

## Serological response to foot and mouth disease and haemorrhagic septicaemia combined vaccine in cattle

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**Abstract:** Foot and mouth disease and haemorrhagic septicaemia diseases are of economic concern to Indian Dairy industry. FMD-HS combined vaccine is the most effective vaccine to prevent both diseases by using single vaccine, and it reduces the vaccination stress to animals and vaccination cost (vaccine and labor costs) to farmers. In the present study, 376 healthy cattle of above 4 months of age (176 cattle at Amul Dairy and 200 cattle at Sabar Dairy) were vaccinated with the FMD-HS combined vaccine. The vaccinated animals were observed for any local and systemic adverse reactions during vaccination and in the follow up period, and evaluated for serological response against the vaccine administered at recommended dose (3 ml) intramuscularly. Blood samples were collected at 0, 30, 90, 180 days post vaccination for Antibody titre examination. The study revealed that the FMD-HS combined vaccine was safe with protective humoral response in cattle of above 4 months age.

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### I. Introduction

The economic losses caused by the foot and mouth disease (FMD) are mainly due to losses in milk production and reduction in working capacity of work animals. The morbidity losses contributed about 97.64% and rest (2.36%) by mortality of animals (Singh *et al.*, 2013). Among different components of losses the maximum loss of 49.83% was observed due to milk loss, followed by opportunity cost (16.15%), reduction in growth (12.20%), loss of work power (9.35%) and treatment cost (8.83%) [1]. Among different components of losses due to haemorrhagic septicaemia (HS), the maximum loss of 76.86 per cent was observed due to mortality and 23.14 per cent due to morbidity in bovines (%) [2]. Among different components of morbidity losses, the highest loss was due to reduction in growth (11.72%), followed by milk loss (4.96%), opportunities cost (2.87%), treatment cost (2.30%) and drop in work power (1.19%) [2].

FMD often leads to immunosuppression in infected animals and many secondary bacterial infections pose threat to the animal, further aggravating the condition of the animal. Certain acute diseases like haemorrhagic septicaemia (HS) can result in mortalities in the infected animals. But, the mortalities in adult animals are generally attributed to FMD, which is not true, as FMD is rarely fatal in adult animals. Simultaneous occurrence of economically devastating diseases like FMD and HS were reported in Moga district of Punjab, India in 1998 [3]. Combined outbreaks of FMD and HS in Haryana, India during 2001 were also reported [4]. In 2013, when widespread FMD outbreaks were reported throughout Southern India, most of the deaths were confirmed to be due to HS infection, which occurred simultaneously with FMD outbreaks [5]. This situation in endemic areas calls for an effective strategy to prevent these co-existing diseases through use of FMD and HS combined vaccine. Epidemiological analysis of FMD and HS in southern states of India during 2002-12, suggests that case fatality rate was high in HS than FMD, whereas prevalence rate was high in FMD than HS [6]. Combination vaccines that protect against FMD and HS diseases can help to simplify the vaccination schedule. Other benefits of these vaccines include reduction of stress to the animals due to handling, and can also solve the problem of animal drop outs for vaccination. Combined vaccines are also convenient to use, save vaccination costs, require less vaccine storage space and help in improved record keeping and tracking.

## II. Material And Methods

**Study design:**The present study was designed to assess safety and immunogenicity of FMD-HS combined vaccine in cattle at Sabar Dairy and Amul Dairy in Gujarat. Animals were selected and enrolled based on the inclusion and exclusion criteria of the study. Healthy cattle of above 4 months old, which were not in the incubating stage for FMD and HS disease, judged to be in good health on the basis of reported medical history and history-directed physical examination were included in the study. The animals which were in advanced stage of pregnancy were excluded from the study. A total of 176 cattle at Amul Dairy and 200 cattle at Sabar Dairy were enrolled for the study.

Before starting the experiment, all the animals were dewormed against endoparasites by recommended doses. Only healthy animals were selected for vaccination. The study animals were monitored and evaluated for 180 days post-vaccination. The blood was collected aseptically in serum vacutainer tubes (Beckton Dickinson, USA) from jugular vein for the estimation of FMD and HS antibodies.

