Indian Journal of Animal Sciences **95** (2): 103–106, February 2025/Article
https://doi.org/10.56093/ijans.v95i2.151036

Effect of a polyherbal immunomodulator on the immunogenicity of booster dose of FMD and FMD-HS-BQ vaccines in dairy cattle

NIDHI MISHRA[1], RASHMI SINGH[1]✉, RAJNEESH SIROHI[1], BHASKAR GANGULY[2], SHREYA GUPTA[2] and AKHIL KUMAR GUPTA[3]

U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura – 281001 (UP)

Received: 29 April 2024; Accepted: 10 February 2025

ABSTRACT

A polyherbal immunomodulator (restobal) was fed orally in dairy cattle (treatment group) to study its effect on the immunogenicity of booster dose of FMD and FMD-HS-BQ vaccines. Serum antibody titres for all three FMD virus serotypes were protective (1.80 $\log_{10}$ to 2.40 $\log_{10}$) in treatment and control groups of vaccinated animals till 90 days of vaccination. Antibody titre did not differ significantly in control and treatment groups. Antibody titres ranging from 1.74 $\log_{10}$ to 1.92 $\log_{10}$ were observed for *P. multocida* and animals were protected on 90 days of vaccination in both control and treatment groups. The Restobal treated group was no different than the control group in antibody response to FMDV and *P. multocida*. Antibody response for FMDV was similar with FMD vaccine and FMD-HS-BQ vaccine.

Keywords: Cattle, Combined vaccine, Dairy, FMD, HS, Monovac, Polyherbal immunomodulator

Foot-and-mouth disease (FMD) and haemorrhagic septicaemia (HS) are major economic diseases of livestock in India. Outbreaks of FMD are reported with serotypes O, A, and Asia-1 across the country, and serotype O being the predominant one (Subramaniam *et al.* 2022). The total estimated loss due to FMD in cattle and buffalo was estimated as INR 20,897 crore during 2013-14 with wide variation in magnitude across the states in India (Govindaraj *et al.* 2020). HS is a highly contagious, fatal septicaemic disease caused by *Pasteurella multocida* serotype B: 2. In India, HS has been estimated to cause economic losses of USD 792 million per year for the livestock industry (Singh *et al.* 2019). More than 25,000 outbreaks of HS were reported in India over three decades (1987-2016) (Chanda *et al.* 2024, Shome *et al.* 2019) and high sero-prevalence was reported in cattle and buffalo (Shome *et al.* 2024).

Regular vaccination has a positive effect on reduction of disease burden (Das *et al.* 2023). India started a systematic, mass vaccination based FMD-Control Program (FMD-CP) in 54 districts in the year 2003 and by 2017-2018 it covered all of the country. Mass vaccination of the bovine population is carried out with inactivated vaccines containing serotypes O, A, and Asia-1 under Livestock Health and Disease Control Programme (LHDCP). Vaccination is done in endemic areas either with alum-precipitated or oil-adjuvant killed bacteria either as monovac for FMD or HS or in combination of FMD-HS or FMD-HS-BQ providing 6 to 9 months of protective immunity in susceptible animals. Serological response for FMD and HS have been shown to be equivalent or elevated in large or small ruminants in combined vaccine as compared to monovac vaccine (Reddy *et al.* 1997, Srinivasan *et al.* 2001, Chhabra *et al.* 2004, Kumar *et al.* 2020, Muenthaisong *et al.* 2021).

Quality of inactivated vaccines may be improved by use of adjuvant, co-stimulatory molecules and feed supplements as immunomodulators/ immunopotentiators. A number of reports are available for use of adjuvants co-injected with vaccine but limited data is available for feed supplements as immunomodulators. The use of an immunopotentiator has been shown to enhance the immune efficacy of FMD vaccine in pigs (Chen *et al.* 2018). Phytogenic feed additives (PFA) from plants in animal feed have been used to improve nutrient utilisation, immunity and livestock health (Lakhani *et al.,* 2019). Antioxidants used along with vaccines in productive animals have shown to attenuate the negative effect of vaccines (Seo *et al.* 2019).

The present study was undertaken to study the effect of a polyherbal immunomodulator at improving the immunogenicity of booster dose of FMD and FMD-HS-BQ vaccines in dairy cattle.

