OPEN ACCESS
Haya: The Saudi Journal of Life Sciences
Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) |ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://scholarsmepub.com/haya/

Original Research Article

Titers of Antibodies to Foot and Mouth Disease Virus in Sera of Vaccinated Cattle and Buffaloes by DIVA and LPB ELISA

H. M Saxena[1*], Pushpinder Kaur[1], Krishan Sharma[1] and Ashwini Kumar[2]
[1]Department of Veterinary Microbiology, College of Veterinary Science, GADVASU, Ludhiana, India
[2]Regional Disease Diagnosis Laboratory, Jalandhar, India

***Corresponding author:** Hari Mohan Saxena
| **Received:** 19.05.2019 | **Accepted:** 25.05.2019 | **Published:** 30.05.2019
DOI:10.21276/haya.2019.4.4.5

Abstract

Sera from 67 bovines (cattle and buffaloes) from villages around Ludhiana city and 10 vaccinated animals from an organized dairy farm were subjected to Foot and Mouth Disease (FMD) DIVA ELISA. Sera from 10/67 field animals and 2/10 farm animals were found positive for the infection. Serial samples at 100 days, 130 days and 145 days post-vaccination from 6 vaccinated buffaloes were assessed for titers of antibody against FMDV serotypes O, A and Asia-1 by Liquid Phase Blocking ELISA. The mean titers were as follows: Antibodies against O at 100 days, 130 days and 145 days = 2.4; Antibodies against A at 100 days = 1.858, at 130 days = 1.866, and at 145 days = 1.85; Antibodies against Asia-1 at 100 days = 2.225, at 130 days = 2.166, and at 145 days = 2.225, respectively. The differences among the mean titers of antibodies against FMDV O, A and Asia-1 at days 100, 130 and 145 were non – significant. However, two animals showed a titer of 1.65 against FMDV A at 100, 130 and 145 days which was below the protective level ($\geq$ 1.8). The findings highlight the need for monitoring larger populations for the level of immunity induced by vaccination.
Keywords: Antibody titers, Foot and Mouth Disease, FMD virus, DIVA, LPB ELISA, cattle.

INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious disease which occurs in Bovidae, sheep, goat, swine, all wild ruminants and suidae. Camelidae have low susceptibility. It is caused by the FMD Virus, an Aphthovirus of the family Picornaviridae. It has seven immunologically distinct serotypes A, O, C, SAT1, SAT2, SAT3, and Asia1 [1]. An epizootic can create extensive losses to the agricultural sector of a country's economy as it affects the animals raised for food or other products. The present study deals with the application of DIVA assay for differentiation of naturally infected animals from those vaccinated with the FMDV and LPB ELISA for estimation of antibody titers in sera of naturally infected animals.

MATERIAL AND METHODS

Blood samples of cattle and buffaloes were obtained from an organized dairy farm of Ludhiana and private farms of Sangroor, Ropar and Kapurthala districts of Punjab state of India. Serum was separated from the blood and 0.05% Merthiolate was added in it to preserve the samples. The sera were kept at -80^{o}C before testing.

DIVA assay

All reagents were equilibrated at room temperature before use. Aliquots of 50 µl of pre-diluted positive and negative control sera were taken in duplicate and put into selected wells and the same quantity of test samples was put in selected wells. Plate was sealed and incubated at 37^{0}C for 30 minutes. The plate was then rinsed three times with PBS Tween Buffer. Then 50µl of HRP conjugate was added in each well and incubated for 30 minutes at 37^{o}C. After 30 minutes again the plate was rinsed three times with PBS Tween Buffer. After rinsing, 50 µl of substrate solution was added to each well and incubated for 30 minutes in a dark room at room temperature. Then 50 µl of stop solution was added to each well and mixed thoroughly. Immediately thereafter, the optical density of control and samples was measured at 405 nm.

LPB ELISA

Test Procedure

Method recommended by OIE [1] was followed. ELISA plates were coated with 50 µl/well rabbit antiserum homologous to the antigen and left overnight in a humid chamber at room temperature. The ELISA plates were washed three times with PBS. In U-bottomed multiwell plates (carrier plates) 50 µl of a duplicate, twofold series of each test serum was

prepared, starting at 1/8. To each well, 50 µl of a constant dose of viral antigen homologous to the rabbit antisera used to coat the plates was added and the mixtures were incubated at 37°C for 1 hour. The addition of the antigen increases the final serum dilution to 1/16. Then 50 µl of serum/antigen mixtures was transferred from the carrier plates to the rabbit-serum coated ELISA plates and the plates were incubated at 37°C for 1 hour on a rotary shaker. After washing, 50 µl of guinea-pig antiserum homologous to the viral antigen used in the previous step (preblocked with NBS and diluted in PBST containing 5% skimmed milk powder) was added to each well. The plates were then incubated at 37°C for 1 hour on a rotary shaker. The plates were washed and 50 µl of rabbit anti-guinea-pig immunoglobulin conjugated to horseradish peroxidase (preblocked with NBS and diluted in PBST containing 5% skimmed milk powder) was added to each well. The plates were incubated at 37°C for 1 hour on a rotary shaker. The plates were washed again three times and 50 µl of substrate solution, containing 0.05% H_2O_2 plus orthophenylene diamine was added to each well. The reaction was stopped after 15 minutes by the addition of 50 µl of 1 M sulphuric acid. The plates were read at 492 nm on a spectrophotometer linked to a computer.

