



RESEARCH ARTICLE

Humoral Immune Response Induced by Various Foot and Mouth Disease Vaccines in Buffalo Calves

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ABSTRACT

Humoral immune response to 5 FMD vaccines (3 imported and 2 locally manufactured) available in Pakistan was studied in 90 buffalo calves over seven months period. All vaccines were trivalent and contained serotypes A, O and Asia-1. Sera were analyzed for the presence of antibodies against non-structural proteins (NSP) and structural proteins (SP) using commercially available 3ABC-trapping indirect ELISA and liquid phase blocking ELISA, respectively. Maternal NSP antibodies waned by 5th month of age in most of the buffalo calves. Vaccine induced NSP antibodies were seen in only two animals, both vaccinated with locally manufactured unpurified vaccines. All three imported vaccines induced significantly higher titers against SP than local vaccines. These titers seemed to stay at protective level for almost six months in two imported vaccines which had aluminum hydroxide and saponin as adjuvant. Peak titers of both local vaccines were highest on day 30 post booster dose but these titers declined sharply and by day 60 post booster dose, the titers were much lower than the protective level. Oil adjuvanted FMD vaccines (both local and imported) did not induce sustained higher immune response in buffalo calves that are normally seen in cattle with these vaccines.

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INTRODUCTION

Foot and mouth disease (FMD) is the most prevalent and economically the most important infectious disease of livestock in Pakistan (Ferrari *et al.*, 2014). The disease can be controlled through zoosanitary measures and/or vaccination (David *et al.*, 2009). Keeping in view the livestock farming systems prevalent in the country, geographical location and veterinary service structure of the country and available resources to the veterinary service, progressive control of FMD through vaccination is the best possible option for Pakistan. This strategy can only succeed if vaccine used in the country is able to successfully protect the animals against clinical disease.

Pakistan is placed in FMD virus pool 3 which also includes Iran, Afghanistan, Turkey and Central Asian States (Gideon and Saraiva-Vieira, 2010). Three serotypes i.e. A, O and Asia-1 are currently prevalent in Pakistan (Abubakar *et al.*, 2012). Sub-serotype or virus topotypes circulating in a given FMD virus pool are usually similar

(OIE, 2014). Pakistan has a huge livestock population (> 74 million cattle and buffaloes and 95 million small ruminants) and total FMD vaccination coverage is very limited i.e. <2.5%. Thus new subtypes/topotypes continue to emerge and determination of subtypes to be incorporated in the vaccine is a constant challenge. At present, at least 5 FMD vaccines are available in the country including 3 imported and two locally manufactured vaccines (Asghar *et al.*, 2007). These vaccines have different formulations of subtypes and adjuvants (Jamal *et al.*, 2013). Field efficacy of these vaccines is variable and there are frequent reports of FMD outbreaks in animals vaccinated with certain products (Ahmed *et al.*, 2002).

Immune response of different formulations of FMD vaccines has thoroughly been studied in cattle (Cloete *et al.*, 2008; El-Sayed *et al.*, 2012). However, similar information in buffaloes (the prime dairy animal of Pakistan) is limited (Jamal *et al.*, 2013). Therefore, the present study was designed to investigate the humoral

immune response of buffalo calves following vaccination with different FMD vaccines available in the country. This study is also expected to yield information on frequent occurrence of clinical FMD in animals vaccinated with certain products in the market.

MATERIALS AND METHODS

Vaccines: Five different types of FMD vaccines available in Pakistan were used in the study. All the vaccines were trivalent containing serotypes O, A and Asia-1. Vaccine A was a purified (did not contain NSP) vaccine having aluminum hydroxide and saponin as adjuvants and contained viral strains O1-Manisa, A/Iraq 64 and Asia 1/Shamir. Vaccine B was a double oil emulsion purified vaccine containing O/TUR/S/2009, A/TUR/20/2006 and Asia-1 Shamir. Vaccine C was also purified vaccine adjuvanted with aluminum hydroxide and saponin and contained A Iran-2005, O PanAsia II and Asia-1/Shamir. Vaccine D contained double oil emulsion as adjuvant and vaccine E had only aluminum hydroxide as adjuvant and did not mention exact strains used in these vaccines. Vaccines A, B and C were imported while D and E were manufactured locally and were not purified.

Experimental design: Ninety buffalo calves of 3 to 18 months age were ear tagged and kept under similar feeding and management conditions. The animals were divided into six groups, each comprising of 15 animals of all age groups. Each of five groups was vaccinated with a separate vaccine as per manufacturer's instructions while 6th group received 3 ml of sterile normal saline and acted as non-vaccinated control. All the animals were vaccinated at day 0 of the study and given booster dose at day 30.

Blood samples were collected from all animals at day 0 (before primary vaccination), 30, 60, 90, 120, 150, 180 and 210 following primary vaccination. Serum was separated and stored at -20°C till analysis.

Before vaccination, body temperature of all animals was recorded. Temperature was again recorded 12 and 24 hours post vaccination to examine any untoward reaction.

