Comparative study on the potency of trivalent vaccine of foot and mouth disease in different cattle breeds and triggered immune response signaling pathway

MOHAMMAD ZEB KHAN[1#], XUWEN GAO[1], XIAOLONG FAN[1], LIYING SONG[1], MUJEEB-UR-REHMAN MEMON[2], DILDAR HUSSAIN KALHORO[2], IQRA BANO[2], SAQIB ALI FAZILANI[2], YIJING LI[1], YIGANG XU[1], LIJIE TANG[1*]

[1] Heilongiang Key laboratory for animal disease control and pharmaceutical development, department of preventive Veterinary Medicine, Northeast Agricultural University, 59 Mucai Street, Harbin-150030, China
[2] Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam-70060, Pakistan

SUMMARY

The Foot and Mouth Disease (FMD) is a very common disease which causes infection in almost all cloven-footed animals including cattle, camel, goat, and sheep. The objectives of current study were to determine the prevalence of FMD in different breeds of Khyber Pakhtunkhwa, Pakistan and to evaluate and compare the potency of locally available trivalent vaccine against FMD in Achai (local breed of Khyber Pakhtunkhwa) and exotic pure breeds i.e. Holstein Friesian and Jersey. In addition, to observed the immune signaling pathway and the involvement of IL-17 cytokine response. A total of 135 sera samples were collected from Achai, Jersey and Holstein Friesian breeds to know the strains of Foot and mouth disease (FMD) circulating in different cattle breeds. The strain-based prevalence against O, Asia 1 and A antibodies against all three tested strains was found. In Holstein Frisian O strain (33.33%) was the highest circulating FMD viral strain followed by Asia1 and A. In Jersey breed, O strain (26.66%) was significantly higher followed by A and Asia 1. Whereas, in Achai breed, O strain (13.33%) was also found to be the highly prevalent FMD viral strain followed by Asia 1. Furthermore, potency of FMD vaccine, prepared at the University of Veterinary and Animal Sciences Lahore, Pakistan besides Deccivac water-based, Germany were used in Cattle, and the potency of the vaccine was evaluated through Complement Fixation Test. The data revealed non-significant difference ($p>0.05$) among the three different groups when evaluated at day 30 and significant difference ($p>0.05$) at day 60 and 90 post vaccination. The difference in antibody titers was found non-significant difference ($p>0.05$) between FMD trivalent preparation of UVAS besides water-based Deccivac on 90th day of immunization screening. A higher titer was recorded in Achai cattle breed followed by Holstein Friesian and Jersey of UVAS made vaccine and water base Desivac vaccine trialed. In addition, initial involvement of IL-17 immune response was observed among challenged groups and the result was supported by IgY antibodies, that showed significantly higher ratio at day 30, 60 and 90.

KEY WORDS

Foot and Mouth Disease, Cattle, IL-17 immune response, Strains, Prevalence, Vaccine.

INTRODUCTION

The Foot and Mouth Disease (FMD) is life threatening viral disease which causes infection in almost all cloven-footed animals including camel, cattle, goat, and sheep. This viral infection is also reported in some wild animals like deer, Asian elephants and wild boar[1]. It causes a considerable loss in terms of decreased productivity[2]. Aphthovirus is causative agent of FMD which belong to family Picornaviridae RNA, non-enveloped and icosahedral virus having a nucleocapsid with diameter 27 to 30 nm, which is surrounded by four capsid coats of proteins enumerated as VP1, VP2, VP3, and VP4. The FMD has seven major immunological serotypes Southern African Territories (SAT) 1, SAT 2, SAT 3, A, O, C and Asia[3].

Foot and Mouth Disease is endemic in Pakistan and its neighboring countries, imposing a substantial negative impact on livestock industries[4]. Cattle and buffalo are a major part and occupy a premier place in the livestock industry, contributing significantly to the economy of the world. Their populations are threatened by a number of health hazards, among the most notable of which are Foot and Mouth Disease. These diseases inflict substantial losses in terms of reduced productivity[5]. The most prevalent serotype of FMD virus in Pakistan is O type followed by Asia1 and A, respectively whiles serotype C has also been reported sporadically in 1954, 1963 and 1995[6,7]. Efforts to control FMD in Pakistan have been limited by the lack of resources available in the country, and many features of the

Corresponding Author:
Lijie Tang (tanglijie@163.com).

transmission and control of FMD in Pakistan are yet to be elucidated. Traditional practices, such as unregulated animal movement between nomadic and transhumant herds, limits the effectiveness of veterinary services. Foot and Mouth Disease vaccines are not used systematically in Pakistan, and thus attributes such as the efficacy, potency, and strain composition of the vaccines are uncertain[6].