MATERIALS AND METHODS

Vaccine and immunomodulator: Two commercially

Present Address: [1]U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura, [2]Zenex Animal Health India Private Ltd., [3]Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS, Hisar. ✉Corresponding author Email: madan_rs@rediffmail.com

available vaccines were used: (a) Oil adjuvant trivalent FMD vaccine (Raksha Ovac, Indian Immunologicals) containing O, A and Asia-1 serotypes of FMDV; and (b) oil adjuvant FMD-HS-BQ combined vaccine (Raksha-Triovac, Indian Immunologicals) containing FMD inactivated antigens of O, A and Asia-1 serotypes, *P. multocida* B:2 and *Clostridium chauvoei.* Restobal (Ayurvet, a division of Zenex Animal Health) was used as a polyherbal immunomodulator.

Experimental animals and trial: Twenty-four lactating cows of similar age, health and body condition, reared under similar managemental practices at Livestock Farm Complex (LFC), DUVASU were included for the trial. The animals had received the Raksha-Triovac vaccine (FMD-HS-BQ) six months prior to the start of experimental study. Experimental protocol was approved by IAEC approval no. IAEC/21/3 dated 18.3.2021, as per the guidelines of CPCSEA, Govt. of India. Animals were dewormed three weeks before the trial. Group I animals received FMD vaccine and Group II animals received FMD-HS-BQ combined vaccine. Each group was subdivided into control and treatment having six animals each. In the treatment group, 50 ml restobal was fed orally twice daily from days 0 to 10. Vaccination was on day 5 (morning) of treatment. Control group did not receive Restobal.

Samples: Blood was collected on 5, 12, 26 and 95 days of the treatment. Serum was separated, heat inactivated at 56°C for 30 min and stored at –20°C till further use.

Estimation of antibody response to FMD virus: Solid-phase competitive ELISA (SPCE) developed by ICAR-DFMD, Bhubaneswar was used to detect serum antibody for FMD virus serotype O, A and Asia-1 (Sharma *et al.* 2015). The test was run in triplicate for each sample and mean value was taken for the analysis. The absorbance was measured at 492 nm in ELISA Reader (Multiscan FC, Thermo Scientific). Optical density (OD) sheet data was converted into MS Excel format and the result was analyzed and interpreted using PDFMD ELISA Analyst v2.0. Antibody titer ≥1.8 $\log_{10}$ was considered as 'protected'.

Estimation of antibody response to P. multocida: Single dilution indirect ELISA (iELISA) developed by the Department of Veterinary Microbiology, COVS, LUVAS, Hisar was used to detect serum antibody for *P. multocida* (Kumar and Kakkar 2006). Whole cell bacterial lysate was the coating antigen and anti-buffalo IgG2 monoclonal antibody was the tracing antibody. OD of each well was measured at 450 nm and the antibody titre ($\log_{10}$) was calculated. The result was interpreted as: antibody titre ≤1.50 $\log_{10}$ as 'not protected'; antibody titre between 1.5 $\log_{10}$ and 1.80 $\log_{10}$ as 'partially protected', and antibody titer ≥ 1.8 $\log_{10}$ as 'protected'.

Statistical analysis: Statistical analysis was performed using SPSS16.0 (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Tukey's b *Post hoc* analysis and Independent T-test was employed to determine statistically significant differences in mean values of each group. Data was presented as mean ± SE, and P<0.05 as significant.

RESULTS AND DISCUSSION

Estimation of antibody response to FMD virus: Serum antibody titre for FMD virus serotype O, A and Asia-1 in SPCE is given in table 1. The $\log_{10}$ titre values are given as Mean ± S.E.

In Group I control and treatment animals, mean antibody titre against serotype O increased significantly (P<0.05) on day 12 as compared to day 5 of treatment and remained the same till day 95 of treatment. The antibody titre against serotype O was significantly (P<0.05) higher on day 5 in the treatment group than the control group and on day 12, 26 and 95 of treatment titre values remained the same in both groups. Similar trend was exhibited for antibody A in control and treatment animals though a significant

Table 1. Antibody titre for FMD virus serotype O, A and Asia-1 in Group I and II dairy cattle.