**Study vaccine:**FMD and HS combined vaccine (RakshaBiovac), manufactured by Indian Immunologicals Ltd. (IIL), Hyderabad, India used in the study. FMD vaccine component IP (vet) contains FMD inactivated antigen against O, A and Asia-1 strains. Haemorrhagic septicaemia vaccine IP (vet) contains formaldehyde inactivated *Pasteurellamultocida* culture strain P52.

**Study procedure:**On day 0, each animal was administered with 3 ml dose of vaccine through deep intramuscular route in the mid neck region using disposable syringe and needle and following aseptic procedures. All the animals were observed for local adverse reactions (local pain, swelling, rashes, skin eruption, sloughing of mucous membrane, redness at administration site) and/or systemic adverse reactions (fever, loss of appetite, restlessness) at the time of vaccination and during the follow-up period. Blood samples were collected on 0, 30, 90 and 180 DPV for humoral antibody response by serology. Sera samples from the animals were screened for FMD antibodies by employing serum neutralization test (SNT) and HS antibodies by indirect ELISA.

**Table 1.** Summary of activities including details of sample collection

S. No.	Days post-vaccination	Activity	Remarks
1.	0	Blood collection; Inoculation of vaccine	Antibody titre examination
2.	30	Blood collection	Antibody titre examination
3.	90	Blood collection	Antibody titre examination
4.	180	Blood collection	Antibody titre examination

**Safety evaluation:**Evaluation of safety was done by observation of animals for any local and/or systemic reactions and recording of the adverse events during the first 30 minutes to 1 hour post vaccination and during the follow-up period.

**Immunogenicity evaluation:**Immunogenicity study was done by determining the humoral immune response of vaccine based on serum antibody titres at different intervals by SNT for FMD antibodies and by indirect ELISA for HS antibodies at different time intervals.

**Evaluation of humoral antibody response to FMD by Serum Neutralization Test (SNT):** SNT was carried out by cell culture method using IB-RS2 cells as described by Golding *et al.*[7]. The method was used with constant virus and decreasing dilution of serum samples. Serum antibody titres were estimated as per the method described by Karber[8]and expressed as log<sub>10</sub> SN<sub>50</sub> values.

**Acceptance criteria:**Protective SN<sub>50</sub> antibody titres against O, A, and Asia -1 strains are Log<sub>10</sub> 1.29, 1.19 and 1.5, respectively.

**Evaluation of humoral antibody response to HS by indirect ELISA:**The humoral antibody response to HS is measured by indirect ELISA method. The method is performed with OMP extracted from *Pasteurellamultocida* and specific dilution of Kaolin treated sera. The percentage positivity is calculated as the ratio between OD of test and positive samples.

Percentage Positivity =  $\frac{\text{Corrected OD of test sample}}{\text{Corrected OD of Positive sample}} \times 100$

Corrected OD of Positive sample

Acceptance Criteria: Percentage positivity  $\leq 36$  is considered as negative and  $>36$  is considered as positive for HS antibodies.

## III. Results

**Safety evaluation:**No local or systemic adverse reactions were observed in the animals at the time of vaccination or during the follow-up period till the end of study. This confirms the safety of FMD-HS combined vaccine in cattle aged 4 months and above.

