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ORIGINAL ARTICLE

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Assessment of immune response in cattle against experimentally prepared trivalent (O, A, and Asia-1) FMD vaccine in Bangladesh

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A B S T R A C T

This research work was conducted to investigate the effects of age, sex and breed on the induction of immune response against experimentally prepared inactivated trivalent (type O, A, and Asia-1) FMD vaccine. Twenty six cattle were divided into four test groups (Group A, B, C, and D; 5 cattle in each group) and one control group (n=6) based on breed (local and cross), age (≤12 months and >12 months), and sex (male and female). Test cattle were vaccinated with the experimentally prepared trivalent FMD vaccine. Pre- and post vaccinated sera from the vaccinated cattle were collected upto 63 days, and the sera were tested using liquid phase blocking enzyme linked immunosorbent assay (LPBE) that was specific for FMD serotypes O, A, and Asia-1. Antibody titers of all the pre-vaccinated serum samples were found to be under protection level. The females were found to be more protected (90%; n=9/10) as compared to males (70%; n=7/10). The titers obtained were statistically analyzed using *t*–test to observe the effects of age, breed and sex. It was observed that the mean values of antibody titer in cattle aging >12 months against O, A, and Asia-1 serotypes were significant ($P<0.05$) at 21, 49 and 63 days as compared to the values obtained from the cattle aging ≤12 months. In conclusion, the local female cattle aging >12 months showed better immune response towards trivalent FMD vaccine.

Keywords

Age, Cattle, ELISA, FMDV, FMD vaccine

ARTICLE HISTORY

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INTRODUCTION

Foot and Mouth Disease (FMD) is an important viral as well as trans-boundary disease affecting almost all cloven-hooved domestic and wild animals such as cattle, goat, sheep, pig, water buffalo and deer (OIE 2012). The disease is identified by fever and blister-like sores on tongue, lips, gum, between the hoove, and teats (Depa et al., 2012).

The FMD virus (FMDV), the causative agent of FMD, is a non-enveloped, positive sense, single stranded RNA virus beloging to *Aphthovirus* genus under Picornaviridae family (Mumford, 2007). The virus has 7 immunologically distinct serotypes (A, O, C, SAT-1, SAT-2, SAT-3, and Asia-1) and >60 subtypes. Vaccine prepared from one type of FMDV does not protect others; thus, each requires a specific vaccine strain to provide immunity to susceptible animal (OIE, 2012).

Among the infectious diseases, FMD is considered as one of the serious problems of livestock population, particularly cattle in Bangladesh (Sil and Taimur, 2000). About $125 million economic losses is occurred per year only due to the outbreaks of FMD in Bangladesh. Currently, FMDV serotypes O, A, and Asia-1 are prevailing in Bangladesh (Zinnah et al., 2010; Sarker et al., 2011; Nandi et al., 2013; Hossen et al., 2014; Alam et al., 2015). Sarker et al. (2011) reported that FMD prevalence in Bangladesh was significantly higher in males as compared to females (Sarker et al., 2011).

To save the animals from FMD vaccination is one of the most effective steps, although it is not ensure protection for longlife (Cloete et al., 2008; Rodriguez and Grubman, 2009). It was shown that protection level conferred by FMD vaccines in prevaccinated animals generally depends on the effectiveness of the vaccine

and antigenic relationship of the vaccine strain and circulating field isolate of FMDV (Goris, 2008). It has been reported that there is a good correlation between antibody response and level of protection in cattle (Pay and Hingley 1992; Barnett et al., 2003).

Considerable difference was observed among animals in terms of their responses to different infectious diseases and vaccination; genetics of host is one of the important considerable factors (Tan et al., 2001; Davies et al., 2009). Doel (1996) stated that species, breed, individuality, age, health, physiological state of cattle and other stress factors (*e.g*., husbandry, climate) might influence in induction of immune response against FMD vaccine. Proper management of cattle helps to decrease the impact of environmental factors by improving the animals' immune system via vaccination and proper nutrition (Hutcheson and Cole, 1986). However, few studies have been reported focusing on the influence of age, sex and breed in inducing immune response against trivalent FMD vaccine in cattle, particularly there is no study emphasizing impact of these parameters in Bangladesh.

