Buffalo Bulletin (October-December 2024) Vol.43 No.4
https://doi.org/10.56825/bufbu.2024.4345591

DETECTION OF ANTI-3AB3 NON-STRUCTURAL PROTEIN ANTIBODIES IN FOOT-AND-MOUTH DISEASE VIRUS VACCINATED BUFFALOES AT A SEMI-ORGANIZED FARM OF MADHYA PRADESH IN INDIA

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Received: 01 October 2023
Accepted: 20 December 2024

ABSTRACT

Foot-and-mouth disease (FMD), one of the most important viral diseases of cloven-hoofed animals in India, is caused by FMD virus (FMDV) which belongs to *Aphthovirus* in the family *Picornaviridae*. There are three serotypes of FMDV (O, A and Asia 1) circulating amongst the livestock population of India. FMD is characterized by the formation of vesicles especially over the tongue and in between the interdigital space. FMD-affected animals with tongue lesions are reluctant to feed and subsequently yield less milk as well as affected animals never regain their production status causing huge economic losses to the animal owners. In the present study, random whole blood samples from FMD-vaccinated 38 adult buffaloes were collected in sterile containers of a semi-organized buffalo farm of Madhya Pradesh in India. Purified FMD vaccines only elicit antibodies (that are protective) against structural proteins of FMDV while natural FMDV infection invokes antibodies against both structural and non-structural proteins. Serum samples were employed in recombinant 3AB3 non-structural protein-based enzyme-linked immunosorbent assay (3AB3 NSP-ELISA kit provided by ICAR-Directorate of FMD, Mukteshwar) for differentiation of FMD-infected and vaccinated animals (DIVA). A total of 10.53% (4/38) serum samples tested positive in DIVA. Largely the vaccinated animals remained protected as no clinical signs of the disease were observed reiterating the importance of regular FMDV vaccination in animals at semi-organized dairy farms.

Keywords: *Bubalus bubalis*, buffaloes, Foot-and-mouth disease virus, 3AB3 NSP ELISA, semi-organized farm, Madhya Pradesh

INTRODUCTION

Foot-and-mouth disease (FMD) is one of India's most important viral diseases of cloven-hoofed animals. It is caused by FMD virus (FMDV) which belongs to the genus *Aphthovirus* in the family *Picornaviridae*. The three serotypes

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of FMDV (O, A and Asia1) are circulating amongst the livestock population of India. FMD is characterized by the formation of vesicles especially over the tongue and in between the interdigital space. It is one of the highly contagious viral diseases of livestock. FMD-affected animals with tongue lesions are reluctant to feed and subsequently yield less milk as well as affected animals never regain their production status causing huge economic losses to the animal owners. India boasts the largest livestock population in the world with a total count of 535.78 million in 2019 as revealed in the 20th livestock census conducted in India. It also tops in milk production in the globe with 24% of global milk production as per the Food and Agriculture Organization Corporate Statistical Database in the year 2021 to 2022. Buffaloes contribute significantly to the total milk production of the country and to the milk production of the state of Madhya Pradesh (Gulati *et al.*, 2021). Generally, trivalent inactivated FMDV vaccine is routinely administered to the livestock population in India for conferring protection. In the present investigation, a herd of FMD-vaccinated buffaloes was investigated for differentiation of FMD-infected and vaccinated animals (DIVA). Foot-and-mouth disease control programme (FMD-CP) was started in India during 2003 to 2004 and by 2010 to 2011, a total of 221 districts were covered under the programme. However, Madhya Pradesh state was not included in the FMD-CP during that period and subsequently, it became a part of the FMD-CP at a later stage. This was followed by the implementation of a nationwide National Animal Disease Control Programme (NADCP) for the control of FMD and Brucellosis in 2019. Since 2022, India's FMD-CP renamed as Livestock Health and Disease Control Programme (LHDCP) endorsed by the World Organization for Animal Health (WOAH), Paris has been run by the Department of Animal Husbandry and Dairying, Government of India.

MATERIALS AND METHODS

Livestock

A FMD-vaccinated (trivalent inactivated FMD oil adjuvant vaccine) herd of buffalo at a semi-organized livestock farm in the state of Madhya Pradesh (Kiratpur in Itarsi tehsil of Hoshangabad) was the focus of the present study. Regular vaccination against FMD was followed as per the routine management practices at the farm. However, in the past few years on the farm whether the FMD outbreak occurred at the farm or not is unknown. More than 150 large ruminants were reared at the farm during the year 2015 to 2016. The majority of these were adult female buffaloes.

Samples

Blood samples from a total of 38 buffaloes were collected in sterile containers after two months of FMDV vaccination, allowed to clot, serum separated and transported to the laboratory of the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Dr. Ambedkar Nagar-Mhow. On receipt, these serum samples were clarified if required for traces of red blood cells by centrifugation at 3,000 rpm for 5 minutes. Serum samples were stored at -20°C till they were further transported in person to the Regional Research Centre on FMD, Department of Veterinary Microbiology, LUVAS, Hisar, Haryana under cold chain for DIVA testing during the year 2017 to 2018.

Recombinant 3AB3 non-structural protein enzyme-linked immunosorbent assay (r3AB3 NSP-ELISA)

The r3AB3 NSP-ELISA was performed to demonstrate antibodies against NSP of FMDV using *Escherichia coli* expressed r3AB3 as developed and standardized by the Project Directorate on FMD (PDFMD), Mukteshwar, Uttarakhand (Mohapatra *et al*., 2011). The r3AB3 NSP-known positive and known negative sera were received from the Central FMD Laboratory, Mukteshwar, Uttarakhand. The test was performed in 96 well ELISA plates as described by Bora *et al*., 2014 and the optical density (OD) of the wells was measured using an ELISA reader (TECAN, Austria) at 492 nm with reference at 620 nm. The test was valid if the mean absorbance of the positive control wells was not less than 0.8. If the mean absorbance of the negative control serum was >0.3, it was rejected. The OD in background control wells should be <0.01. The result is the percent positivity (PP) value (for each test serum) calculated by dividing the OD of the test serum by that of the positive control serum and then multiplying by 100. A sample with a PP value of more than 40% was considered as positive and that with less than 40% negative.

