Haryana Vet. (December, 2021) **60(2),** 176-178

Research Article

DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS ANTI-3AB NON-STRUCTURAL PROTEIN ANTIBODIES IN MULTIPLE VACCINATED HARDHENU CATTLE AT ORGANIZED FARM

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Received: 13.01.2021; Accepted: 10.06.2021

ABSTRACT

Forty apparently healthy, newly evolved Hardhenu crossbreed cattle, maintained at Animal Farm, Department of Animal Genetics and Breeding, LUVAS, Hisar were divided into four (I-IV)groups of 10 animals each on the basis of age (<1.0; 1.5-3.0; 3.0-5.0 and >5.0 years, respectively) that received different number of foot-and-mouth disease virus (FMDV) vaccine (from multiple manufacturers at different times) shots (1-2, 3-5, 6-9 and >10, respectively) since birth at every six months interval. The pre- and post- FMDV vaccination (up to five months) serum samples were tested in 3AB3 non-structural protein (NSP) ELISA for the presence of FMDV antibodies against 3AB NSP antibodies. The animals from group IV (100%), III(30%), II(20%) and I(0%) were positive for 3AB NSP antibodies. The peak mean3ABNSP antibody response was observed at four months post-vaccination for group I and at one month post-vaccination for groups II, III and IV. There was a significant increase in the proportion of animals demonstrating 3AB NSP antibodies with increase in the number of FMDV vaccine shots. These observations indicated that the available FMDV vaccines (from multiple manufacturers) were possibly having residual amounts of NSPs as contaminating antigen(s), resulting in the development of anti-3AB NSP antibody response in multiple vaccinated Hardhenu cattle.

Keywords: 3AB non-structural protein, DIVA ELISA, Foot and mouth disease virus, Hardhenu cattle, Multi vaccinated

How to cite: Kumar, V., Kakker, N.K., Magotra, A., Mohapatra, J.K. and Dahiya, S. (2021). Detection of foot-and-mouth disease virus anti-3AB non-structural protein antibodies in multiple vaccinated Hardhenu cattle at organized farm. *Haryana Vet.* **60(2)**: 176-178.

Foot-and-mouth disease (FMD) caused by FMD virus (FMDV), a member of the genus Aphthovirus within the family Picornaviridae is a highly contagious disease affecting cloven-hoofed animals leading to severe economic consequences. Considering the economic importance of the disease, Govt. of India launched FMD-Control Program (FMD-CP) during 2003-04 through biannual FMD vaccination in the cattle and buffaloes accompanied by regular sero-monitoring and disease monitoring through active surveillance (Pattnaik *et al.*, 2012). As per the OIE guidelines (in regions adopting vaccination to control FMD), sero-surveillance should be performed by an assay capable of differentiating infected from vaccinated animals (DIVA strategy). In India, DIVA ELISAs have been developed employing 3AB3, 3ABC, truncated 2C (2Ct) non-structural proteins (NSPs), etc. (Mohapatra *et al.*, 2011; Sharma *et al.*, 2012; Mahajan *et al.*, 2013). Of these, the 3AB3 ELISA is being used countrywide for sero-surveillance (Mohapatra *et al.*, 2011). These indigenous assays have also been validated in field (Bora *et al.*, 2014; Lather *et al.*, 2014). Apart from development of antibodies to structural proteins of FMDV, the anti-NSP antibodies can also be induced by residual NSPs contained in commercially available inactivated FMD vaccines (Clavijo *et al.*, 2004). For the purpose of identifying infected animals by detecting antibodies to NSPs induced by contaminated residual NSPs contained in less pure FMD vaccine can be problematic for serological screening. Further, the anti-NSP antibodies have also been detected in multiple vaccinated animals (Lee *et al.*, 2006).

Hardhenu (*Bos taurus* × *Bos indicus*) is a newly evolved dairy crossbreed cattle (62.5% exotic inheritance), best suited to agro-climatic conditions of Haryana (Yadav *et al.*, 2020). These animals have been regularly vaccinated bi-annually with FMDV vaccine from multiple manufacturers since birth. The present communication describes the possible effect of repeated FMDV vaccination on the generation of anti-3AB NSP antibodies in multiple vaccinated Hardhenu cattle which can interfere with accurate identification of FMDV infected animals.