Serological analysis: For the detection of antibodies to NSP in serum samples, 3ABC-trapping indirect ELISA kit manufactured by IZSLER, Italy (Dekker *et al.*, 2008) was utilized. Briefly, 1:100 diluted serum was added to microtiter plates pre-coated with 3ABC antigen captured by the monoclonal antibodies. Plates were incubated for one hour at room temperature. After washing, horse radish peroxidase conjugated anti-ruminant IgG (MAb) was dispensed and plates incubated for another hour. Unbound conjugate was removed by washing and TMB chromogen was added. Optical density (OD) was measured after addition of 0.6 N sulphuric acid as stop solution at 450 nm using an ELISA reader. Positive, weak positive and negative control sera were run on each plate. Test results were interpreted using manufacturer's instructions.

Antibodies to SP of FMD virus were measured using liquid phase blocking ELISA manufactured by BDSL UK (Hamblin *et al.*, 1986). Briefly, microtiter plates were coated with trapping FMD serotype specific rabbit antibody by incubating overnight at 4°C . Mixture of 1:16

diluted test sera and antigen was allowed to react in another microtiter plate overnight at 4°C and then transferred to antibodies coated plate. The plates were incubated at 37°C with continuous shaking for one hour. After washing, homologous guinea pig anti-FMDV antibodies were added and incubated at 37°C for one hour with continuous shaking. Horse radish peroxidase labeled anti-guinea pig IgG was added after washing and plates incubated with continuous shaking for one hour at 37°C . After thorough washing, substrate/chromogen ($\text{H}_2\text{O}_2 + \text{OPD}$) was added. Reaction was stopped by using 1.25 M sulfuric acid and OD value of each well was recorded at 492 nm. Percent inhibition was calculated as per manufacturer's formula.

Statistical analysis: Statistical analysis of the data was undertaken by using software (STATISTIX 8.1).

RESULTS

Body temperature of all experimental animals after vaccination remained within normal range when measured at 12 and 24 hours post vaccination (data not shown).

Antibodies against NSP of FMDV were detected in buffalo calves included in all 6 experimental groups. Before vaccination, a total of 17 buffalo calves were positive for NSP antibodies with 2 to 4 animals in each group (Figure 1). Most of the calves which were positive for NSP antibodies were less than three months of age (12 out of 17). Number of animals showing NSP antibodies, however decreased in subsequent samplings and all except 2 animals became negative for NSP antibodies by day 120. Among these NSP positive animals, two animals remained positive throughout the study period (one each belonging to group D & group E). No new animal was found positive for NSP antibodies in any group during the course of study.

A similar trend for the development of antibody titer was seen in the vaccinated groups for all three serotypes of FMDV. Following primary vaccination at day 0, there was an increase in antibody titer which further increased following booster vaccination. This humoral response then started decreasing after day 30 post booster. The peak titers produced by local vaccines were much lower than the peak titers produced by imported vaccines. Locally produced FMD vaccines generally induced significantly lower immune response as compared to imported vaccines (Figure 2 A, B & C). Furthermore the titers induced by local vaccines were not maintained after one month post booster vaccination.

Among imported FMD vaccines, the vaccines containing aluminum hydroxide plus saponin as an adjuvant produced a higher immune response and the titers were maintained throughout the experiment. While on the other hand, the vaccine that had double oil emulsion as an adjuvant had lower immune response. However, this difference was not statistically significant.

Out of 75 vaccinated animals in the trial, 25 animals had been previously vaccinated with a local FMD vaccine three months before the trial. This vaccination did not interfere with the development of titers as antibody titers in these animals were similar to those which has not been previously vaccinated. Statistically, there was no significant

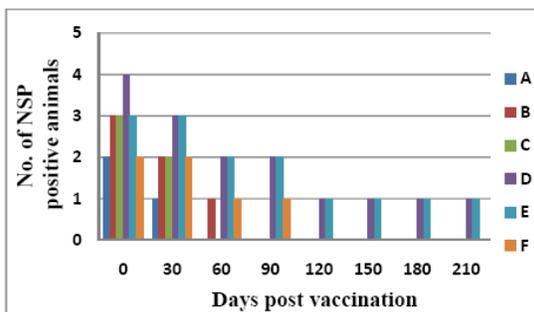


Fig. 1: Number of animals showing antibody titers against non-structural proteins (NSP) of FMDV before and after vaccination. Letters A, B, C, D, E and F indicate the vaccination groups.

