are not included in vaccination coverage in India under FMD-control programme. A total of 43 animals including sheep (16) and goat (27) were used for vaccination study by combined vaccine (FMD, HS and BQ) and FMD vaccine alone. Humoral immune response was evaluated by monitoring serum antibody titres against FMDV serotypes O, A and Asia 1 on 30, 60, 90 dpv. In both vaccinated groups, peak antibody titre for all 3 serotypes was obtained on 30 dpv, it remained constant up to day 60 and after that there was gradual decrease at 90 dpv. As in both groups there was no significant difference and protective titre remained upto 90 dpv, use of combined vaccine can be suggested as cost effective strategy.

Key words: Combined vaccine, FMD, Goat, LPB ELISA, Sheep

Foot and mouth disease (FMD) is a highly contagious disease of domestic and wild cloven hoofed animals including cattle, sheep, goat, deer and pig. The disease is endemic in India with outbreaks reported regularly causing great economic loss (Singh *et al.* 2013). Despite representing largest part of the world's foot-and-mouth disease virus (FMDV)-susceptible domestic livestock, sheep and goats have generally been neglected with regard to their epidemiological role (Parida 2009). The disease is generally subclinical in nature in small ruminants and they may become FMDV carriers acting as reservoir of infection. The possible risks of spread of FMD from them have gained prominence by several works in past (Sharma 1981, Pay 1988, Barnett and Cox 1999, Uppal *et al.* 1972). Small ruminants have been incriminated in the transboundary spread of the disease (Madhanmohan *et al.* 2011) on several occasions thus, presenting a major risk in trade of live sheep and goats in FMD-free countries.

FMD-control program (FMD-CP) that is being carried out in India is basically targeted to induce herd immunity in large ruminants (cattle/buffalo). Serological investigations in India indicated sheep and goat positive for 3AB NSP antibodies from as low as 3.93% and 0.01% to as high as 20.35% and 38.33%, respectively providing

an evidence of FMDV circulation especially in areas of FMD outbreaks in large ruminants (Ranabijuli *et al.* 2010, Rout *et al.* 2014a, 2014b, Mohanty *et al.* 2015, Hegde *et al.* 2016). Similarly, protective antibody titres were observed against structural proteins of all 3 FMDV types in sheep and goat in a range of 4.54% and 6.27% to 17.32% and 38.87%, respectively (Ranabijuli *et al.* 2010, Rout *et al.* 2014a, 2014b, Mohanty *et al.* 2015, Hegde *et al.* 2016). As small ruminants are not included in vaccination coverage it indicates they are frequently exposed to FMDV infection and remain as subclinical host. All these facts stress upon the inclusion of small ruminants in the ongoing FMD-control program.

Currently, inactivated vaccines are in use in India against FMDV serotype O, A and Asia 1. Immune response following FMD vaccination has been extensively studied in cattle, wherein the vaccine has been found to induce a protective immune response for about 6 months. There is limited data on immune response of sheep and goat against FMD vaccination (Patil *et al.* 2002, Madhanmohan *et al.* 2009, Selim *et al.* 2010, Madhanmohan *et al.* 2011). Combined vaccines against FMD, Haemorrhagic Septicaemia (HS) and Black Quarter (BQ) available for susceptible livestock for cost effective vaccination strategy has been studied in cattle (Reddy *et al.* 1997, Srinivasan *et al.* 2001, Chhabra *et al.* 2004). However, no such report on use of combined vaccine is available in case of small ruminants in India.

Therefore, the present study was designed to compare the serological response for FMDV in monovac vaccine

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(FMD) and combined vaccine (HS+BQ+FMD) in sheep and goat.

MATERIALS AND METHODS

Experimental animals: Apparently healthy indigenous non-descript sheep (16) and goat (27) aged more than 4 months were selected from the Department of Physiology, Veterinary College, Mathura. The animals were not vaccinated against FMD. Three weeks before the vaccination, all animals were dewormed (Fenbendazole @ 10 mg/kg). IAEC approval was sought from the concerned authority of the University. The animals were divided in to 3 groups. In sheep; Gr 1 contained 6 animals, Gr 2 contained 7 animals in goats; Gr 1 and 2 had 12 animals each while, 3 animals each were in control group.

