

EFFECT OF DIFFERENT ADJUVANTS USED IN FOOT AND MOUTH DISEASE VIRUS VACCINE ON ANTIBODY RESPONSE OF BUFFALO CALVES

K. Muhammad, M. A. Tariq, I. Altaf, *M. Rabbani, **R. M. Khawaja, *Q. Akram, *M. A. Ali and *K. Hanif

Department of Microbiology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan

*University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan

**Veterinary Research Institute, Ghazi Road, Lahore-Cantt., Pakistan

Corresponding author E-mail: drkhushi.muhammad@uvas.edu.pk

ABSTRACT

Foot and Mouth Disease (FMD) is a viral problem of cloven footed animals such as buffaloes, cattle, sheep, goats, camels, wild animals, etc. The disease outbreaks have been recorded even in the vaccinated dairy animals. The disease is causing heavy economic losses to the dairy industry. Efforts were made to monitor effect of different adjuvants used in FMD vaccine on antibody response of buffalo calves. In each type of vaccines, local FMD virus "serotype "O" having 10^6 units/ml of mean tissue culture infective dose (TCID₅₀) was used. The binary ethylene imine inactivated FMD virus suspension was mixed with either of montanide-ISA-70 (OB), lanolin and aluminum hydroxide gel (AHG) for production of homogenized vaccines. Each of the adjuvant containing vaccines (3 ml dose) induced detectable level of anti-FMD virus neutralizing antibody titer (Anti-FMDV-VN) in buffalo calves. Subcutaneous injection of the OB vaccine induced sterile abscess or granuloma formation at the inoculation site while deep intramuscular injection showed un-detectable reactions. In contrast to AHG based vaccines, the OB vaccine induced antibody response latter and showed higher boosting response. The antibody titer (>10 units of anti-FMDV-VN) in serum of the animals vaccinated with OB vaccine persisted for more than 240 days post priming. It is concluded that montanide ISA-70 based FMD virus vaccine induces protective antibody titer that persists for more than eight months and vaccination of dairy animals with the OB FMD vaccine twice a year could be an effective way of immunoprophylaxis.

Key words: Foot and Mouth Disease vaccine, Buffalo, Adjuvant, Antibody response

INTRODUCTION

Foot and Mouth Disease (FMD) is a contagious virus vesicular disease of cattle, buffaloes, sheep, goats, and wild ungulates like giraffe, deer, etc. It is characterized by rise in body temperature with saliva drooling from its mouth due to vesicular lesions on the tongue, gums, cheeks and hard palate. The sick animals also show vesicular lesions in the cleft of feet, at their coronary bands and on teats of the milking animals. The vesicles soon rupture to form ulcerative lesions. There is significant reduction in milk production, working efficiency and weight gain and abortions in infected animals. There is 100 percent morbidity in susceptible animal population and negligible mortality in adults. However, high mortality in sucklers is due to the virus induced necrotic lesions and myocardial degeneration (Sharma and Adhlakha, 1995; Murphy *et al.*, 1999 and Rowland, 2003).

In Pakistan, formaldehyde inactivated gel based FMD vaccine is being used to control the disease. However, sporadic outbreaks are not uncommon even in the vaccinated animals. Lack of causative FMDV serotype in the in-use vaccine, poor information on the prevalence of FMDV serotype in the country, inadequate quantity of immunogen per dose of vaccine, lack of cold

chain system, prevailing concurrent diseases and biological and physicochemical immuno-suppressive agents are incriminated to be cause of failure of immunoprophylaxis (Meyer and Knudersen, 2001).

Keeping in view the poor efficacy of available vaccine and FMD induced heavy economic losses to the livestock farmers, investigations are required to improve the existing vaccines. This project was, therefore, designed to monitor the effect of different adjuvants used in FMD ("O" serotype) virus vaccine on antibody response of buffalo calves.

