Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 4 Issue 2 February 2022

Research Article

Serotype Specific Antibody Avidity in Multiple Vaccinated Animals Against Foot-and-Mouth Disease

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Received: January 24, 2022

Published: January 31, 2022

Abstract

In endemic countries FMD outbreaks are controlled by vaccinating the animals by using a trivalent FMD vaccine. The aim of this study was to compare the avidity of antibodies developed against O and Asia 1 FMDV serotypes in multiple vaccinated animals against FMD. In this study, thirty-three serum samples from multiple vaccinated animals, against Foot-and-mouth disease (FMD) were collected from field buffaloes. Samples with titre more than 1.4 in LPBE were selected for measuring antibody avidity. The study was conducted to know the post vaccination avidity index of antibodies against O and Asia 1 serotype of FMDV. For this purpose, thirty three serum samples from multiple FMD vaccinated animals were tested by O and Asia 1 serotype specific indirect avidity ELISA.

It was found that not all samples having high avidity antibodies for O serotype had high avidity antibodies for Asia 1 serotype. Similarly, not all samples having high avidity antibodies for Asia 1 serotype had high avidity antibodies for O serotype. However, no significant difference was observed in the mean avidity indices of antibodies for both O and Asia 1 serotype. As the avidity indices of antibodies may differ against different serotypes in an individual animal, hence, it is paramount to conduct regular post vaccination monitoring using avidity ELISA.

Keywords: Avidity ELISA; Foot-and-Mouth Disease; High Avidity Index; Post-Vaccination Monitoring

Abbreviations

LPBE: Liquid Phase Blocking ELISA; AI: Avidity Index; FMD: Foot-and-Mouth Disease; FMDV: Foot-and Mouth Disease Virus; PVM: Post-Vaccination Monitoring

Introduction

Foot-and-mouth disease (FMD) is an economically important disease of domestic and wild cloven-hoofed animals. It is caused by the Foot-and-mouth disease virus of the family *Picornaviridae*. In endemic countries, the annual loss incurred by FMD outbreaks and cost of vaccination amount to US$ 6.5 to 21 billion [1]. Additionally, FMD outbreaks in FMD free countries cause an annual loss of more than US$ 1.5 billion [1]. FMD outbreaks in endemic countries are controlled by biannual vaccination. Both pre-vaccinated and post-vaccinated sera are collected and are subjected to Liquid Phase Blocking ELISA (LPBE) to measure and compare the protective antibody titer generated. The antibody generated after vaccination is considered to be protective and is considered to prevent future outbreaks, although, it does not provide sterile immunity.

In an endemic setting, along with the measurement of antibody titer, it is also necessary to measure the binding affinity of

the antibodies with the antigen, particularly in the case of multiple vaccinated animals where the booster dose of FMD vaccine is administered in every six months. The avidity of an antibody refers to the strength of its bonding with antigen and is related to the antigen-antibody site. Avidity ELISA has also been applied for the assessment of heterologous protection against FMDV in cattle [2]. The avidity of antibody responses against FMDV and its relationship with protection has not been investigated, although the idea of relevance of avidity ELISA in complementing quantitative assessments has been already proposed in previous reports [3-6].

In India, the maximum number of cases are caused by O serotype followed by Asia 1 serotype [7]. Therefore, in an endemic setting it is paramount to conduct the post vaccination monitoring for measuring the strength of neutralizing antibodies developed. In this regard, we have previously developed an avidity ELISA for the measurement of avidity indices [8].

In this study, we have compared the avidity of antibodies developed against O and Asia 1 FMDV serotypes in multiple FMD vaccinated animals. The current study emphasizes the importance of regular post vaccination monitoring and checking the antibody status against FMDV serotypes to achieve the target of FMD eradication by 2030 as set by OIE.

Materials and Methods

Serum samples

In this study, thirty-three serum samples from multiple vaccinated animals were collected randomly from the field. These animals were already vaccinated by a trivalent inactivated FMDV vaccine (comprising of O, A and Asia 1 antigens) by the field veterinarians. Multiple vaccinated animals are those animals which have been vaccinated more than two times by using FMDV trivalent vaccine. The serum samples were collected after consent from the animal owners. Proper history of FMD vaccination was taken for multiple FMDV vaccinated animals. Only samples showing LPBE titer more than 1.4, suggestive of strong antibody titer were tested for their avidity.

Avidity ELISA

The samples were tested using an avidity ELISA described previously [8]. The test was interpreted as an avidity index.

$$\text{Avidity Index} = \frac{\text{Optical density (OD) of Urea treated samples}}{\text{OD of Urea untreated samples}} \times 100$$

Cut-off value of avidity ELISA

The cut-off value of O serotype specific avidity ELISA was calculated by measuring the average avidity index of the hundred negative samples and for Asia 1 serotype specific ELISA using thirty-six negative serum samples. The mean value of avidity indices + 2SD was taken as the cut-off value for each of the ELISAs. Samples having avidity index more than the cut-off value were considered as having high avidity antibodies and less than the cut-off were considered as having low avidity antibodies.