Vaccine: Two FMD vaccines available in the local market were used. These include, Combined Triovac-Oil-Adjuvanted vaccine (FMD, HS and BQ) containing inactivated *Pasteurella multocida*, *Clostridium chauvoei* and FMDV serotype O, A and Asia 1, and Oil-Adjuvanted Polyvalent FMD vaccine containing inactivated FMDV serotype O, A and Asia 1.

Vaccination of sheep and goats: Gr 1 animals received combined vaccine against FMD, HS and BQ and Gr 2 received FMD vaccine alone. One ml dose of each vaccine was given subcutaneously (s/c) as per the manufacturer's instruction. Gr 3 animals served as control.

Serum samples: Blood samples were collected on 0, 30, 60 and 90 days post-vaccination (dpv). Zero day sample was collected prior to vaccination. Serum was separated, heat inactivated at 56°C for 30 min and stored at -20°C till further use.

3AB3 NSP ELISA: An indirect-ELISA was performed as per Mohapatra *et al.* (2011) using in-house r3AB NSP-ELISA kit from Directorate on FMD, Mukteswar to assess antibodies against 3AB NSP of FMDV on serum samples collected on day zero before vaccination.

Liquid phase blocking ELISA (LPBE): LPBE kit (PDFMD, Mukteswar) was used for the measurement of serotype-specific SP antibodies as described previously (Ranabijuli *et al.* 2010).

Data analysis: Two tailed unpaired Student's t-test was

used to calculate differences in ELISA antibody titres between groups of animals.

RESULTS AND DISCUSSION

In developing country like India, to make the vaccination programme more cost effective in terms of money and manpower, it will be better if two or more vaccines can be given as combined vaccine. Early reports by different researchers suggested that FMD vaccine can be given simultaneously with other vaccine(s) (Joseph and Hedger 1984, Hedger *et al.* 1986, Clercq *et al.* 1989, Srinivas *et al.* 1996, Trotta *et al.* 2015) or can be combined with other vaccine(s) (Palanisamy *et al.* 1992, Reddy *et al.* 1997, Srinivasan *et al.* 2001, Chhabra *et al.* 2004, El-Bagoury *et al.* 2014) without causing any difference in immune response to individual antigenic components; though, these studies have been carried out in cattle.

In the present study, all goat samples were found negative for antibodies against 3AB3 NSP of FMDV whereas, two sheep samples were positive having PP value more than 40%. These two sheep were excluded from the study. 3AB3 NSP ELISA results are indicative of past exposure or ongoing virus activity in susceptible animals. Negative animals in study ruled out prior exposure to virus as well as any concurrent viral activity though sheep and goat have been found as sero-reactors in NSP-ELISA.

In LPBE, antibody titre against all 3 FMDV serotypes were deduced from the final plate OD value with the help of PD-FMD analyst software at day 0, 30, 60 and 90 days of post vaccination (Tables 1–2; Figs 1–6).

In goat, following vaccination with combined vaccine (Gr 1), protective antibody titre ($> 1.8 \log_{10}$) for serotype O was obtained on 30 dpv and then it remained almost constant at 60 dpv while showing a decline at 90 dpv, though statistically non-significant. Similar pattern was observed for serotype A and Asia 1. Mean antibody titre was highest against serotype Asia 1 as compared to serotypes O and A, though it was statistically nonsignificant. In Gr 2 (monovac vaccine), protective antibody titre was on 30 dpv that remained constant at 60 dpv while showing a decline at 90 dpv, though statistically nonsignificant. Similar pattern was observed for serotype A and Asia 1. Immune response was

Table 1. Mean antibody titre against serotype A, O and Asia 1 of FMDV in monovac and combined vaccine in goat