Days of vaccination	Days of treatment	Group I		Group II	
		Control	Treatment	Control	Treatment
		Serotype O			
0	5	1.80^{aA} ±0.134	2.30^{aB} ± 0.031	2.13^{aA} ± 0.145	2.10^{aA}± 0.134
7	12	2.40^{bA} ±0.000	2.40^{bA} ± 0.000	2.40^{aA} ±0.000	2.37^{abB}± 0.030
21	26	2.40^{bA} ±0.000	2.40^{bA} ± 0.000	2.40^{aA} ±0.000	2.40^{bA} ±0.000
90	95	2.40^{bA} ±0.000	2.40^{bA} ± 0.000	2.40^{aA} ±0.000	2.40^{bA} ±0.000
		Serotype A			
0	5	1.80^{aA}±0.1710	2.20^{aA}±0.083	2.16^{aA}±0.130	1.86^{aA}±0.130
7	12	2.37^{bA}±0.030	2.40^{bB}±0.000	2.40^{aA}±0.000	2.40^{bA}±0.000
21	26	2.40^{bA}±0.000	2.40^{bA}±0.000	2.40^{aA}±0.000	2.40^{bA}±0.000
90	95	2.40^{bA}±0.000	2.40^{bA}±0.000	2.40^{aA}±0.000	2.40^{bA}±0.000
		Serotype Asia-1			
0	5	2.13^{aA}±0.110	2.25^{aA}±0.094	2.16^{aA}±0.168	2.22^{aA}±0.073
7	12	2.40^{bA}±0.000	2.40^{aA}±0.000	2.37^{aA}±0.030	2.37^{abA}±0.030
21	26	2.40^{bA}±0.000	2.40^{aA}±0.000	2.40^{aA}±0.000	2.40^{bA}±0.000
90	95	2.40^{bA}±0.000	2.40^{aA}±0.000	2.40^{aA}±0.000	2.40^{bA}±0.000

*Means bearing different superscript letters differ significantly (P<0.05) difference was observed between control and treatment animals on day 12. Antibody titre against serotype Asia-1 was similar as for serotype O and A in control and treatment animals though, no significant difference was observed between control and treatment animals on day 5, 12, 26 and 95.

In Group II control animals, antibody titre was protective (≥1.8 log_{10}) on day 5 and no significant increase was observed on day 12, 26 and 95 for all serotypes. In Group II treatment animals, mean antibody titre against all three serotypes increased significantly (P<0.05) on day 12 as compared to day 5 and remained the same till day 95 of treatment. No significant difference was observed between control and treatment animals on day 5, 12, 26 and 95.

Serum antibody titres ranging from 1.80 log_{10} to 2.40 log_{10} were observed by SPCE in both Group I and Group II animals and all animals showed protective levels of antibodies. In comparison study of Group I and Group II, there was statistically no significant difference in antibody titres for serotype O, A, Asia-1 on day 5, 12, 26 and 95 of treatment except for serotype O, where antibody titre was significantly higher on day 5 in group II as compared to group I. No significant difference was observed in antibody titre among all three serotypes in control and treatment animals in group I and group II individually.

Estimation of antibody response to P. multocida: Serum antibody titre for *P. multocida* estimated by single dilution indirect ELISA is given in table 2. The log_{10} titre values are given as Mean ± S.E.

Table 2. Antibody titre for *Pasteurella multocida* in Group I and II dairy cattle.

Days of vaccination	Days of treatment	Control	Treatment
0	5	1.79^{aA}±0.083	1.77^{aA} ±0.055
7	12	1.81^{aA} ±0.084	1.74^{aA} ±0.082
21	26	1.78^{aA} ±0.094	1.74^{aA} ±0.088
90	95	1.92^{aA} ±0.095	1.89^{aA} ±0.132

Serum antibody titres ranging from 1.74 log_{10} to 1.92 log_{10} were observed by iELISA. In Group II control animals mean antibody titre was 1.79±0.083 on day 5 of treatment that is considered in partial protection level. It increased to protected level (1.81 ±0.084) on day 12 and remained protective till day 95 (1.92±0.095) though the values differed non significantly. In Group II treatment animals, antibody titre remained at partial protected level till day 26 (1.74±0.088) and then increased to protected level on day 95 (1.89±0.132), though the values remained statistically non-significant. Between control and treatment groups, there was no difference in antibody titre statistically.

In the present study, serum antibody titres were ≥ 1.8 log_{10} (protective levels) on the day of vaccination for FMDV serotypes O, A, Asia-1 in FMD and FMD-HS-BQ vaccinated animals. FMDV titre for all three serotypes increased to 2.4 log_{10} by day 21 of vaccination and were not different significantly in both control and treatment group. Serum antibody titres ranging from 1.80 log_{10} to 2.40 log_{10} were observed by SPCE in both Group I and Group II animals and all animals showed protective levels of antibodies against all these serotypes (≥ 1.8 log_{10}). For *P. multocida* antibody titre was <1.8 log_{10} (partial protection levels) on the day of vaccination and increased to >1.8 log_{10} (protective levels) on 90 days of vaccination in both control and treatment animals though, statistically all the values were same and did not differ significantly. As the present study was aimed for dairy animals, the animals had received the Raksha-Triovac vaccine (FMD-HS-BQ) six months prior to the start of experimental study and none of the animals were seronegative on day 0 of vaccination.

