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Antibody Response of Buffalo Calves to Different Levels of Foot and Mouth Disease Virus Immunogen

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Antibody response of buffalo calves to different levels of Foot and Mouth Disease (FMD) virus immunogen was investigated. Vaccine containing $10^{6.2}$ units of immunogen/$TCID_{50}$ of FMD virus (O, A and Asia-1) serotypes induced $\log_2$ (1.3± 0.4) units of anti-FMD O Complement Fixing Geometric Mean antibody (FMD O CFT-CGM) titer, $\log_2$ (1.4±0.3) units of anti-FMD A CFT-CGM titer and $\log_2$ (2.0±0.7) units of anti-FMD Asia-1 CFT-CGM titer. The vaccine containing $2x10^{6.2}$ units of immunogen of each of the virus serotypes induced $\log_2$ (2.2±0.2) units of anti- FMD O CFT-CGM titer, $\log_2$ (2.1±0.25) units of anti- FMD A CFT-CGM titer and $\log_2$ (3.4±0.8) units of anti-FMD Asia-1 CFT-CGM titer. The vaccine containing $3x10^{6.2}$ units of $TCID_{50}$ of each of the virus serotypes

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induced log_2 (5.3 ± 2.0) units of anti-FMD O CFT-CGM titer, log_2 (4.6±1.9) units of anti-FMD A CFT-CGM titer and log_2 (5.0±2.2) units of anti- FMD Asia-1 CFT-CGM titer. Moreover, buffalo calves (n=3) which were primed and boosted with 60 days interval using vaccine containing $2x10^{6.2}$ units of immunogen of each of the virus serotype, showed $log_2$5.0 and $log_2$6.3 units of anti FMD O CFT-GMT antibody titer, $log_2$4.6 and $log_2$6.0 units of anti FMD A CFT GMT antibody titer, $log_2$5.6 and $log_2$6.0 units of anti FMD Asia-1 CFT GMT antibody titer, on 30 and 120 days post boosting.
Antibody response of buffalo calves was directly proportional to amount of FMD virus immunogen serotypes in the vaccine.

Keywords: FMD virus; immunogen; buffalo calves; antibody response.

1. INTRODUCTION

Foot and Mouth Disease (FMD) is a viral problem of cloven hoofed animals such as buffalo, cattle, goat, sheep, deer and camel [1]. The disease is characterized by pyrexia, vesicles on tongue, feet and udder [2]. It causes heavy economic losses in dairy animals that are deliberated in terms of high mortality, morbidity, loss in milk production, working efficiency, quality of hide, weight gain, abortions, cost of treatment and consternation to the farmers [3,4]. Causative agent of the disease belongs to genus *Apthovirus* of family *Picornaviridae*. There are seven distinct known serotypes of FMD virus i.e. O, A, C, ASIA 1, SAT 1, SAT 2 and SAT 3. None of these serotypes have cross immunity against each other. Distribution pattern of FMD virus serotypes and strains vary within geographical regions [5]. Serotype O is the most common serotype worldwide. The disease is endemic in Pakistan and outbreaks are mainly caused by O, A and Asia-1 serotypes [6,5,4].

Disease can be controlled by implementation of strict bio-security measures, culling of infected animal, passive immunization and mass scale vaccination. Keeping in view the poor economic condition of farmers and without any compensation from the government, culling of infected animals is not possible in developing countries. Mass vaccination of the susceptible population is the only effective way to control and eliminate the disease from the country. Thus killed oil based trivalent vaccines has been developed and used successfully to vaccinate the animals [7,8]. The amount of immunogen of FMD virus per vaccine dose is a critical requirement for the production of such killed vaccines. This project was therefore planned to investigate the antibody response of buffalo calves to different levels of immunogen per dose of prevalent serotype of FMD virus in oil based trivalent vaccine.

2. MATERIALS AND METHODS

2.1 Source of Baby Hamster Kidney (BHK-21) Cells and its Propagation

Baby Hamster Kidney (BHK-21) cells were procured from Department of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. The cryopreserved cells were revived using standard procedure as devised by Altaf *et al.* [8]. The BHK-21 cells were grown in Glasgow minimum essential medium (GMEM) with Earl's salts (Biomedical; USA). The medium was supplemented with 5 % Fetal Calf Serum (FCS) and was sterilized using filter of 0.2 µ porosity. The liquefied cells (10^7 cells) were transferred to roller bottle (480 cm^2) containing 100 mL of the growth medium. The bottle was incubated at 37°C with 5% CO_2 for 60 hours and was propagated further.