**Table 2.** Adverse events profiling in different animal groups at Amul and Sabar Dairy

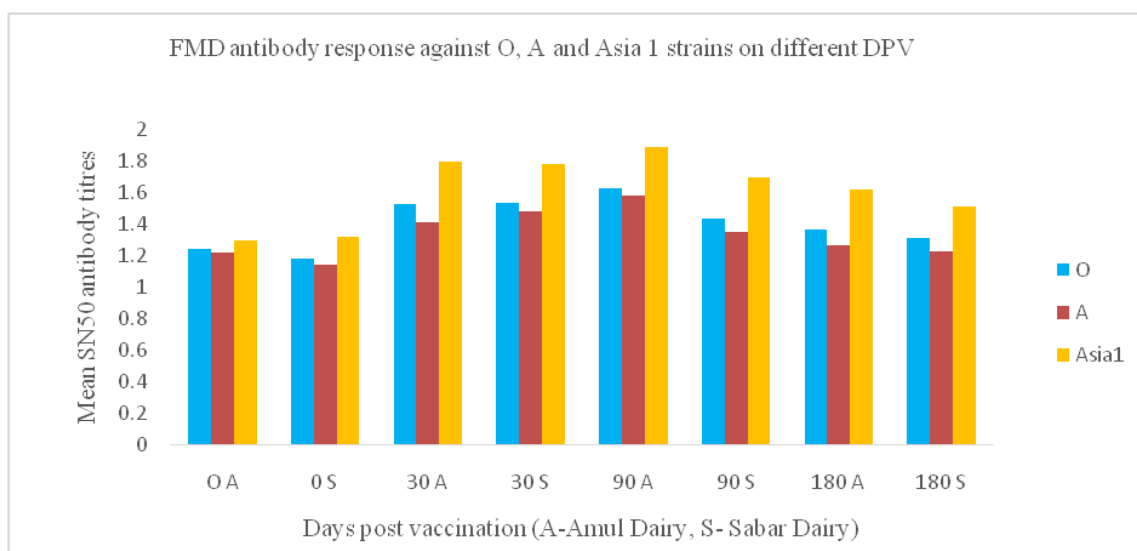
Adverse Events	Amul Dairy Cattle (N=176) n (%)	Sabar Dairy Cattle (N=200) n (%)
<b>Local</b>		
local pain	0 (0)	0 (0)
swelling, rashes, skin eruption, sloughing of mucous membrane	0 (0)	0 (0)
Redness at administration site	0 (0)	0 (0)
<b>Systemic</b>		
Fever	0 (0)	0 (0)
loss of appetite	0 (0)	0 (0)
Restlessness	0 (0)	0 (0)
Diarrhoea	0 (0)	0 (0)

N – No. of animals in study group; n- percentage of animals exhibiting AE

**Immunogenicity evaluation:** Humoral immune response elicited against the FMD and HS components of vaccine were measured in the form of serum antibody titre at the both study centers.

**Evaluation of FMD antibody titres:**

At Amul Dairy, on 0<sup>th</sup> day, the mean Log10 SN<sub>50</sub> antibody titres for O, A, and Asia 1 strains were 1.24, 1.22 and 1.30, respectively. However, by 30<sup>th</sup> DPV, the animals showed good seroconversion and titres were found to be 1.53, 1.41 and 1.80 for O, A and Asia 1 strains respectively, which were above the protective titres for respective strains (O: 1.29, A: 1.19 and Asia 1: 1.50). This trend continued till 180<sup>th</sup> day, at which the animals showed protective titres of 1.37, 1.27 and 1.62 for O, A Asia 1 strains, respectively. Hence, it is concluded that all the vaccinated animals were protected against all the three FMD strains (O, A, and Asia1) up to 180 days post-vaccination.