Controls

Four wells each of strong positive, weak positive and negative bovine reference sera at a final dilution of 1/32 were included on each plate together with an equivalent number of reaction (antigen) control wells containing antigen in diluent alone without serum. For end-point titration tests, duplicate two-fold dilution series of positive and negative homologous bovine reference sera were included on one plate of every run.

Interpretation of the Results

Antibody titres are expressed as the 50% end-point titre, i.e. the dilution at which the reaction of the test sera resulted in an optical density equal to 50% inhibition of the median optical density of the reaction (antigen) control wells. The median was calculated as the mean of two mid-values of the reaction control wells, eliminating from the calculation the highest and the lowest values.

RESULTS AND DISCUSSION

Titers of antibodies in vaccinated animals at an organized dairy farm by DIVA ELISA

Serum samples of 10 vaccinated animals (6 cattle and 4 buffaloes) from an organized dairy farm were subjected to FMD DIVA ELISA. Two (1 cattle and 1 buffalo) out of the 10 animals were found to be positive for FMD (Table-1). Since the dairy farm animals were annually vaccinated for FMD, the finding raises concerns about the effectiveness of the vaccination.

Table-1: DIVA ELISA of sera of FMD vaccinated animals from an organized dairy farm

Sr. No	Animal No	Positive (+) / Negative (-)
1.	***C1234***	-
2.	***B2587***	+
3.	***B2465***	-
4.	***B2406***	-
5.	***C0993***	+
6.	***C1346***	-
7.	***C1421***	-
8.	***B2519***	-
9.	***C1346***	-
10.	***C1423***	-

One of the major difficulties in controlling FMD despite regular vaccination programs is the inadequate immunity generated by vaccination in animals. Insufficient antigenic mass in the vaccine could be one of the possible reasons for this discrepancy and this need to be checked out before commencing vaccination programs in endemic areas.

Titers of antibodies to FMDV in sera of field animals by DIVA ELISA

Serum samples from 67 animals from various villages around Ludhiana were subjected to FMD DIVA ELISA. Sera from 10 animals were found positive for FMDV infection. It is believed that all the cattle and buffaloes in villages are vaccinated annually for FMD (Table-2).

Table-2: FMD DIVA ELISA of sera of field animals

Sr. No	Animal No	FMD Positive	Sr. No	Animal No	FMD Positive
1	*MP1*	-	35	*KS-10*	-
2	*MP2*	-	36	*KS-11*	-
3	*MP3*	-	37	*KS-12*	-
4	*MP4*	-	38	*KS-13*	+

5	*MP5*	-	39	*KS-14*	-
6	*MP6*	-	40	*KS-15*	+
7	*MP7*	-	41	*KS-16*	-
8	*MP8*	-	42	*KS-17*	-
9	*MP9*	-	43	*KS-18*	-
10	*MP10*	-	44	*KS-19*	-
11	*MP11*	-	45	*KS-20*	+
12	*MP12*	-	46	*Sangrur-1*	-
13	*MP-13*	-	47	*Sangrur-2*	+
14	*MP-14*	-	48	*Sangrur-3*	-
15	*MP-15*	-	49	*Sangrur-4*	+
16	*MP-16*	-	50	*Sangrur-5*	-
17	*MP-17*	-	51	*Sangrur-6*	+
18	*MP-18*	-	52	*Sangrur-7*	-
19	*MP-19*	-	53	*Sangrur-8*	+
20	*MP-20*	-	54	*Sangrur-9*	-
21	*MP-21*	-	55	*Sangrur-10*	+
22	*MP-22*	-	56	*RS-1*	-
23	*MP-23*	-	57	*RS-2*	+
24	*MP-24*	-	58	*RS-3*	-
25	*MP-25*	-	59	*RS-4*	-
26	*KS-1*	-	60	*RS-5*	-
27	*KS-2*	-	61	*MW-1*	-
28	*KS-3*	-	62	*MW-2*	-
29	*KS-4*	-	63	*MW-3*	-
30	*KS-5*	-	64	*Rop-1*	-
31	*KS-6*	-	65	*Rop-3*	-
32	*KS-7*	-	66	*Rop-4*	-
33	*KS-8*	+	67	*Cl-1/HS*	-
34	*KS-9*	-			

Titers of Antibodies to different serotypes of FMDV in vaccinated animals by LPB ELISA