Therefore, this study was undertaken with an objective to determine the impact of age, sex and breed to induce antibody response against experimentally prepared inactivated trivalent (O, A, and Asia-1) FMD vaccine in cattle.

MATERIALS AND METHODS

Vaccine: The FMD vaccine was experimentally prepared (data not shown) as water-in-oil emulsion that contained partially purified inactivated FMDV strain (O, A, and Asia-1), Montanide and saponin. Three serotypes of FMDV had been isolated from field samples of different districts of Bangladesh during outbreak in May 2014, and the virus was adapted to grow in BHK-21 cell cultures. Sterility and safety of the vaccines are under-way; all the techniques followed the requirements described in OIE Manual (OIE, 2012). The vaccine batch was stored at 4-8°C and used in vaccine efficacy trials subsequently.

Experimental cattle: A total of 26 local and cross breed cattle aging between 6 to 24 months were randomly selected for this experiment. The cattle were reared by the farmers at their houses at Boyra, Mymensingh. The cattle were classified into four test groups (five cattle in each group) based on sex and breed; group A, B, C and D represented as local male, local female, cross male and cross female, respectively, whereas Group E was considered as non-vaccinated group (control; n=6). The experimentally prepared trivalent FMD vaccines were administered subcutaneously at neck region (dosed at 6 mL/animal) to the cattle of groups A, B, C and D, except control cattle. In vaccinated cattle, 11 animals aged ≤12 months, and 9 aged >12 months. There were no reports of clinical FMD from any of the experimental cattle before and during the course of the study. The cattle were reared under similar supervision and nutrition during trials.

Ethical guidelines: For the animal experimentation, international as well as institutional guidelines were followed.

Serum sample: About 3 mL of blood was collected without anticoagulant from the jugular vein of experimental cattle with the sterile syringe at 21, 35, 49 and 63 days post-vaccination. The syringes were placed in a slanting position at room temperature for clotting. After traction, syringes were placed in the incubator at 37°C for 1 h to retract the clot properly. Then the sera were collected in sterile eppendorf tube. The tubes were centrifuged for 15 min at 1000 rpm to get more clear serum. The serum was then kept in sterile eppendorf tube and was preserved at -20°C until used.

ELISA test: Liquid phase blocking ELISA was carried out for the detection of FMDV antibodies in serum using FMD Antibody Detection Kit (BDSL, UK), as described by Hamblin et al. (1986 a, b).

Statistical analysis: The data obtained were subjected to *t*-test used to analyze the effect of age, breed and sex in vaccinated cattle. Statistical analysis was done with SPSS 20 version.

RESULTS AND DISCUSSION

Among the 20 cattle, ELISA antibody titers of 9 cattle aging >12 months were above protective level where the titers of 4 out of 11 cattle of ≤12 months were below protective level. The immune responses in >12 months aged cattle was significant (P<0.05) at 21, 49 and 63 days in vaccinated cattle against all serotypes comparing to ≤12 months aged cattle. The ELISA antibody titers in cattle >12 months against O, A, and Asia-1 were 250.00±0.00, 234.67±62.47 and 219.33±60.85, respectively (**Table 1**) on 49 days post vaccination where antibody titer in cattle of ≤12 months were 233.64±186.88, 233.64±186.88 and 221.09±190.28, respectively (**Table 2**) on 63 days post vaccination. This indicates that >12 months aged cattle responded well and quickly against FMD vaccine comparing to ≤12 months aged cattle.

The mean and standard deviation of antibody titers against O, A, and Asia-1 serotype were 105.80±19.61 for local breed and 93.40±29.95 for cross breed at 21 days. The highest antibody titers in local breed against O, A, and Asia-1 were 416.00±194.66, 385.00±193.86 and 371.20±208.87, respectively. At the same time, antibody titers in cross breed were 314.00±226.92, 300.20±235.29 and 314.00±226.92 against O, A, and Asia-1, respectively. The protective antibody level in local breed cattle was higher than cross breed, however, it was not significant against all serotypes (O, A, and Asia-1) (**Figure 1**). The titer of all control cattle were at unprotective level throughout the study period, and the value of which was considered as zero.