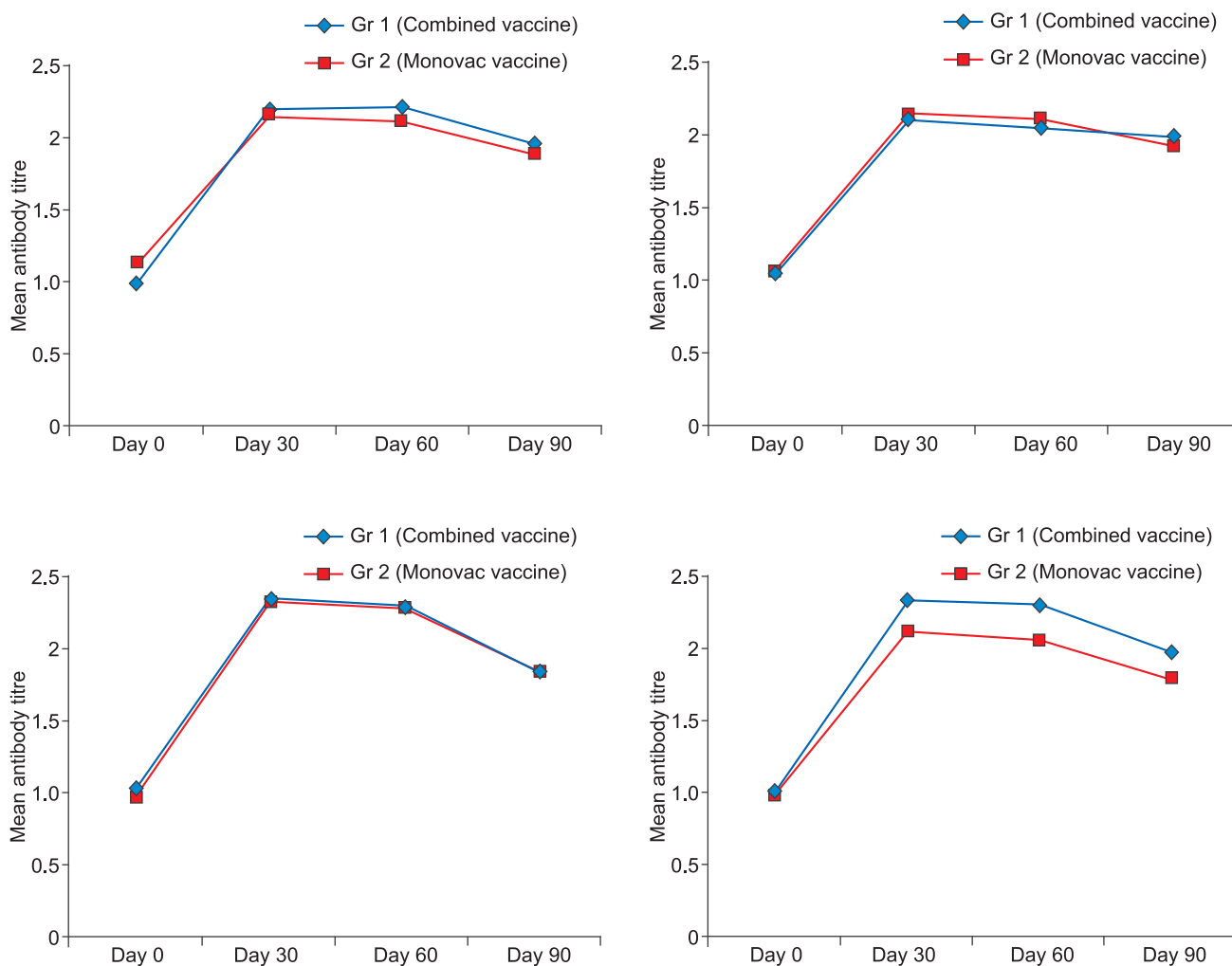
Serotype	Group	Mean antibody titre			
		Day 0	Day 30	Day 60	Day 90
A	Gr 1 (Combined vaccine)	1.0448 ^a	2.1026 ^b	2.0494 ^b	1.9908 ^b
	Gr 2 (Monovac vaccine)	1.0733 ^a	2.1508 ^b	2.114 ^b	1.9229 ^b
	Gr 3 (Control)	1.0117 ^a	1.0233 ^a	1.0071 ^a	1.0112 ^a
O	Gr 1 (Combined vaccine)	0.9896 ^a	2.1912 ^b	2.2093 ^b	1.9563 ^b
	Gr 2 (Monovac vaccine)	1.1348 ^a	2.1544 ^b	2.1122 ^b	1.889 ^b
	Gr 3 (Control)	0.8511 ^a	0.969 ^a	1.0008 ^a	1.0041 ^a
Asia 1	Gr 1 (Combined vaccine)	1.038 ^a	2.3551 ^b	2.2946 ^b	1.8348 ^b
	Gr 2 (Monovac vaccine)	0.9726 ^a	2.3306 ^b	2.2886 ^b	1.8459 ^b
	Gr 3 (Control)	0.9897 ^a	1.0012 ^a	1.0123 ^a	1.0044 ^a