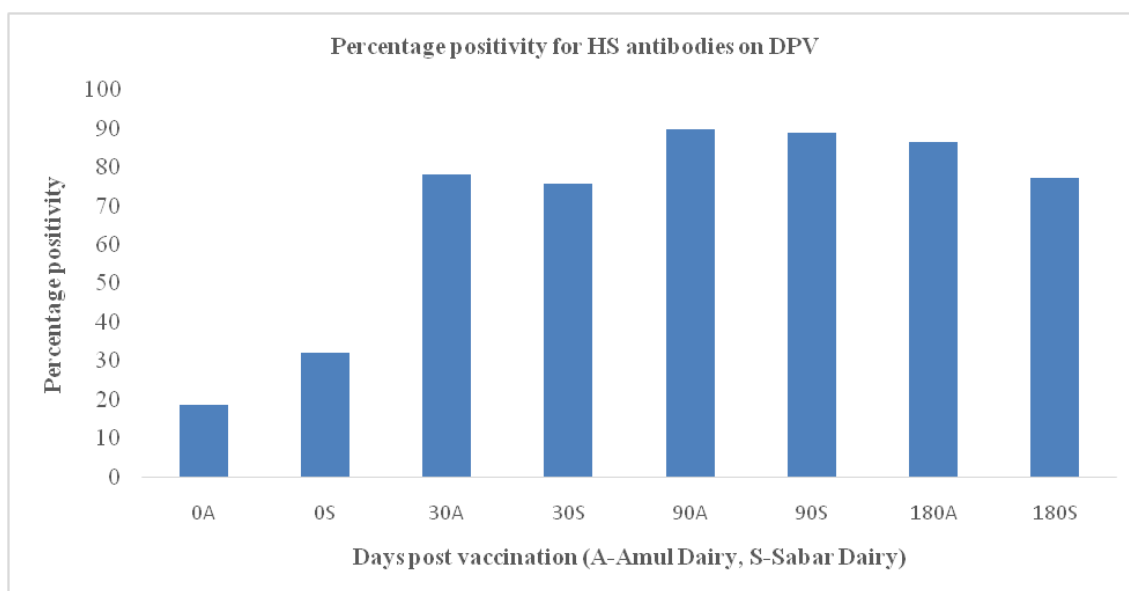


**Fig. 1.** FMD antibody response against O, A and Asia 1 strains on different days post vaccination

At Sabar Dairy, on 0<sup>th</sup> day, mean log 10 SN<sub>50</sub> antibody titres for O, A, and Asia 1 strains were 1.18, 1.14 and 1.32, respectively. Whereas, on 30<sup>th</sup> DPV, the animals showed good seroconversion and the titres were found to be 1.54, 1.48 and 1.78 for O, A and Asia 1 strains, respectively. The titres observed were well above the protective titres for respective strains (O: 1.29, A: 1.19 and Asia 1: 1.50). The protective titres continued till 180<sup>th</sup> day, at which the titres were 1.31, 1.23 and 1.51 for O, A Asia 1 strains, respectively. This indicates that all the vaccinated animals were protected against all the three FMD strains (O, A, and Asia1) up to 180 DPV.

**Evaluation of HS antibodies:**

At Amul Dairy, the percentage positivity values at 0, 30, 90 and 180 DPV were 18.6%, 78.00%, 89.88%, and 86.53% respectively. All the vaccinated animals showed good humoral response against HS.



**Fig. 2. Percentage positivity for HS antibodies on different days post vaccination**

At Sabar Dairy, the percentage positivity values at 0, 30, 90 and 180 DPV were 31.98%, 75.78%, 89.00% and 77.33%, respectively. All the vaccinated animals showed good humoral response against HS.

#### **IV. Discussion**

In the present study, the FMD-HS combined vaccine (RakshaBiovac) was found to be safe without any local or systemic adverse events and was able to induce a good seroconversion against FMD virus and *P. multocida* components as evidenced by protective antibody titres observed on 30, 90, and 180 DPV. The present study results are similar to the findings of Reddy *et al.* [9], which showed protective level immune response in cattle vaccinated with FMD+HS+BQ combined vaccine containing FMD virus, *P. multocida* and *Clostridium chauvoei* antigens. The combined FMD-HS vaccine was found to be effective in eliciting an excellent immune response against both FMD and HS disease and duration of protection (upto 6 months) against both diseases is also similar to single vaccine. Both routes (subcutaneous and intramuscular) of administration of combined FMD, HS and Black Quarter vaccine produce satisfactory level of immune response on the 21<sup>st</sup> day post vaccination [10]. Srinivasan *et al.*[11]also found that combined vaccine (FMD virus antigens with *P. multocida*, *C. chauvoei*, and Rabies virus antigens) triggered good immune response. The antibody response of the rabbits against each immunogen of combined FMD+HS vaccine is higher than that of rabbits receiving monovalent vaccines [12](Altafet *et al.*, 2012).

#### **V. Conclusion**

Based on the study findings, it can be concluded that the FMD-HS combined vaccine is safe and highly immunogenic in vaccinated cattle of over four months of age, when administered through intramuscular route. FMD-HS combined vaccine can be effectively used in FMD and HS endemic areas.

#### **Acknowledgement**

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