Better immune response was observed in females (90%; n=9/10) than males (70%; n=7/10), but the observed difference was small and statistically not significant consistently at 21, 35, 49 and 63 days (P>0.05) against O, A, Asia-1 serotypes (**Figure 2**).

Results of the present study closely resembles the findings of Gowane et al. (2013) who stated that adult animals (>1-year; P<0.05) were obtain more protective level than the younger animals. They also reported that impact of sex in vaccinated animals on the vaccine-induced antibody titre was non-significant (P>0.05) for all the three serotypes (O, A, and Asia-1) consistently. There was contradictory evidence that calves responded considerably less well to FMD vaccination than adult animals (Doel, 1996). This variation might be influenced by difference of breed, as described by Giacomo et al. (2013) who compared the response in different sires and breeds. Giacomo et al. (2013) also reported that developed immune response in the offspring of Jersey sires after vaccination significantly lower (P<0.05) than offspring of Holstein sires for the three FMDV strains analyzed at 45 days after vaccination, but there was no significant difference in intra-breed immune responses. However, in our study, significant variation in immune response was not

Table 1: Antibody titers in cattle of >12-months after vaccination with experimentally prepared trivalent vaccine.

Vaccine serotype	Sera collection at day post vaccination	Mean	SD	P-value*
O	21	112.00	0.00	**0.045**
	35	158.00	69.00	**0.008**
	49	250.00	0.00	**0.001**
	63	525.56	103.33	**0.001**
A	21	112.00	0.00	**0.045**
	35	142.67	60.85	**0.021**
	49	234.67	62.47	**0.011**
	63	475.78	170.65	**0.008**
Asia-1	21	112.00	0.00	**0.045**
	35	142.67	60.85	**0.021**
	49	219.33	60.85	**0.013**
	63	491.11	136.70	**0.002**

P = Probability, SD = Standard deviation, * = P<0.05.

Table 2: Antibody titers in cattle of ≤12 months after vaccination with experimentally prepared trivalent vaccine.

Vaccine serotype	Sera collection at day post vaccination	Mean	SD	P-value*
O	21	89.45	31.28	**0.045**
	35	89.45	31.28	**0.008**
	49	127.09	83.67	**0.001**
	63	233.64	186.88	**0.001**
A	21	89.45	31.28	**0.045**
	35	89.45	31.28	**0.021**
	49	164.73	99.53	**0.011**
	63	233.64	186.88	**0.008**
Asia-1	21	89.45	31.28	**0.045**
	35	89.45	31.28	**0.021**
	49	127.09	83.67	**0.013**
	63	221.09	190.28	**0.002**

P = Probability, SD = Standard deviation, * = P<0.05.

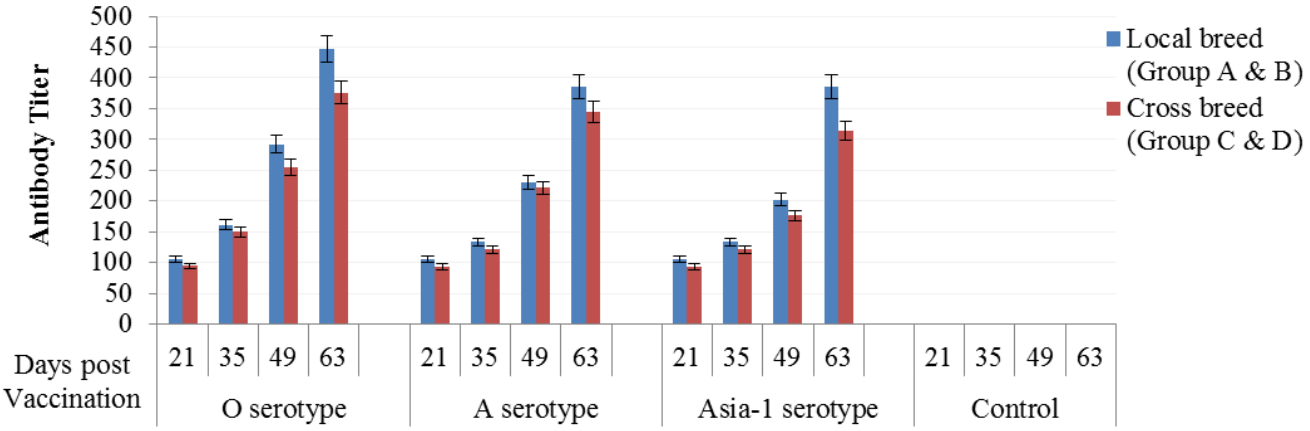

Figure 1: Graphical presentation of antibody titer based on breed against O, A, and Asia-1. The titer of all control cattle were at unprotective level throughout the study period, and the value of which was considered as zero.