MATERIALS AND METHODS

Animals: Forty apparently healthy Hardhenu crossbreed cattle maintained at Animal Farm, Department of Animal Genetics and Breeding, LUVAS, Hisar were divided into four age groups (I to IV of 10 animals each) on the basis of age and FMDV vaccine shots received since birth (Table 1). The pre-vaccination data from group I calves were used as control (baseline data). The Animal Farm had no history of FMDV outbreak for the last more than ten years. The approval for animal experimentation was granted by the Institutional Animal Ethics Committee (IAEC), LUVAS vide VPHE/IAEC/1702-33 dated 28/05/2016.

Vaccine and vaccination: The animals were vaccinated with trivalent inactivated FMDV vaccine (containing

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FMDV serotypes O, A and Asia-1) in July 2016 as per the manufacturer's instructions (dose and route) and vaccination schedule at the farm. The calves in group I were also administered booster dose of vaccine two months after the first injection.

Blood and serum: Blood samples were collected without anticoagulant pre-vaccination and then every month post-vaccination up to 5 months from July to December 2016; serum was separated using standard protocol, labeled and stored at –20 ºC for further use.

FMDV 3AB3 NSP ELISA: The *E. coli* expressed FMDV recombinant 3AB3 NSP based indirect ELISA kit containing known positive and negative sera, designed, developed and evaluated completely by ICAR-Project Directorate on FMD, Mukteswar (Mohapatra *et al.*, 2011) was used as described earlier (Bora *et al.*, 2014). The final result for each test serum was expressed as the percent positivity (PP) value, calculated by dividing the Optical Density (OD) of the test serum by that of the positive control serum and then multiplying with 100. The result was interpreted as 3AB NSP-antibody positive, if PP value was more than 40%; and negative, if PP value was less than 40%.

Statistical method: Statistical analysis was performed using the IBM SPSS version 23. The frequency of positive and negative cases in between groups was checked by Chi square analysis. However, between groups OD values variation was checked by repeated measures ANOVA at different time intervals.

$$X^2 = \sum (\text{Observed} - \text{Expected})^2 / \text{Expected}$$

X^2 = Chi squared; O = observed value; e = expected value

RESULTS AND DISCUSSION

The serum samples from all the ten (100%), three (30%), two (20%) and none (0%) of the animals in groups IV, III, II and I, respectively having received >10, 6-9, 3-5 and 1-2 FMDV vaccine shots, respectively demonstrated anti-3ABNSP antibodies in pre- and up to five months post-vaccination. The data from pre-vaccinated calves was taken as control (baseline data).

The findings in the present study are in agreement with Lee *et al.* (2006), who observed an increase in the humoral response to 3ABC NSP in repeatedly vaccinated calves and demonstrated that incomplete vaccine purification may lead to transient induction of NSP antibodies in vaccinated animals. The immune responses to NSPs of FMDV have also been observed in cattle vaccinated with concentrated vaccines (Lubroth *et al.*, 1998). In addition, Mackay *et al.* (1998) reported that the animals which had received more than ten vaccinations had NSP antibody profiles similar to those in some animals several months after infection. These occasional, multiple-vaccinated animals could not therefore be differentiated from infected animals on the basis of antibody to NSPs alone. Hayer *et al.* (2018) reported an increase in FMDV NSP-antibody in 3AB3 NSP ELISA following repeated vaccinations. The findings in the present study are consistent with the above observations as all the ten (100%) animals in group IV demonstrated ≥ 40 percent positivity (PP) value for FMDV 3AB NSP antibodies despite the fact that the present study didn't include the FMDV infected and recovered (convalescent) animals.