Statistical analysis

The avidity indices of antibodies for both O and Asia 1 serotype were compared by Student's t-Test using Graph Pad Prism Software.

Results

Cut-off value of avidity ELISA

Cut-off values for both O and Asia 1 serotype specific ELISA were calculated separately. For O serotype specific antibodies hundred negative samples were subjected to testing by O serotype specific avidity ELISA and their avidity index was calculated. The mean avidity index was 25.96 with a standard deviation of 6.40. The cut-off value calculated was 38.77 for O serotype specific avidity ELISA. Similarly for Asia 1 serotype specific ELISA, thirty-six serum samples were subjected to testing by Asia 1 serotype specific avidity ELISA. The mean avidity indices were 27.97 with a standard deviation of 5.59. The cut-off value calculated for Asia 1 serotype specific avidity ELISA was 39.16.

Avidity Index of multiple vaccinated animals

Out of thirty-three samples, sixteen samples turned to be positive in both ELISAs. The mean avidity indices of these samples didn't differ significantly in paired t tests (p value = 0.224). Eight samples scored negative in both ELISAs (Table 1). However, three samples which scored positive in O serotype specific ELISA were negative in Asia 1 serotype specific ELISA. Similarly, six samples which scored positive in Asia 1 serotype specific ELISA scored negative in the O serotype specific ELISA.

The mean avidity indices of the serum samples for O serotype specific ELISA was 43.19 and for Asia 1 serotype specific ELISA was 44.23. However, no significant difference was found between both avidity indices (p = 0.293).

ELISA for Asia 1 serotype specific antibodies	ELISA for O serotype specific antibodies		
	Negative	Positive	Total
Negative	8	3	11
Positive	6	16	22
Total	14	19	33

Table 1: 2 X 2 contingency table for samples scored positive and negative by ELISA for detection of O serotype and Asia 1 serotype specific antibodies.

Comparison between avidity indices of naïve animals and multiple vaccinated animals

The mean avidity indices of naïve animals and multiple vaccinated animals was calculated and compared by Student's t-Test in a Graph pad prism software. The mean AI of naive animals in O serotype specific ELISA was 25.96 and of multiple vaccinated animals was 43.19(Figure 2). Similarly in Asia 1 serotype specific ELISA the mean AI of naïve animals was 27.97 and of multiple vaccinated animals was 44.23 (Figure 3). A significant difference was obtained between avidity indices of naïve and multiple vaccinated animals (p value = 0.000) in both O serotype specific and Asia 1 serotype specific ELISAs.

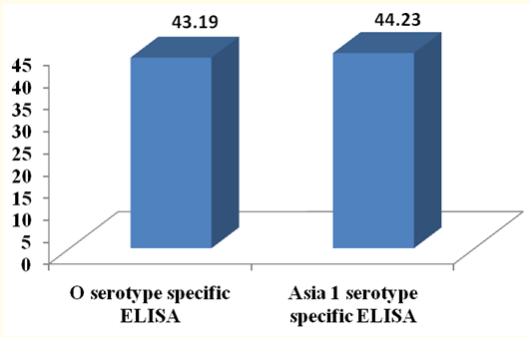

Figure 1: Mean Avidity Index of antibodies in serum sample of multiple FMD vaccinated animals has been depicted for O serotype and Asia 1 serotype specific ELISAs. Values on the Y axis denote the avidity index. No significant difference was observed between the mean avidity indices of antibodies in O serotype and Asia 1 serotype specific ELISAs.

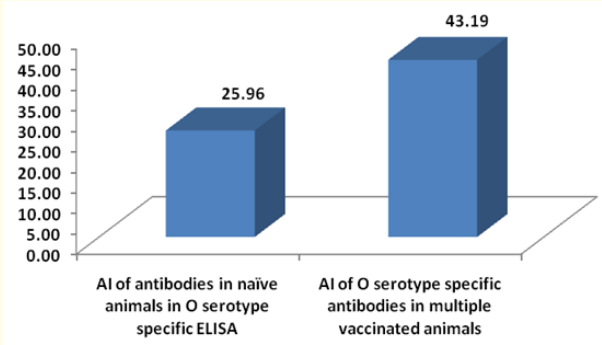

Figure 2: Comparison of avidity indices of antibodies in naïve animals and FMD multiple vaccinated animals after testing in O serotype specific ELISA. The Y axis denotes the Avidity indices. The mean avidity indices are denoted above the bar diagram. The difference between the mean avidity indices between the naïve animals and FMD multiple vaccinated animals was found to be significant (p=0.000).