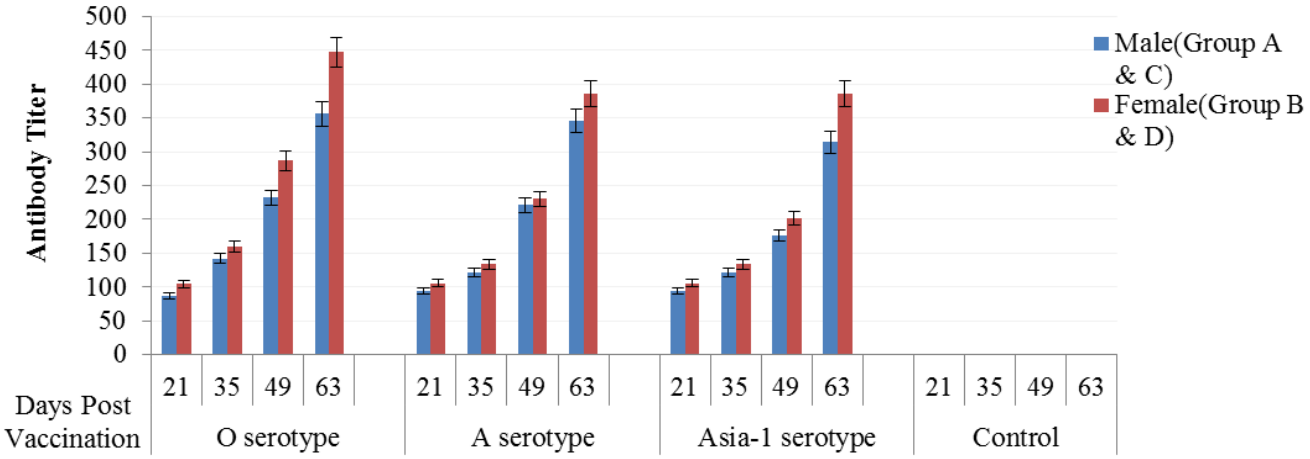

Figure 2: Graphical presentation of antibody titer based on sex against O, A, and Asia-1 serotypes. The titer of all control cattle were at unprotective level throughout the study period, and the value of which was considered as zero.

found between local and cross breeds. This might be due to adaptibility of the cross breeds in Bangladesh.

Şevik (2013) found higher antibody response (P<0.05) in female animals (older than 11 months) as compared to males of same age. No significant correlation between antibody response was found at the age of 0-11 months. Findings of the present study are in partially inclined with the report of Şevik (2013). There are some reports indicating that male reproductive hormones are responsible for suppression of immune cells' activity, reduction of immunoglobulin and cytokine production, and limited proliferation of lymphocyte (Rettew et al. 2008). Several reports have been found about X-chromosome gene that indicate its involvement as a regulators of immune response (Fish 2008; Pinheiro et al., 2011; Dai and Ahmed, 2011). According to these reports, females produce more immune response comparing to males for its X-chromosome. These reports not only differred with our study but also with other investigations (Jakel et al., 2008; Gowane et al., 2013). Jakel et al. (2008) found an insignificant effect of breed and sex against rabies vaccine. So it is not understood how much effects of X-chromosome have to induce significant immune response. Further study is needed to explain the differences in immune response between male and female animals as a result of FMD vaccination.

CONCLUSION

The results of the prevailing study indicate that induction of protective antibody level against FMD vaccine is delayed in cattle aging below 12 months of

age as compared to the cattle aging older than 12 months. So, it can be concluded that age of cattle influence significantly to produce good immune response against FMD vaccine.

CONFLICT OF INTERESTS

First (MMRC) and second (MLH) authors of this article contributed equally. All other authors declare that they have no conflict of interests.

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