*Means bearing different superscript letters differ significantly ($P < 0.05$).

Table 2. Mean antibody titre against serotype A, O and Asia 1 of FMDV in monovac and combined vaccine in sheep

Serotype	Group	Mean antibody titre			
		Day 0	Day 30	Day 60	Day 90
A	Gr 1 (Combined vaccine)	1 ^a	2.199 ^b	2.242 ^b	1.9663 ^b
	Gr 2 (Monovac vaccine)	1 ^a	1.888 ^b	2.0135 ^b	1.8077 ^b
	Gr 3 (Control)	1 ^a	1 ^a	1 ^a	1 ^a
O	Gr 1 (Combined vaccine)	1 ^a	2.3327 ^b	2.3054 ^b	1.9783 ^b
	Gr 2 (Monovac vaccine)	0.9809 ^a	2.115 ^b	2.0633 ^b	1.7914 ^b
	Gr 3 (Control)	1 ^a	1 ^a	1.2373 ^a	1 ^a
Asia 1	Gr 1 (Combined vaccine)	1.0838 ^a	2.171 ^b	2.3799 ^b	1.8253 ^b
	Gr 2 (Monovac vaccine)	1 ^a	1.9546 ^b	1.9098 ^b	1.706 ^b
	Gr 3 (Control)	1 ^a	1 ^a	1 ^a	1 ^a

*Means bearing different superscript letters differ significantly ($P < 0.05$).