2.2 Source of Foot and Mouth Disease (FMD) Virus and its Cultivation

Each of the FMD virus serotype (O, A and Asia-1) was obtained from the Department of Microbiology, UVAS, Lahore and cultivated on monolayer of BHK-21 cells in the roller bottles. The virus was then harvested, titrated and processed for vaccine production as per standard procedure given by Altaf I et al., 2012.

2.3 Preparation of Montanide Oil Based Trivalent FMD Virus Vaccine

Each of the FMD virus serotype was inactivated by using binary ethylene imine. The safe and sterile inactivated virus was emulsified in Montanide ISA-70 at 2:3. The mixture was turned into white colored emulsion [8]. The vaccine was packed aseptically in sterilized bottles, labeled properly and transported to Buffalo Research Institute (BRI), Pattolki, district Kasur for trials in experimental buffalo calves. Each dose (3 mL)

from any of the three vaccines (#1, 2 and 3) contained either $10^{6.2}$, $2x10^{6.2}$ or $3x10^{6.2}$ units of immunogen ($TCID_{50}$) of the candidate FMD virus serotypes and 1.8 mL Montanide ISA-70. The vaccine contained 0.05% thiomersal sodium and stored at 4ºC till use.

2.4 Evaluation of FMD Trivalent Vaccines

Twenty experimental buffalo calves (6 months of age) were selected from BRI, Patokki, district Kasur and were divided into four groups A, B, C and D (each group contained five calves). Each of the five calves from group A, B, C and D were vaccinated (3 mL: deep intra muscular) using vaccine # 1, 2 and 3, respectively. The calves of group D were kept as control (non-vaccinated). Similarly six calves of the same age were divided into two groups (E and F: each containing three calves) and primed with vaccine # 2 using the same route and dose of the vaccine. Each of the three calves of group F were boosted 60 days post priming using the same dose and route. Blood (5 mL) was collected from jugular vein of each of the calves on 0, 1, 2, 3 and 6 months post priming. Each of the blood sample was stored at 4ºC for one hour followed with incubation at 37ºC for two hours. Serum from each blood sample was separated and transferred into properly labeled serum vials. Each of the serum samples was stored at – 40ºC till required for monitoring anti FMD virus Complement Fixation Antibody [5]. The antibody titer in each of these samples were determined by Complement Fixation Test (CFT) using multi channel micro titrating 96 well plastic plates [9].

2.5 Statistical Analysis

The serum antibody titer of each calf of each group on 0, 1, 2, 3 and 6 months post priming was processed for calculation of its geometric mean titer (GMT) [10]. The GMT data of each group on 0, 1, 2, 3 and 6 months post priming was processed for calculation of GMT and SD values. These GMT values of the antibody titer of each vaccinated group were compared using one way analysis of variance (ANOVA and subsequently Duncan multiplication test by using SPSS: 13.0 software.

3. RESULTS AND DISCUSSION

The FMD progressive Control Pathway is a serial tool to access endemic countries, developed and endorsed by Food and Agriculture organization (FAO) and World Organization for Animal Health (OIE) Sumption. [11]. Keeping in view the importance of vaccine in the control of FMD, in 2015 the FAO and OIE published Post Vaccination Monitoring (PVM) guidelines to suggest various principles and procedures for monitoring various aspects of FMD vaccines G Ferrari et al., [12]. The assessment of vaccine quality is practiced through small scale immunogenicity studies.