The statistical analysis revealed significant differences amongst all the four groups in the mean FMDV anti-3AB NSP antibodies response during pre- and all the five months post-vaccination (Fig. 1). None of the animal in group I exhibited PP value more than 40 during pre- and all through five months post-vaccination but there were indications that at four and five months post-vaccination, the mean PP value of FMDV anti-3AB NSP antibodies response (18.0 and 18.3, respectively) was higher than the pre-vaccination response (5.8). The calves in group I demonstrated increasing trend in the mean PP values of anti-3AB3 response during all the six months post-vaccination which could be due to the FMDV booster vaccine (given after two months).The mean PP value was <40 throughout the experiment in group I and II while it increased to >40 after four months of vaccination in group III. All the ten animals in group IV exhibited PP value more than 40 during pre- and at all sampling time points up to five months post-vaccination. The number of animals exhibiting ≥ 40 PP value remained the same i.e. 2 and 3 in group II and III, respectively throughout the experiment. The peak mean PP value of FMDV anti-3AB NSP antibodies response for group IV was 86.3at one month post-vaccination whereas for group III, it was 42.6 and 42.5 at four and five months post-

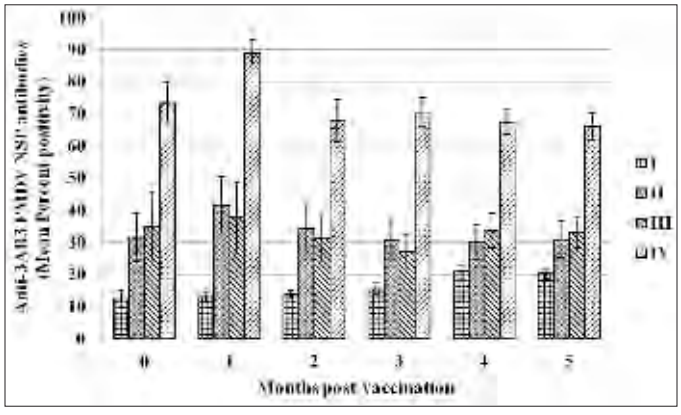

Fig. 1. FMDV anti-3AB3 NSP antibody response (Mean PP value ± S.E.) in pre(0)- and post-vaccination serum samples of Hardhenu cattle. Groups I, II, III and IV: <1.0, 1.5-3.0, 3.0-5.0 and >5.0 years, respectively and 1-2, 3-5, 6-9 and >10 number of FMDV vaccine shots received since birth, respectively.

Table 1

Experimental design depicting different groups of Hardhenu cattle on the basis of age and number of FMDV vaccine shots received since birth

Groups	Animals	Age of animals (years)	No. of FMDV vaccine shots received since birth	No. of animals
I	Calves*	< 1.0	1-2	10
II	Heifers	1.5-3	3-5	10
III	Young Adults	3-5	6-9	10
IV	Adults	> 5.0	> 10	10

* The data from pre-vaccinated naïve calves was taken as control (baseline data)

vaccination, respectively. Espinoza *et al.* (2004) revealed that reactivity to NSPs in the population approached the cut-off value following number of vaccinations, consistent with the results of present study.

The increase in number of animals positive for 3AB NSP antibodies with increase in number of FMDV vaccine shots received and absence of clinical signs development during the period of study and history of no FMD outbreak in the farm for more than 10 years prior to the study probably indicated that the available FMDV vaccines were having residual amounts of NSPs as contaminating antigens resulting in development of anti-3AB NSP antibodies response in multiple vaccinated Hardhenu cattle. From the findings of the present study, it can be concluded that if the cattle had received multiple doses (more than three) of FMDV vaccine from which NSPs were not completely removed, a proportion of these animals might test positive (depending upon the number of vaccine shots received) without actual exposure to FMDV. However, more studies are required to substantiate the data. Thus, it is of utmost importance that the inactivated FMDV vaccines being used in FMD-CP throughout the country are certified as "NSP Free" by the manufacturers for successful adoption of NSP ELISA based DIVA strategy for FMD sero-surveillance which in turn aids in the assessment of effectiveness of vaccination based FMD control programme.

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar and Prof. and Head, Department of Animal Genetics and Breeding, LUVAS, Hisar for providing the necessary permission and infrastructure facilities for undertaking the study. The authors are also thankful to the ICAR-Directorate of FMD, Mukteswar for supplying the 3AB3 NSP ELISA kit.

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