In the present study, antibody titre against FMDV serotype O, A, and Asia-1 in FMD vaccinated animals were not significantly different than FMD-HS-BQ vaccinated animals. The finding is similar to Reddy *et al.* (1997) that observed no significant difference in mean antibody titres of calves vaccinated with FMD vaccine and FMD-HS-BQ vaccine. Similarly, Muenthaisong *et al.* (2021) found no statistically significant difference in mean antibody titre against HS in the combined FMD-HS vaccine than in the HS vaccine groups. Likewise, the mean antibody titre to FMDV serotypes O and Asia-1 in the combined vaccine were not statistically different when compared to the FMD vaccine group. In contrast to this, Chhabra *et al.* (2004) found higher LPB-ELISA antibody titres and virus specific IgM titres in buffalo calves receiving combined vaccine as compared to calves immunized with FMD vaccine alone.

Restobal is a herbal anti-stressor product that contains *Ocimum sanctum, Withania somnifera, Phyllanthus emblica* and many more herbs and it has been recommended as a supportive therapy during vaccination programmes in dairy animals (Sivajothi *et al.* 2018). In the present study, the effect of Restobal on the immunogenicity of booster dose of FMD and FMD-HS-BQ vaccines in dairy cattle could not be found. The inactivated vaccines have potent adjuvants for generating strong immune response and therefore, the effect of a polyherbal immunomodulator supplemented for a shorter duration could not be ascertained to have any significant enhancement on the immunogenicity or the immune response. But nevertheless, in this experimental group of animals, the treatment with Restobal was found to significantly increase the cell population involved in immune response especially, total leukocyte count and monocytes and it was also found to significantly reduce the cortisol level produced after vaccination (Mishra *et al.* 2023).

Immune response in terms of antibody production for FMD virus was equivalent in animals vaccinated either with FMD vaccine or FMD-HS-BQ vaccine. Restobal as a polyherbal product may be used as a supportive therapy during vaccination programmes in dairy animals, though it may not have direct any effect on immunogenicity of the vaccines but it increases the total leukocyte count and reduces stress.

ACKNOWLEDGMENTS

The authors are thankful to Project Director, ICAR Directorate on FMD, Bhubaneswar and Head, Department of Veterinary Microbiology, LUVAS for providing test kits for estimation of antibodies for FMD and HS vaccines, respectively. The financial support provided by the Dean, College of Veterinary Science and Animal Husbandry, DUVASU Mathura for master's thesis research of first author and Zenex Animal Health India Private Ltd., Baddi, Himachal Pradesh for carrying out the testing of immunomodulator in a project to corresponding author is greatly acknowledged.

REFERENCES

Chanda M M, Purse B V, Hemadri D, Patil S S, Yogisharadhya R, Prajapati A and Shivachandra S B. 2024. Spatial and temporal analysis of haemorrhagic septicaemia outbreaks in India over three decades (1987–2016). *Scientific Reports* **14**: 6773.

Chen J, Yu X, Zheng Q, Hou L, Du L, Zhang Y, Qiao X, Hou J and Huang K. 2018. The immunopotentiator CVC1302 enhances immune efficacy and protective ability of foot-and-mouth disease virus vaccine in pigs. *Vaccine* **36**: 7929-35.

Chhabra R, Sharma R, Kakker NK. 2004. Comparative immunogenicity of foot-and-mouth disease virus antigens in FMD-haemorrhagic septicaemia combined vaccine and FMD vaccine alone in buffalo calves. *Indian Journal of Experimental Biology* **42**: 259-64.

Das S, Pal S, Rautaray S S, Mohapatra J K, Subramaniam S, Rout M, Rai S N and Singh R P. 2023. Estimation of foot-and-mouth disease virus sero-prevalence rates using novel computational approach for the susceptible bovine population in India during the period 2008–2021. *Scientific Reports* **13**: 22583.

Govindaraj G, Ganesh Kumar B, Krishnamohan A, Hegde R, Nandakumar S, Prabhakaran K, Mohan V, Kakker N, Lokhande T, Sharma K, Kanani A, Limaye A, natchimuthu K, Khan T A, Misri J, Dash B B, Pattnaik B and Rahman H. 2020. Economic Impact of FMD in cattle and buffaloes in India. *Indian Journal of Animal Sciences* **90**(7): 971–6.