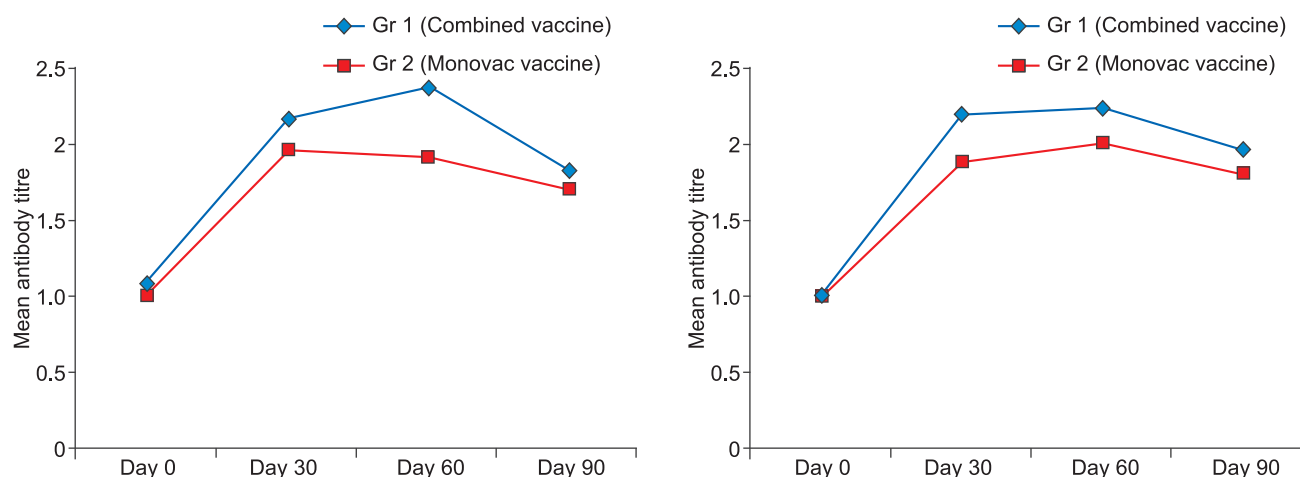


Figs 1–4. **1.** Comparison of mean antibody titre against serotype O of FMDV in Gr 1 (combined vaccine) and Gr 2 (monovac vaccine) in goat. **2.** Comparison of mean antibody titre against serotype A of FMDV in Gr 1 (combined vaccine) and Gr 2 (monovac vaccine) in goat. **3.** Comparison of mean antibody titre against serotype Asia 1 of FMDV in Gr 1 (combined vaccine) and Gr 2 (monovac vaccine) in goat. **4.** Comparison of mean antibody titre against serotype O of FMDV in Gr 1 (combined vaccine) and Gr 2 (monovac vaccine) in sheep.

highest for serotype Asia 1 as compared to serotype O and serotype A, though it was statistically nonsignificant.

In sheep, following vaccination with combined vaccine (Gr 1), the mean serotype O antibody titre was highest on

30 dpv and then it decreased slightly at day 60 and at day 90 a rapid decrease was seen though statistically nonsignificant. Against serotype A and Asia-1 antibody response was highest at day 60 and then it decreased at day



Figs 5–6. 5. Comparison of mean antibody titre against serotype A of FMDV in Gr 1 (combined vaccine) and Gr 2 (monovac vaccine) in sheep. 6. Comparison of mean antibody titre against serotype Asia 1 of FMDV in Gr 1 (combined vaccine) and Gr 2 (monovac vaccine) in sheep.

90. Mean antibody titre was highest against serotype O than serotype A and Asia 1, though it was statistically non-significant. In Gr 2 (monovac vaccine), antibody response was maximum at day 30 for serotype O and Asia 1, and then at day 60 and 90 it decreased. The mean antibody titre against serotype A was maximum at day 60 and then it decreased at day 90. The immune response was maximum against serotype O as compared to serotype A and Asia 1 though statistically nonsignificant.

In both species, on day zero, there was no significant difference in mean antibody titre against all the serotypes (O, A and Asia 1) of FMDV in monovac, combined triovac and control group ($P < 0.05$). However, on 30, 60 and 90 dpv, a significant rise in antibody titre was noticed in both monovac and combined groups in comparison to control group ($P < 0.05$). As no protective antibody titre was obtained at day zero in monovac as well as combined vaccine group it confirmed the history of no vaccination. On 30, 60 and 90 dpv, there was significant difference between the mean antibody titre against serotype O, A, Asia 1 of FMDV between control group and the vaccinated groups ($P < 0.05$) in sheep and goat indicating seroconversion in vaccinated groups.

While comparing the seroconversion pattern of combined triovac and monovac vaccine in sheep and goat, it was found that both the vaccines showed maximum immune response at day 30 and it was nearly constant up to day 60 and after that there was gradual decrease in antibody titre for all these serotypes though protective titre remained upto 90 dpv. On 30, 60 and 90 dpv, there was no significant difference between the mean antibody titre against serotype O, A and Asia 1 of FMDV in combined vaccine group and monovac group ($P < 0.05$).

In present study, the peak immune response was obtained on day 30 against all three serotypes of FMDV in monovac vaccine in goat. The result was similar to Madhamohan *et al.* (2009) who obtained antibody titre at 21 dpv with oil adjuvant adjuvant FMD vaccine in goats and Chhabra *et*

al. (2004), who found maximum immune response on day 30 in case of monovac vaccine in buffalo calves.