The FMD virus causes viral problem in cloven footed animals. The virus belongs to Apthovirus of Picornaviridae. The virus has single stranded, positive-sense, non-enveloped RNA genome of 8.5 kb. The RNA of FMD virus translates four structural and nine non-structural proteins. The FMD virus genome is surrounded by three external (VP-1, VP-2 and VP-3) and one internal (VP-4) protein [13]. The VP-1 is the most significant protein for virus attachment and serotype specificity. The FMD virus binds on the surface of BHK-21 cells, replicates and induces cyto-pathic effects like lysis or clumping, aggregation and detachment. In the experiment, each of the virus serotypes (O, A and Asia-1) showed high infectivity titer (more than 10^7 units of $TCID_{50}$) on BHK-21 cells. The BHK-21 cells were nourished with cell culture medium having 2% FCS. The FMD virus doesn't attain attenuated form so live attenuated form of vaccine is not available. Each serotype of FMD virus was inactivated using binary ethyleneamine (BEI) that might have inactivated viral nucleic acid and inhibited its replication in the host cells. Such BEI inactivated FMD virus suspension qualified safety and sterility test. The BEI molecules do not bind with the capsid protein so may not decrease its immunogenicity [14]. Inactivated virus suspension without adjuvant absorbs quickly from inoculation site of the vaccinated animals and does not elicit the antibody response. The viral suspension is usually adsorbed on aluminium hydroxide gel or admixed with mineral oil such as montanide ISA-70. The adjuvant enhanced its retention time and induced mild form of granuloma at the injection site. The antigen is removed through antigen presenting cells (APC) for production of cell mediated as well as humoral immune responses [15,16,8]. The humoral response of the buffalo calves is measured by virus neutrilization test (VNT), CFT [17,8] and ELISA [18]. The calves (n=5) primed with montanide ISA-70 based vaccine containing $10^{6.2}$ units of $TCID_{50}$ of each of the three serotypes of FMD virus induced $\log_2 1.3 \pm 0.4$ units of anti-FMD O CFT Cumulative Geometric mean antibody (FMD O CFT-CGM)

Table 1. Effect of immunogen level on antibody response of buffalo calf to Foot and Mouth Disease virus vaccine

Immuogen amount /dose n=5	Serotype of FMD virus	Anti FMD virus Compliment fixation antibody titer on days post priming					
		0	30	60	90	180	CGMT (Log_2) ±SD
$10^{6.2}$	O	0,0,0,0,0 (0)	2,2,2,2,2 (1)	2,2,4,2,4 (1.4)	4,4,4,2,4 (1.8)	2,2,4,2,2 (1.2)	1.3^{a} ± 0.4
	A	0,0,0,0,0 (0)	2,2,4,2,2 (1.2)	2,2,2,2,4 (1.2)	2,4,4,4,2 (1.6)	4,4,4,2,4 (1.8)	1.4 ^{a}±0.30
	Asia-1	0,0,0,0,0(0)	2,2,2,2,2 (1)	4,4,8,8,8 (2.6)	4,4,4,8,4 (2.2)	4,4,4,8,8(2.4)	2.0^{a} ± 0.71
$2x10^{6.2}$	O	0,0,0,0,0 (0)	4,4,4,4,4(2)	4,4,8,4, 8 (2.4)	4,4,4,4,8 (2.2)	4,4,4,8,8 (2.4)	2.2 ^{b}± 0.2
	A	0,0,0,0,0 (0)	4,4,2,4,4 (1.8)	4,4,4,4,4 (2)	8,4,8,4,4 (2.4)	4,4,4,4,8 (2.2)	2.1^{b}± 0.25
	Asia-1	0,0,0,0,0(0)	4,4,8,4,8 (2.4)	8,8,8,16,16(3.4)	16,16,16,16,16 (4)	8,8,8,16,8 (3.2)	3.4^{b} ± 0.83
$3x10^{6.2}$	O	0,0,0,0,0 (0)	8,8,8,8,8 (3)	32,32,16,32,32 (4.8)	32,64,64,64,64(5.8)	256,256,128,256,256 (7.8)	5.3^{c} ± 2.0
	A	0,0,0,0,0 (0)	8, 8, 8, 8, 8 (3)	16,,8,16,8,32 (3.8)	16,64,64,64,64 (5.6)	64,64,64,64,64(6)	4.6^{c}± 1.88
	Asia-1	0,0,0,0,0(0)	4,8,8,8,8(2.8)	8,16,16,16,16(3.8)	64,64,128,16,128(6)	128,128,256,256,128(7.4)	5.0^{C} ± 2.15