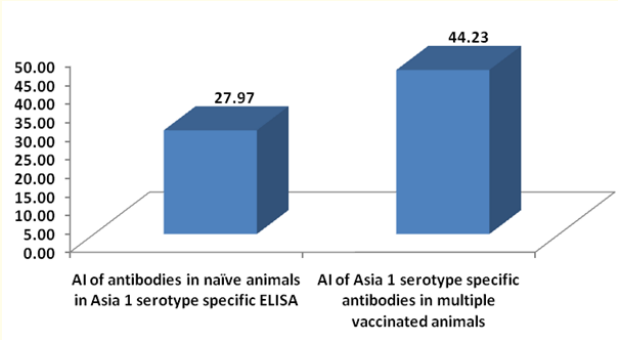

Figure 3: Comparison of avidity indices of antibodies in naïve animals and FMD multiple vaccinated animals after testing in Asia-1 serotype specific ELISA. The Y axis denotes the Avidity indices. The mean avidity indices are denoted above the bar diagram. The difference between the mean avidity indices between the naïve animals and FMD multiple vaccinated animals was found to be significant (p=0.000).

Discussion

In endemic countries biannual FMD vaccination plays a major role to control FMD every year. The animals are vaccinated of commercially obtained inactivated vaccine. Immune response generated against one serotype does not protects animal from infection by another serotype or variants within same serotype [9]. A large sum is invested in FMD vaccination project every year. Therefore, monitoring of the FMD vaccination program and herd immunity becomes crucial in an endemic setting. It also becomes important for countries seeking official recognition by the World Organisation for Animal Health (OIE) as FMD free with vaccination status.

A strong antibody titer generated post-vaccination is suggestive of a successful vaccination regime. Further evidence of the protective nature of elicited antibodies can be confirmed by Virus neutralization test which is time-consuming. Avidity ELISA is an alternative to measure the generated immune response which in past has been used for other diseases [2,10,11].

In the present study, when the qualitative analysis of antibodies generated after multiple vaccinations was done by using avidity ELISA it was found that the avidity of antibodies in an an individual animal varied for both O and Asia 1 serotype of FMDV. Few animals which had high AI for O serotype had low AI for Asia 1 serotype. Similarly, few samples having high AI for Asia 1 serotype had low avidity index for O serotype. This could be due to the difference of the host's immune response generated in animals. However, it becomes paramount to conduct regular PVM for estimating the immune status of animal against various FMD serotypes, despite of the fact that the animal has been vaccinated multiple times. There was no significant difference in the mean AI for both groups which could be because not all animals showed high AI for both O and Asia 1 serotype due to which the decrease in the AI for each of the serotype was compensated in the mean AI.

Some of the animals had high avidity antibodies against both O and Asia 1 serotypes. These animals play a vital role in providing a strong herd immunity for reducing the disease transmission and less shedding of the virus. Not necessarily, these animals may be completely protected because FMDV vaccine does not provide sterile immunity. However, due to stronger immune response developed these animals can act as disease trasnmission stopper against multiple FMD serotypes. Therefore, advocating PVM plans using avidty ELISA may help in developing new strategies for segregating zones based on herd immunity level. Moreover, it may substantiate the OIE target for FMD eradication by 2030.

In this study the avidity indices of antibodies generated against both O and Asia 1 serotype were compared. It was found that not all samples had high avidity indices generated against both O and Asia 1 serotypes, hence, it emphasizes the importance of regular post vaccination monitoring against foot and mouth disease. More precisely, the post vaccination avidity indices of antibodies generated against both O and Asia 1 serotype should be on the higher side so as to combat any unprecedented FMDV infection in cattle and buffaloes. It is a simple and rapid test which can be performed easily by following the aforesaid protocol.

Acknowledgments

This work performed in this work received funding from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India.

Declarations

Funding

The work received funding from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

All data generated or analysed during this study are included in this published article.

Code Availability

Not applicable.

Authors' Contribution

All authors have made considerable contribution to this work. Beenu Jain conducted research, wrote first draft of manuscript and designed research. Anuj Tewari designed research, analysed data, edited manuscript. Surender Kadian supervised the work. All authors reviewed and approved the manuscript.

Ethical Approval

The study protocol was assessed and approved by Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, India. All

protocols designed by Institutional Animal Ethics Committee, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, India were followed while collecting serum samples from field buffaloes. Serum samples used in this study were collected by veterinarians in veterinary practices after taking prior consent from the animal owners, with minimized stress to the animal.

Consent to Participate

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

Consent for Publication

All authors gave their consent for research publication.

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