MATERIALS AND METHODS

Preparation of Foot and Mouth Disease Virus Vaccine: Local serotype "O" of FMD virus (7th passage) was obtained from Department of Microbiology, University of Veterinary and Animal Sciences, Lahore and characterized using serum neutralization test with serotype specific rabbit antiserum. The virus was propagated on monolayer of BHK-21 cell line in Roux flasks containing 100 ml of maintenance medium with 1 % fetal calf serum (Muhammad *et al.*, 2011). One ml of the freshly grown virus suspension was processed for calculation of its mean tissue culture infective dose (TCID₅₀) titer. The virus suspension was serially diluted

as 10 fold in maintenance cell culture medium and each dilution is labeled as 1 to 9. From 10^{-1} dilution of the virus, 200 μ l was added to each of the 8 wells of the first column of the 96 well flat bottomed well cell culture plate containing washed monolayer of BHK-21 cell line and from the 10^{-2} dilution, the same quantity was added to each of 8 wells of the second column. This procedure was repeated with every dilution of the virus till dilution of 10^{-9} was reached. All wells of the 10th column were kept as cell control and no virus was added. Eight wells worked as replicates for each dilution of the virus. The test plate was incubated at 37°C for 72 hours and observed at 40X under inverted microscope after every 24 hours. Cells in each well were examined for the presence of any cytopathogenic (CPE) effects which formed the bases of TCID₅₀ calculation of the virus following formulation as described by Villegas (1988).

The virus suspension with known biological titer inactivated by treating with 0.004M concentration of binary ethyleneimine for 48 hours and subsequently with 2% sodium thiosulphate for 24 hours (Bahnmann, 1975).

The chemically inactivated FMD virus suspension was processed on laboratory bacteriological media (tryptose soya and Sabouraud's agar) for sterility test and on monolayer of BHK-21 cell line for safety test. The safe and sterile virus suspension was admixed with thiomersal sodium at rate of 0.05 percent (Kitching, 2000).

The virus suspension was mixed with one of the adjuvants such as Montanide ISA-70 (OB), aluminium hydroxide gel and lanolin base, for preparation of adjuvant containing FMD vaccines (Iyer *et al.*, 2000).

Monitoring of Antibody Response of Buffalo Calves:

A total of 32 un-vaccinated male buffalo calves (one year old) were de-wormed and treated against coccidiosis. The calves were vaccinated against hemorrhagic septicemia and were divided randomly into A, B, C, D, E, F, G and H groups each containing four animals. In experiment I, each calf of group A, B, C and D was primed and boosted with 19 days interval (dose 3 ml: deep intramuscular at neck) with respective vaccines and in experiment II, each calf of group E, F, G and H was primed and boosted with 120 days interval using above mentioned dose and route with respective vaccines. Blood sample from each calf of each group was collected from the jugular vein on day 0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 post priming. Each of the sera was heat inactivated at 56 °C for 30 minutes, and stored in properly labeled vials at -20 °C till required for Virus Neutralization (Golding *et al.*, 1976) test. The test was performed using 2 fold serial dilution up to 1:256 of each serum from each of the FMD vaccinated buffalo calves. Fifty microliter of each dilution of each serum sample was admixed with equal volume of 100 units of TCID₅₀ of "O" serotype of FMD

virus and the filtered mixture was transferred on to respective well containing monolayer of BHK-21 cell line in 96 well cell culture plates, aseptically. Well number 9, 10, 11, and 12 served as control for BHK-21 cell line alone, FMD virus+ BHK-21 cell line, antiserum and the cell line and the diluent with the cell line, respectively. The plates were incubated at 37°C for 48 hours. The highest dilution of the serum or well number of each row showing no CPE was the anti-FMD "O" VN antibody titer of the serum.

Statistical Analysis: Data of antibody response of each calf of each group was processed for calculation of geometric mean titer (GMT) of anti-FMD ("O" virus) VN antibodies (Villegas, 1998) and the GMT values were expressed in form of graphs.

RESULTS AND DISCUSSION

Adjuvants such as montanide ISA-70, aluminum hydroxide gel or lanolin in the inactivated virus suspensions enhance immunogenicity of veterinary vaccines. Adjuvant containing FMD virus "O" type vaccine when injected to one year old buffalo calves induced detectable level of anti-FMD virus neutralizing (VN) antibodies (Fig 1 and 2). In first experiment, when the calves were boosted with oil based vaccine on 19 days post priming, the anti-FMDV-VN antibody titer attained maximum level up to 128 days and declined thereafter. In the second experiment, when the calves were boosted with oil based FMD vaccine 120 days post priming, the antibody titer gradually increased up to 180 days and started declining thereafter. The anti-FMD "O" antibody titer in serum of the vaccinated animals is determined using complement fixation test and virus neutralization test (Grist *et al.*, 1979; Doel, 1999; Iyer *et al.*, 2000 and Doel, 2003). Deep intramuscular injections of oil based vaccines resulted into higher titer of the antibodies that were maintained for longer period of time as compared to that of AHG based vaccines. Oil based vaccines protect vaccinates for longer time and are more suitable for ring vaccination programs to control of FMDV infections (Barteling and Vreeswijk, 1991, Barteling, 2002 and Suttmoller *et al.*, 2003). Moreover, antibody response is directly proportional to the biological titer of the vaccine virus serotype. The 10^6 units of biological titer of the virus in oil based vaccine showed optimum antibody response in the vaccinated rabbits (Muhammad *et al.*, 2011). Antigen adsorbed on aluminum hydroxide gel or emulsified in oils can not directly enter in to circulation from the inoculation site. The gel is least toxic for animal tissues therefore, is commonly used in human, canine, bovine, caprine and equine vaccines. Oil based FMD virus vaccine when injected to buffalo or cattle subcutaneously induced severe granuloma or sterile abscess formation that