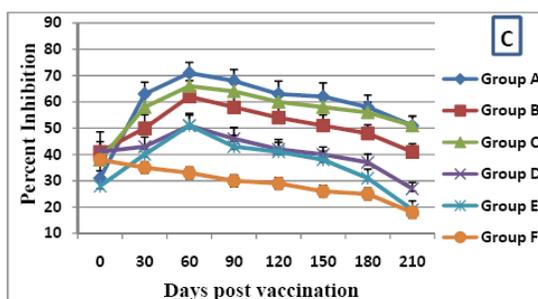
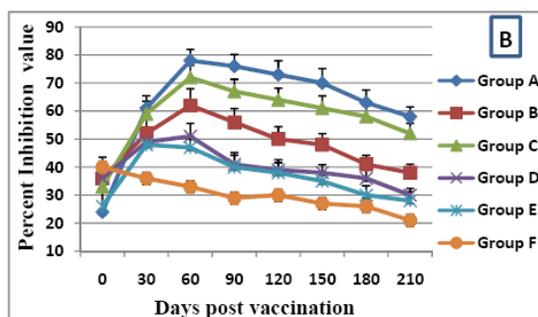
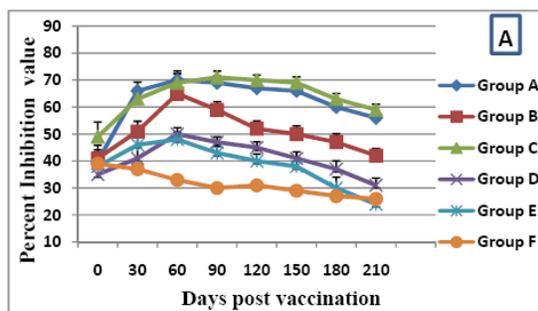


Fig. 2: Immune response of buffalo calves following different FMD vaccines. A shows serotype A titers, B shows titers for serotype Asia-1 and C indicates titers for serotype O. Mean \pm SE of percent inhibition (PI) values in ELISA are plotted. Primary vaccination was given on day 0 followed by booster dose on day 30. Antibody titers were measured using liquid phase blocking assay. Percent inhibition (PI) \geq 50 is normally considered as protective titer.

difference ($P > 0.05$) among these groups. Similarly no difference was observed in the immune response of animals from different age groups (<6 months, 6-12 months, >12 months) against all three serotypes of FMDV.

DISCUSSION

All vaccines used in the study did not induce any untoward reaction following primary or booster vaccination indicating that the vaccine lots used in the study had no safety issue. Almost all vaccine manufacturers undertake safety test on each batch to avoid immediate untoward reaction as recommended by OIE (2014).

Maternally acquired NSP antibodies waned in most of the buffalo calves by fifth month of age. Only two animals vaccinated with local vaccines had continuous titers against NSP. As local vaccines were not purified and had NSP, these vaccines may have induced the antibodies in these animals. Non-purified vaccines are known to induce NSP antibodies in the vaccinated animals (Park, 2013). This is why all OIE/WHO approved FMD vaccine manufacturers remove NSP from the vaccines and animals vaccinated with the purified vaccines do not exhibit NSP antibodies, thus these animals could be differentiated from recovered animals from infection using this DIVA technology.

The study demonstrated that both locally manufactured vaccines induced lower humoral immune response than imported vaccines from well established producers that are following OIE standards. The titers induced by local vaccines neared 50 per cent PI only on 30 days post booster indicating probable protection if any, for a very limited time. Imported vaccines on the other hand seem to provide protective titers for 6 months following booster vaccination. As locally manufactured vaccines are standardized on TCID₅₀ basis and not internationally accepted criteria of mass of 146S antigen, these are not expected to induce the required protective level of SP antibodies. These results also corroborate the results of Jamal *et al.* (2013) who used serum neutralization test to measure antibodies. The results also correlate well with field observations in which many outbreaks of FMD have been observed in herds routinely vaccinated with locally manufactured vaccines (Ahmed *et al.*, 2002; Abubakar *et al.*, 2014).

It was interesting to note that oil adjuvants used in the vaccines (both local and imported) did not induce sustained higher immune response in buffalo calves that are normally seen in cattle (Saravanan *et al.*, 2014). This finding contradicts an earlier study (Muhammad *et al.*, 2013) that reported persistence of FMD antibody titers in buffalo calves following oil based Foot and Mouth disease vaccine when compared to alum hydroxide adjuvant. This could be due to difference in adjuvants as aluminum hydroxide + saponin was used in the vaccines used in present study. This needs to be explored further to determine whether this phenomenon is only limited to oil adjuvants used with FMD vaccine or these oil adjuvants do not induce sustained immune response with other antigens also.

Quality of FMD vaccines mostly depends upon presence of relevant strains of circulating FMD serotypes, quantity of FMD protective antigen (ratio of 146S to 12S in the vaccine) and adjuvant present in the vaccine. There is need to constantly monitor the prevalent FMD topotypes and include the most relevant strains in the

vaccine. Furthermore, for each vaccine strain, the quantity of 146S required for protection has to be determined and incorporated in the vaccine. For FMD vaccine, aluminum hydroxide with saponin work satisfactorily as adjuvant. For use in FMD vaccine intended for buffaloes, oil adjuvants should be evaluated in this species before incorporating these in the vaccine.

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Author’s contribution: AJ undertook experimental work and lab analysis, RZ assisted in designing the experiment, MA guided the lab work and assisted in data analysis, MJA and EK assisted in sample collection, TA managed the experimental animals and MA planned the experiment and did final write-up of the manuscript.

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