Figures in parenthesis indicate the geometric mean Anti FMD O Compliment fixation antibody titer. The mean values having similar superscript are not significantly different (p< 0.05)

Table 2. Effect of boosting on antibody response of buffalo calves to oil based FMD trivalent (O, A and Asia-1) vaccine # 2

Days Post-priming n=3	Anti FMD virus Compliment fixing antibody response (GMT) of buffalo calves to trivalent oil based FMD vaccine					
	FMD Type O		FMD Type A		FMD Type Asia-1	
	Primed calves	Primed and boosted calves	Primed calves	Primed and boosted calves	Primed calves	Primed and boosted calves
0	0,0,0 (0)	0,0,0 (0)	0,0,0 (0)	0,0,0 (0)	0,0,0 (0)	0,0,0 (0)
30	4,4,4 (2)	4,4,4 (2)	4,4,2 (1.6)	4,4,4 (2)	4,4,4 (2)	4,4,8 (2.3)
60 (Boosting)	4,8,4 (2.3)	4,8,4 (2.3)	4,2,4 (1.6)	4,4,4 (2)	8,8,4 (2.6)	4,8,8 (2.6)
90	8,8,16 (3.3)	32,32,32(5)	8,8,8 (3)	32,32,16 (4.6)	32,32,32 (5)	32,64,64(5.6)
180	32,16,32(4.6)	64,128,64(6.3)	16,64,16(4.6)	128,128,128(6)	64,32,32(5.3)	128,128,128(6)

Figures in parenthesis indicate the geometric mean Anti FMD Asia-1Compliment fixation antibody titer

titer, log_2(1.4± 0.3) units of anti-FMD A CFT-CGM titer and log_2(2.0±0.7) units of anti-FMD Asia-1 CFT-CGM titer. The calves (n=5) primed with montanide ISA-70 based vaccine containing $2x10^{6.2}$ units of $TCID_{50}$ of each of the three serotypes of FMD virus induced log_2(2.2± 0.2) units of anti- FMD O CFT-CGM titer, log_2(2.1± 0.2) units of anti- FMD A CFT-CGM titer and log_2(3.4±0.8) units of anti-FMD Asia-1 CFT-CGM) titer. The calves (n=5) primed with montanide ISA-70 based vaccine containing $3x\ 10^{6.2}$ units of $TCID_{50}$ of each of the three serotypes of FMD virus induced log_2(5.3±2.0) units of anti-FMD O CFT-CGM titer, log_2(4.6±1.9) units of anti-FMD A CFT-CGM titer and log_2(5.0±2.2) units of anti- FMD Asia-1 CFT-CGM titer (Table 1). The antibody response of the vaccinated animals is directly proportional to biological titer of the immunogen per dose of the vaccine [17,8,7]. Immunogen amount of each serotype of FMD virus per dose of the vaccine provides guideline for production of multivalent vaccine against common animal diseases. Multivalent vaccines are commonly prepared against infectious diseases of bovine, caprine, canine and poultry [8].

In the present study, the buffalo calves primed with vaccine containing $2x10^{6.2}$ units of immunogen level of each of the three serotypes of FMD virus and boosted on 60 days post priming with the vaccine containing same level of immunogen induced $log_2$5 and $log_2$6.3 units of anti FMD O CFT GMT antibody titer, $log_2$4.6 and $log_2$6.0 units of anti FMD A CFT GMT antibody titer, $log_2$5.6 and $log_2$6.0 units of anti FMD Asia-1 CFT GMT antibody titer, on 30 and 120 days post boosting (Table 2). In the boosted animals, the memory immunocytes are triggered and show speedy response to the immunogen of the second dose of the vaccine. In the boosted animals the higher titers of specific antibodies are detected in serum in shorter period post injection than that of primed animals [8].

4. CONCLUSION

It was concluded that $2x10^{6.2}$ units of immunogen of each of the three serotypes of FMD virus per dose of the vaccine induces more than log_2 2.2 units of CFT antibodies against each of the serotypes. This antibody titer persists for more than six months. Boosting of animals on 60 days post priming show more than $log_2$5 units of anti FMD virus CFT antibodies on 30 days post boosting which showed increasing trend of the antibody titer thereafter.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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