disappeared automatically over period of less than 2 months where as deep intra-muscular injection of the vaccine induced un-noticeable granuloma. Oils are irritant and induce skin necrosis that could be plausible reason for inflammatory response such as granuloma or abscess formation at the inoculation site (Barteling and Vreeswijk, 1991 and Tizzard, 1998).

The antigen depot or granuloma recruits immunocompetent cells (antigen presenting cells, APC and lymphocytes) by causing irritation at the injection site. The antigen processing cells phagocytose, process and present the antigen on their surface in association with self immune associated (Ia) molecules. The thymus dependent lymphocytes (T-cells) can only recognize the antigen when presented on surface of APC with Ia antigen. The antigens stimulated T-cells transform into lymphoblast and populate in different lymph nodes and other lymphoid organs such as spleen, mucus membrane associated lymphoid tissues such as Peyer's patches, etc. (Vanio *et al.*, 1988; Muhammad *et al.*, 1993) and secrete lymphokines which potentiate the activity of bursal dependent lymphocytes (B cells) and induce cell mediated as well as humoral immunity (Barteling and Vreeswijk, 1991; Tizzard, 1998). Oil based vaccines some times may produce adverse anaphylactic reaction if the emulsifier particularly Tween 80 is added in higher concentration in oil base (Toman *et al.*, 1992). The liquid paraffin (oil adjuvant) is used in many veterinary vaccines as mineral oil. The emulsifiers such as Arlacel-A (Mannide mono-oleate) and Span-80 (Sorbitan mono-oleate) are used in 10 percent concentration in vaccines (Dalsgaard, 1987). Addition of these mixtures in aqueous phase of antigen results in milky white product. Addition of the surfactant like tween-80 in oil base reduces its viscosity and improves the syringe ability. The stability of the vaccine depends upon balanced concentration of the emulsifiers.

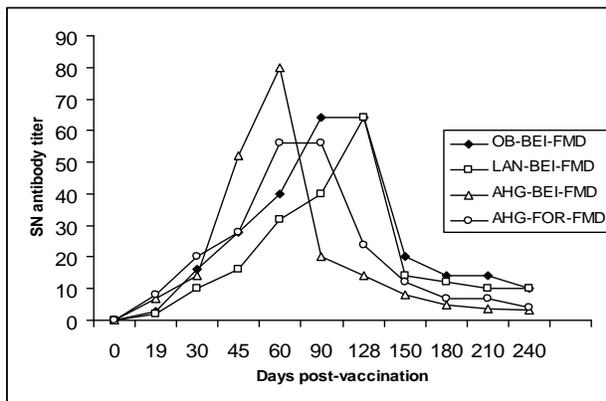


Fig 1. Antibody response of buffalo calves to Foot and Mouth Disease type “O” virus vaccines (Boosting on day 19 post-priming)

It is concluded that oil based vaccines induce antibody response in buffalo calves latter than that of gel adsorbed vaccine and the immunity was maintained for more than 6 months. Deep intramuscular injection of oil based FMD vaccine bi-annually is therefore recommended for effective immunoprophylaxis.

