



Randomised field trial to evaluate serological response after foot-and-mouth disease vaccination in Uttar Pradesh, India

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The foot-and-mouth disease (FMD), a highly contagious, notifiable disease of animals (cattle, sheep, pigs, goats and wild cloven hoofed animals), is an economically important disease in India with a maximum loss of 49.83% in milk production followed by opportunity cost (16.15%) and reduction in growth (12.20%) (Singh *et al.* 2013). Vaccination of susceptible animals against FMD and antibody monitoring with regular active surveillance is a well established control strategy undertaken by All India Coordinated Research Project on FMD (AICRP-FMD). Vaccination based FMD-Control Programme (FMD-CP) which involves 6 monthly vaccinations of all cattle and buffaloes, is currently being done in many states of India including Uttar Pradesh in all districts using a trivalent (O, A, Asia 1) inactivated and oil adjuvant vaccine at 3 PD₅₀/dose. In regularly vaccinated areas, there has been progressive build up of herd immunity and substantial decline in the occurrence of disease, severity of clinical signs and non structural protein (NSP) reactors/converters (Biswal *et al.* 2012). Despite this, FMD still remains a major threat and the rapid decline in immunity post-vaccination may leave the animal susceptible long before the next 6-monthly round of vaccination.

The serological investigations in endemic countries like India are significant for epidemiological surveys for estimating the efficacy of vaccination programs. Since the protective immune response to FMD virus is heavily dependent on humoral antibody, so the serum antibody level is an indicator of the immune status of the animals.

Keeping this in view, the present study was done with the objective to evaluate vaccine protection in a randomised field trial in Uttar Pradesh.

Area and period of study: Villages (6) from different blocks of Mathura district were selected for the study during 16th phase of vaccination under FMD-CP (2014–15). The

study included traditional small-holdings within villages, where most households keep a few animals.

Vaccine: NSP purified inactivated tissue culture oil adjuvant FMD Trivalent (O, A, Asia 1) vaccine supplied to UP State Animal Husbandry Department under FMD-CP was used. All susceptible animals in each village were vaccinated, with a dose of 2 ml intra-muscularly.

Serum samples: At least 20 serum samples in a volume of 2 ml were collected from cattle and buffaloes randomly from each village before vaccination and 30 days post vaccination. Each household was visited in late October to mid November, 2014 and again in late November to late December 2014 by the project staff of Collaborating Centre of AICRP-FMD, DUVASU, Mathura.

Serological kits: Recombinant r3AB3 NSP-ELISA and single dilution-liquid phase blocking-ELISA (sdLPB-ELISA) kits provided by Directorate on FMD (D-FMD), Mukteswar, Uttarakhand, were used to detect the antibody against NSP of FMDV and sero-conversion to FMDV antigen to all 3 serotypes O, A and Asia-1 serotype, respectively as per instructions on the kit. Testing of the samples was done at Collaborating Centre, DUVASU, Mathura.

r3AB3 NSP-ELISA: The final result for each test serum was expressed as the percent positivity (PP) value, calculated by dividing the OD of the test serum by that of the positive control serum and then multiplying with 100. The result was interpreted as r3AB3 NSP positive, if PP value was more than 40%, and negative, if PP value was less than 40%.

sdLPB-ELISA: The test was performed using optimally diluted rabbit sera specific for all three FMDV serotypes as coating antibodies and anti-FMDV guinea pig serum as tracing antibodies. Indirect estimation of the log₁₀ SN₅₀ values which equates with 50% protection (PD) is referred to as 50% protective antibody (PA). The PA₅₀ values for type O, A, and Asia ≥ 1.8 is considered protective, as per the instruction manual provided along with the kit.

Statistical analysis: Mean log₁₀ antibody titre were compared to detect significant differences in antibody titres between the various vaccine strains separately for pre and post vaccinated sera samples using one-way analysis of

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variance (ANOVA) followed by the Bonferroni multiple comparison test. The differences between the mean \log_{10} antibody titre of pre and post sera for individual vaccine strain types were tested for significance using two tailed paired t-test ($P < 0.05$).