Serial samples of serum at 100 days, 130 days and 145 days post-vaccination from 6 FMD vaccinated buffaloes were assessed at RDDL, Jalandhar for titers of antibody against FMDV serotypes O, A and Asia-1 by liquid phase blocking ELISA. The mean titers were as follows: Antibodies against O at 100 days, 130 days and 145 days were 2.4; Antibodies against A at 100 days = 1.858, at 130 days = 1.866 and at 145 days = 1.85; Antibodies against Asia-1 at 100 days = 2.225, at 130 days = 2.166 and at 145 days = 2.225, respectively. The differences among the mean titers of antibodies against FMDV O, A, and Asia-1 at days 100, 130 and 145 were non – significant. However, two animals showed a titer of 1.65 against FMDV A at 100, 130 and 145 days which was below the protective level of 1.8 (Table-3).

Table-3: Titers of antibodies to FMDV at various intervals post-vaccination by LPB ELISA

S. No	Animal No	Post-vaccinal titers of antibodies against FMD Virus serotypes								
		O			A			Asia-1		
		0 d	30 d	45 d	0 d	30 d	45 d	0 d	30 d	45 d
1	2172	2.4	2.4	2.4	2.0	2.0	2.0	2.25	2.25	2.2
2	2211	2.4	2.4	2.4	1.9	1.95	1.95	2.2	2.25	2.25
3	2215	2.4	2.4	2.4	1.65*	1.65*	1.65*	2.2	2.2	2.25
4	2310	2.4	2.4	2.4	2.0	2.0	1.85	2.25	2.2	2.2
5	2360	2.4	2.4	2.4	1.95	1.95	2.0	2.25	2.25	2.2
6	2369	2.4	2.4	2.4	1.65*	1.65*	1.65*	2.2	1.85	2.25
Mean ± SD		**2.4**	**2.4**	**2.4**	**1.858± 0.165**	**1.866± 0.169**	**1.85± 0.164**	**2.225± 0.027**	**2.166± 0.157**	**2.225± 0.027**

* = Non protective titer (protective titer is > 1.8); Differences among 0, 30- & 45-days titers of FMDV O, A & Asia-1 serotypes are not significant.

LPB ELISA is a prescribed test for international trade. In general, sera with titres greater than or equal to 1/90 are considered to be positive. A titre of less than 1/40 is considered to be negative. For

certification of individual animals for the purposes of international trade, titres of greater than 1/40, but less than 1/90 are considered to be doubtful. Results are considered to be positive if the second sample has a titre of 1/40 or greater. For the purposes of herd-based serosurveillance as part of a statistically valid serological survey, a cut-off of 1/90 may be appropriate [1].

The duration of immunity (DOI) is an important characteristic of the efficacy of a vaccine. In FMD-endemic countries it is desirable to use FMD vaccines that provide as long a DOI as possible in order to avoid frequent re-vaccination. For effective vaccination, it is desirable that the immune response conferred by vaccines is strong and homogeneous, inducing high levels of protective antibodies and a long duration of immunity in most vaccinated animals. The immune response of goats to two commercial foot-and-mouth disease vaccines was compared [2]. Highest mean antibody titre was observed on days 60 and 21 in goats vaccinated with two doses of algel (group 1) and oil adjuvant (group 2) quadrivalent vaccines, respectively. There was no significant ($P > 0.05$) difference in mean antibody titre between the two vaccine groups. However, the antibody titres for type O fell below the protective titres by day 180 and 270 for groups 1 and 2, respectively. Greater variation in the antibody response among individual cattle has been observed [3] than in individual pigs. This may simply be a species difference or may be due to a combination of factors: vaccine formulation, FMDV strain and animal species.

CONCLUSION

Two out of ten vaccinated bovines of an organized farm and ten out of 67 animals from house holds in villages were found to be positive for FMD by DIVA ELISA. The titers of two out of six vaccinated animals were found to be below the protective level by LPB ELISA 130 days after vaccination. The findings raise suspicion over the efficacy of vaccine or vaccination.

REFERENCES

1. OIE. (2009). Chapter 2.1.5. Foot and mouth disease. OIE Terrestrial Manual 2009, Paris.
2. Madhanmohan, M., Tresamol, P. V., & Saseendranath, M. R. (2009). Immune Response in Goats to Two Commercial Foot-and-Mouth Disease Vaccines and the Assessment of Maternal Immunity in Their Kids. *Transboundary and emerging diseases*, *56*(1-2), 49-53.
3. Smitsaart, E., Espinoza, A. M., Sanguinetti, R., Filippi, J., Ham, A., & Bellinzoni, R. (2004). Addition of saponin to double oil emulsion FMD vaccines enhances specific antibody responses in cattle and pigs. *Report on the European Commission for the control of foot-and-mouth disease. Session of the Research Group of the Technical Standing Committee, Food and Agriculture Organization of the United Nations, Chania, Greece, 11–15 October 2004*, 344-351.