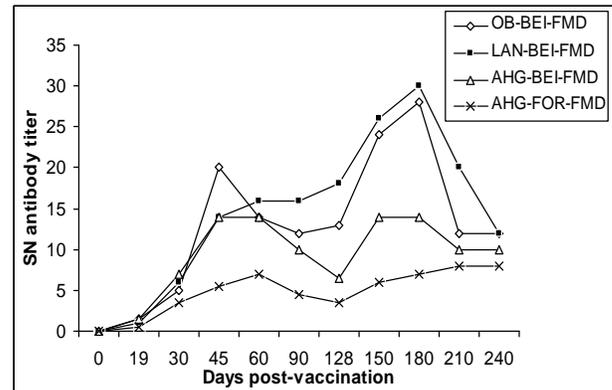


Fig 2. Antibody response of buffalo calves to Foot and Mouth Disease type “O” virus vaccines (Boosting on day 120 post-priming)

Acknowledgements: The authors of the project have enormous honor to express their deep sense of obligations to Directorate of Agriculture Linkage Program, Pakistan Agriculture Research Council (ALP-PARC), Islamabad, Pakistan for providing funds (Rs 5.9 millions) for research proposal “ Immunoprophylaxis of Foot and Mouth Disease in Bovine” in the best interest of Dairy Industry, Pakistan.

REFERENCES

Bahnemann, H. G. (1975). Binary ethylenimine as an inactivant for foot and mouth disease virus and its application for vaccine production. *Archive Virology*, 47: 47-56.

Barteling, S. J. (2002). Development and performance of inactivated vaccines against foot and mouth disease. *Rev. Sci. Tech.*, 21(3): 577-88.

Barteling, S. J. and J. Vreeswijk (1991). Developments in foot and mouth disease vaccines. *Vaccine*, 9(2):75-88.

Dalsgaard, K. (1987). Adjuvants. *Vet. Immunol. Immunopathol.*, 17:145-152.

Doel, T. R.. (1999). Optimization of the immune response to foot and mouth disease vaccines. *Vaccine*, 17 (15-14):1767-71.

Doel, T. R. (2003). Foot and Mouth Disease Vaccines. *Virus Research*, 91: 81-99.

Golding, S. M., R. S. Hedger and P. Talbot (1976). Radial immunodiffusion and serum neutralization techniques for the assay of

- antibodies to swine vesicular disease. *Res. Vet. Sci.*, 20: 142-147
- Grist, N. R., E. L. Bell, E. A. C. Follett, and G. E. D. Urquhart (1979). *Diagnostic Methods in Clinical Virology*. 3rd ed. Blackwell Scientific Publications, London. 95 p
- Iyer, A. V., S. Gosh, S. N. Singh, and R. A. Deshmukh (2000). Evaluation of three ready to formulate oil adjuvants for foot and mouth disease vaccine production. *Vaccine*, 19 (9-10):1097-1105.
- Kitching, R. P., P. V. Barnett, and A. I. Donaldson (2000). *Foot and Mouth Disease. OIE Manual of Standards for Diagnostic Tests and Vaccines*. 77-92 p
- Meyer, R. F. and R. C. Knudersen (2001). Foot and mouth disease: A review of the virus and the symptoms. *J. Environ. Health*, 64 (4):21-23.
- Muhammad, K. (1993). *In vitro* studies of chicken macrophages and their role in salmonella related immunization failure. Ph. D. Thesis. School of Biological Sciences, Univer. of Surrey, Surrey, U.K.
- Murphy M. L., R. F. Meyer, C. Mebus, A. A. Schudel, and M. Rodriguez (1999). *Veterinary Virology*. 3rd ed. Academic Press, New York. 521-527 p
- Rowland, R. (2003). *Foot and Mouth Disease*. Elsevier Australia, 1 p
- Sharma, S. N. and S. C. Adlakha (1995). *Veterinary Virology*. Vikas Publishing House, New Delhi. 212-223 p
- Sutmoller, P., S. S. Barteling, R. C. Blascoaga, and K. J. Sumption (2003). Control and eradication of foot and mouth disease. *Virus Research*, 91: 101-144.
- Tizzard, I. R., (1998). *Veterinary Immunology, An Introduction*. 5th Ed. Harcourt Brace and Company Asia Pte Ltd. New Delhi, India.
- Toman, M., J. Krejci, K. Pinka, and P. Mansik (1992). Causes of anaphylactoid reactions in cattle after administration of lipoid preparations. *Vet. Med. (Praha)*, 37 (8): 417-26.
- Vanio, O., T. Veromaa, E. Verola, P. Toivanen, and M. J. H. Ratcliffe (1988). Antigen presenting cell- T-cell interaction in chicken in MHC-Class II antigen restricted. *J. Immunol.*, 140: 2864-2868.
- Villegas, P. (1998). Titration of biological suspensions. *In: A Laboratory Manual of Isolation and Identification of Avian Pathogens*. Edited by The American Association of Avian Pathologists, USA. 248-253 p.