The maximum immune response against different serotypes of FMDV in combined vaccine in goat was observed on day 30. It was similar to Chhabra *et al.* (2004) who found maximum immune response on day 21 in combined vaccine in buffalo calves. However, no report on study of combined vaccine in goats is available.

In both monovac and combined vaccine groups in goat, the immune response obtained against serotype Asia 1 of FMDV was higher than that of serotype O and A though statistically nonsignificant. Similar results were obtained by Chhabra *et al.* (2004) in both monovac and combined vaccine groups in buffalo calves and Madanmohan *et al.* (2009) using monovac vaccine in goats.

In sheep, maximum immune response against serotype O and serotype Asia 1 of FMDV in monovac vaccine was obtained on day 30. Immune response against serotype A was maximum on day 60. In a study of monovac vaccine in sheep, Patil *et al.* (2002) found similarly peak immune response against serotype A on day 60 and serotype O on day 30.

In sheep, peak immune response against serotype A and Asia 1 of FMDV in combined vaccine, was obtained on day 60 while the peak response against serotype O was obtained on day 30. It seems there is no study of combined vaccine in sheep which includes FMDV antigen. But it can be compared with the response of combined vaccine in the buffalo calves by Chhabra *et al.* (2004). They found serotype O, A and Asia 1 giving peak immune response on day 30 in buffalo calves.

If we compare the mean antibody titre of serotype A, O and Asia 1 of FMDV of monovac vaccine in sheep and goat there was no significant difference ($P < 0.05$). There was no significant difference between the different serotypes (O, A, and Asia 1) of FMDV in combined vaccine in sheep and goat ($P < 0.05$).

In comparison study of monovac and combined vaccine,

the results obtained in present study were similar to Reddy *et al.* (1997) where they could not find any significant difference between the mean antibody titre against FMDV in combined (FMD+HS) and monovac vaccinated groups in cattle. Similarly, in studies carried out using simultaneous vaccine with other antigens no significant difference was observed (Joseph and Hedger 1984, Hedger *et al.* 1986). However, Chhabra *et al.* (2004) found combined vaccine (FMD+HS) to be better than monovac vaccine in buffalo calves.

In early reports on monovac vaccine studies in goats, Patil *et al.* (2002) found double oil emulsion vaccine to be superior than aluminium hydroxide gel vaccine. Madhanmohan *et al.* (2009) found no difference in immune response in goats to inactivated quadrivalent aluminium hydroxide gel and oil adjuvant vaccine until 180 dpv, whereas oil adjuvant vaccine elicited satisfactory immune response upto 270 dpv. Madhanmohan *et al.* (2011) found that goats vaccinated with oil adjuvant FMD vaccine resisted virulent challenge at 21 days post-vaccination.

In the present study, protective antibody titre was obtained on 30 dpv that remained constant at 60 dpv while showing a decline at 90 dpv (though protective) both for monovac and combined vaccines. Madhanmohan *et al.* (2009) found the antibody titre to be maintained till 120 dpv thereby declining to nonprotective level at 180 dpv with inactivated quadrivalent aluminium hydroxide gel vaccine whereas, in oil adjuvant vaccines the titres were maintained until 270 dpv with no difference in both vaccines until 180 dpv.

As compared to cattle, where protective antibody titers following FMD vaccination is well known to persist for about 6 months, both combined and monovac FMD vaccines used in this study induced an antibody response, protective titers of which persisted for only about 3 months. This is an interesting observation from this study and it will certainly help in devising strategically FMD control program in small ruminants.

The present study highlights the usefulness of combined vaccine as a viable alternative to the present vaccination regime involving vaccination with individual antigens. However, comparative immunogenicity of antigens other than FMDV, i.e. *Clostridium chauvoei* and *Pasteurella multocida* need to be studied, to ascertain any possible difference in immunogenicity of these antigens when combined.

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