Kumar A and Kakkar N K. 2006. Annual Report-Monitoring Immune Status of Cattle and Buffaloes Vaccinated against Haemorrhagic Septicaemia in the state of Haryana. Department of Veterinary Microbiology, CCSHAU, Hisar. Print

Kumar Y, Singh A P and Singh R. 2020. Serological response against foot-and-mouth disease virus to FMD-haemorrhagic septicaemia-black quarter combined vaccine and FMD vaccine alone in sheep and goat. *Indian Journal of Animal Science* **90**(2): 141-6.

Lakhani N, Kamra D N, Lakhani P and Alhussien M N. 2019. Immune status and haemato biochemical profile of buffalo calves supplemented with phytogenic feed additives rich in tannins, saponins and essential oils. *Tropical Animal Health and Production* **51**: 565–73.

Mishra N, Singh R, Sirohi R, Ganguly B, Yadav S K. 2023. Effect of polyherbal immunomodulator on milk production, haematological parameters and cortisol level in dairy cattle vaccinated against the foot-and-mouth disease and haemorrhagic septicaemia. *Indian Journal of Veterinary Public Health* **9**(1): 32-6.

Muenthaisong A, Nambooppha B, Rittipornlertrak A, Tankaew P, Varinrak T, Muangthai K, et al. 2021. Immune response in dairy cattle against combined foot and mouth disease and haemorrhagic septicemia vaccine under field conditions. *BMC Veterinary Research* **17**:186.

Reddy G S, Antony P X, Srinivasan V A. 1997. Serological response to combined vaccination of cattle against foot-and-mouth disease, hemorrhagic septicaemia and black quarter. *Indian Journal of Animal Sciences* **67**(7): 585-6.

Seo J, Song M, Jo N, Kim W, Jeong S, Kim J, Lee S and Seo S. 2019. The co-injection of antioxidants with foot-and-mouth disease vaccination altered growth performance and blood parameters of finishing Holstein steers. *Asian-Australasian Journal of Animal Sciences* **32**(6): 792-9.

Sivajothi S, Reddy V V P, Reddy S B, Vani S, Chandel S, Ravikanth K and Ganguly B. 2018. Evaluation of herbal anti-stressor product (Restobal) during the vaccination stress in buffaloes. *International Journal of Current Microbiology and Applied Sciences* **7**(7): 723-7.

Sharma G K, Mahajan S, Matura R, Subramaniam S, Ranjan R, Biswal J, Rout M, Mohapatra JK, Dash B B, Sanyal A and Pattnaik B. 2015. Diagnostic assays developed for the control of foot-and-mouth disease in India. *World Journal of Virology* **4**(3): 295–302.

Shome R, Deka R P, Sahay S, Grace D and Lindah J F. 2019. Seroprevalence of hemorrhagic septicemia in dairy cows in Assam, India. Infection Ecology and Epidemiology **9**: 1604064.

Shome R, Kanani A, Gurrappanaidu G, Subbanna N KG, Mohandoss N, Prajapati A, Baskar K, Skariah S, Shanmugam G, Maharana S M, Vijayalakshmy K and Habibur R. 2024. Sero-prevalence of hemorrhagic septicaemia in cattle and buffalo population of Indian States Karnataka and Gujarat. *Veterinary Science* **24**(11): 386.

Singh R K, Sharma G K, Mahajan S, Dhama K, Basagoudanavar S H, Hosamani M, Sreenivasa B P, Chaicumpa W, Gupta V K and Sanyal A. 2019. Foot-and-Mouth Disease Virus: Immunobiology, advances in vaccines and vaccination strategies addressing vaccine failures- An Indian perspective. *Vaccines* **7**(3): 90.

Srinivasan V A, Reddy G S, Rao K A, Kihm U. 2001. Serological response of bovines to combined vaccine containing foot and mouth disease virus, rabies virus, *Pasteurella multocida* and *Clostridium chauvoei* antigens. *Veterinary Archives* **71**(1): 37-45.

Subramaniam S, Mohapatra J K, Sahoo N R, Sahoo A P, Dahiya S S, Rout M, Biswal J K, Ashok K S, Mallick S, Ranjan R, JanaC and Singh R P. 2022. Foot-and-mouth disease status in India during the second decade of the twenty-first century (2011–2020). *Veterinary Research Communications* **46**: 1011–22.