In the present study, 2 heifers (out of 51 cattle) were found sero-positive for anti-NSP antibody in r3AB3 NSP-ELISA indicating prior infections. They were excluded from the study. FMD structural protein (SP) antibody levels are strongly correlated with protection. In sd-LPB ELISA, 120 pre vaccinated and equal number of post vaccinated serum samples comprising 71 samples from buffalo and 49 samples from cattle were tested. The overall percent of animals demonstrating protective antibody titres ($\geq 1.8 \log_{10}$) against FMDV serotypes O, A and Asia-1 in pre-vaccinated cattle and buffalo was 65.8, 53.3 and 49.2%, respectively. A significant rise in herd protection level was noticed after vaccination as the percentage of animals protected against serotypes O, A and Asia-1 virus after vaccination showed an increase to 78.3, 67.5 and 74.2%, respectively. The overall vaccination response against serotype O, A and Asia-1 was comparable in both cattle and buffalo and no significant difference was observed. A higher level of anti 'O' antibody was observed both in pre and post vaccinated serum samples in comparison to serotype A and Asia-1. There was a significant rise in the percent of animals with protective titre ($\geq 1.8 \log_{10}$) for all the three serotypes from 31.6% for pre-vaccinated samples to 63.3% for post vaccination samples. Again no significant difference was observed between cattle and buffaloes in overall protection level for all the three serotypes. It is interesting to note that despite there was little (nonsignificant) change in mean antibody titre in pre and post vaccinated sera samples, significant rise in the percent protected animals after vaccination was observed for all three serotypes together. It is to be worth mentioning that in Western Uttar Pradesh including Mathura, 15 rounds of vaccination were already completed before this trial (Singh *et al.* 2015a). It has been noted earlier that minimal increase of neutralizing antibody titre occurred by additional vaccination of animals vaccinated at least four times (Elnekave *et al.* 2016). In a study in Turkey, 4 to 5 months after vaccination, only a third of single-vaccinated cattle had antibody levels above a threshold associated with protection and it was not until animals had received three vaccine doses in their lifetime, vaccinating every six months, that most (64 to 86% depending on serotype) maintained antibody levels above this threshold (Jones *et al.* 2015).

No significant difference was observed in mean \log_{10} antibody titre in pre-vaccination serum samples between the different serotypes ($P < 0.05$; ANOVA test). However, the mean \log_{10} antibody titre against A-serotype were significantly lower ($P < 0.05$; ANOVA test), from the other two serotypes in post-vaccination serum samples. In the present study, a significant difference was observed in the mean antibody titre of pre and post vaccinated sera samples

for serotype A, however, the change in neutralizing antibody titre was nonsignificant for O and Asia-1.

Ahuja and Sen (2002) reported that for effective control of FMD about 60–80% of animals need to be covered under vaccination so as to control the outbreak of diseases. Efforts to achieve sustained herd immunity, through six monthly vaccination of cattle are compromised by rapid population turnover and waning protection which is evident by occurrence of outbreaks in FMD-CP districts where biannual vaccination (13th and 14th phase) was carried out in 2013–14 (Singh *et al.* 2015b). Serological response to FMD vaccine has been evaluated earlier (Reddy *et al.* 1997, Chhabra *et al.* 2004) in India, but it appears to be first report on randomised field trial.

SUMMARY

Vaccination was done in 6 villages in Mathura by oil adjuvant FMD trivalent vaccine, selecting twenty cattle and buffaloes from each village for the study. Pre and post vaccination serum samples were tested for antibody against O, A and Asia-1 serotype by sdLPB- ELISA. The overall percent of animals demonstrating protective antibody titre against FMDV serotypes O, A and Asia-1 in pre-vaccinated animals was 65.8, 53.3 and 49.2%, respectively. A significant rise in herd protection level was noticed after vaccination as the percentage of animals protected against serotypes O, A and Asia-1 virus was 78.3, 67.5 and 74.2%, respectively. A higher level of anti 'O' antibody was observed both in pre and post vaccinated serum samples in comparison to serotype A and Asia-1. There was a significant rise in the percent of animals with protective titre for all the three serotypes from 31.6% for pre-vaccinated samples to 63.3% for post vaccination samples.

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