RESULTS AND DISCUSSIONS

In the present investigation, a herd of FMD-vaccinated buffaloes was investigated for differentiation of FMD-infected and vaccinated animals (DIVA). Of the 38 buffaloes tested, four (10.53%) buffaloes demonstrated the presence of FMDV anti-non-structural protein antibodies. In India, DIVA testing in vaccinated animals has earlier been reported by various researchers (Bora *et al*., 2014; Audarya *et al*., 2017; Audarya *et al*., 2019; Kumar *et al*., 2021). Kumar *et al*., 2021 opined that the presence of anti-NSP antibodies in multiple vaccinated Hardhenu cattle could be due to possible residual amounts of NSPs as contaminating antigen(s) in FMD vaccines since there was no history of the FMD at the organized farm. In the present study, the history of the FMD at the semi-organized farm in Madhya Pradesh was unknown. However, the FMD vaccination is being done regularly following routine management practices. Taken together, the results in the present study also indicated that the presence of anti-NSP antibodies in multiple vaccinated adult buffaloes could be due to possible residual amounts of NSPs as contaminating antigen(s) in FMD vaccines and/or possible circulation of FMDV in the buffalo population reared at the farm due to introduction of new animals with uncertain health status.

FMD is of utmost economic importance to livestock industries worldwide and in India (Audarya, 2021; Krishnamoorthy *et al*., 2022). The disease causes a draught power loss to the tune of at least INR 3,400 per animal. Additionally, an amount of INR 800 to 2,500 was spent towards treatment purposes per animal. The total farm-level economic loss projected due to FMD in cattle and buffaloes in India was INR 10,610 million to 221,110 million depending on the severity of the disease (Govindaraj *et al*., 2021). Previously, the highest number of affected villages and disease incidence was observed in non FMD-CP implemented states and one of those was Madhya Pradesh (Govindaraj *et al*., 2021). Soni *et al.* (2012) reported FMD in large ruminants from Jabalpur where 42% of animals were found affected. Krishnamoorthy *et al.* (2022) estimated low prevalence rates of FMD in the central zone of India of which Madhya Pradesh state is also a part. However, the central zone is sandwiched from all

sides by the north, east, west and south zones and all these surrounding zones individually showed higher prevalence rates for FMD than the central zone. Some of these zones (west and east) had more than double the prevalence rates for FMD compared to the central zone. Very few reports are available reporting FMD from the state of Madhya Pradesh. This can be due to protection conferred against FMD because of the inclusion of Madhya Pradesh in FMD-CP and measures adopted for control and prevention including vaccination or underreporting of the events to the authorities or unexplained reasons.

There was a decline in annual outbreaks reported after 2016 except in 2018 because of the implementation of vaccination in livestock populations and movement restrictions. Disease symptoms in ruminant populations were less severe. Consistent vaccination at every six-month interval minimizes the disease incidence and must be implemented along with intensive disease surveillance (Subramaniam *et al*., 2022). Generally, trivalent inactivated FMDV vaccine is routinely administered to the livestock population in India for conferring protection. However, Haryana was the first state in India where the government of India permitted the use of oil-adjuvanted FMD+HS combined vaccine in cattle and buffaloes as a pilot experiment to confer protection against the two most economically important diseases of livestock (Dahiya *et al*., 2022; Rustagi *et al*., 2023). FMDV isolates are categorized into seven major serotypes. Infection with one serotype does not confer immunity against other serotypes (Quinn *et al*., 2011).

The FMDV remains infective on soil for 3 to 28 days depending on the season. Winter season favors survival of the FMDV on soil more than summer. In the present investigation, serum samples were collected from the vaccinated buffaloes in the post-monsoon season. The FMDV can persist in vaccinated animals infected with a subtype different from the vaccinal subtype. The virus can persist in the pharyngeal region of carrier animals that have recovered from FMD (Quinn *et al*., 2011). Considering the above-mentioned facts, the introduction of the virus into the farm may be due to various possible reasons such as the presence of carrier animals in the herd that can harbor the FMDV, the introduction of infected animals into the farm and the entry of the virus by various means through inanimate objects. Such DIVA-tested positive animals can be segregated and further examined for isolation of the virus in the pharyngeal fluid to ascertain their carrier status for the FMDV. Largely the vaccinated animals remained protected as they never showed noticeable clinical signs of the disease reiterating the importance of regular FMDV vaccination in large ruminants of semi-organized dairy farms. Strict adherence to the bi-annual vaccination schedule, timely vaccination coverage, standard preventive and quarantine measures related to animal movement and interaction with farmers to sensitize them about the disease and to vaccinate their animals may prove vital in reducing the incidence of FMD (Govindaraj *et al*., 2021). However, truthfulness in reporting the disease event today and having a holistic approach at the time and during the vaccination regime will safeguard the future of the livestock industries including the dairy sector in India.

CONCLUSION

Based on the results obtained in the present study, the importance of regular FMDV

vaccination in animals of semi-organized dairy farms can be reiterated since the vaccinated animals remained protected largely as they did not show clinical signs of the disease.

ACKNOWLEDGEMENTS

The authors wish to thank all the staff members and supporting hands at the buffalo farm, especially Dr. A. Kirar and laboratory staff members at respective laboratories in this study for their help.

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