Buffalo and cattle are the main contributor in the livestock sector and play a vital role directly and indirectly in the livestock sector contributing drastically to world economy. In Pakistan, the breeds of cattle are Red Sindhi, Thari, Rohjan, Dhanni, Lohani, Bhag Nari, Sahiwal, Achai[2]. Globally FMD is the main concern in improvement of the socio-economic situation[8]. Vaccination is the most proper method for the control of disease in China, India and certain African countries where FMD is endemic. The World Organization for Animal Health (OIE) officially documented Argentina and Uruguay as free countries from FMD with the proper schedule vaccination and management[9]. The efficacy of vaccination program will largely depend on the quality (purity, safety, and potency) and suitability of the chosen vaccine as well as selection of vaccine on basis of suitable adjuvants like oil or gel-based. The choice of adjuvant in vaccine development determines its effectiveness or ineffectiveness.

The adjuvant can be classified on the basis of chemical nature, origin and physical properties as well as on the basis of activation of innate or adaptive immune response[10]. The OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals describes two methods for assessing FMD vaccine potency in cattle, namely the Ph. Eur. 50% protective dose (PD50) test and the South-American "Protection against Podal Generalization" (PPG) tests OIE[11]. In addition, relations between pathogen PAMPs and TLRs trigger a torrent of indicating actions that result in the stimulation of dendritic cells and cellular reactions, such as the difference of naive T helper (Th) cells into mature effector Th1, Th2, Th17, and Treg cells[12,13]. These signals actions terminate in the secretion of different cytokines, including proinflammatory interleukin (IL)-1 Th cells, interferon-γ (IFN-γ) by Th1 cells, IL13 by Th2 cells, IL-17 by Th17 cells, and transmuting growth factor (TGF)-β and IL-10 by Treg cells[14,15]. Cytokines are the effector fragments that linkage information among cells of the immune system.

Currently in Pakistan, various types of inoculations are used for FMD but still outbreaks of FMD have been reported in various regions even in vaccinated animals which points towards the facts to be investigated. Keeping in view the above-discussed problem, the current study was intended to determine the prevalence of FMD in District Swat Pakistan and evaluate the potency of locally/imported prepared trivalent vaccine in Achai, Jersey and Holstein Friesian breed. Furthermore, it illuminated the host role in characterization of antibodies and immune response of different cytokines in the vaccinated animals.

MATERIAL AND METHODS

Study Area and Epidemiological Study

The study was conducted in Khyber Pakhtunkhwa, Pakistan. Data for the study was collecting on the basis of Tag number, sex, age, breeds, previous vaccination, manure disposal and interaction of animals with each other.

Experimental design

A total number of 36 animals of 1-3 years of age from Achai (A), Jersey (B) and Holstein Friesian (C) breeds were used to determine vaccine potency. Each group was comprised of 12 animals which were divided into 3 subgroups, A1, A2 A3, B1, B2, B3, C1, C2, and C3. Each subgroup A1, B1, and C1 were comprised of 4 animals, and was injected with manufacturer recommended dose of oil-based vaccine, A2, B2, and C2 subgroups were injected according to the manufacturer recommended dose of water-based vaccine while; subgroups A3, B3 and C3 were kept as control.

Prevalence percentage

The total number of 135 sera samples, 45 samples from Achai, 45 samples from Jersey and 45 from Holstein Friesian breeds were collected from suspected animals of those farmers visited in the study area respectively. Data were collected regarding the suspected risk factors of these animals through a questionnaire. The sample size was calculated by considering the expected Seroprevalence to be 8-10% based on previous studies conducted in Pakistan[16] with confidence limits at 95%.

Vaccine procedure and Sample collection

Sampling was started by day 0 i.e. preparing dosage in all three assemblies, each group contains 4 animals and the booster dose was given after 30 days. The Immunization of animals was performed with a master line sterile BD syringe in the early morning. The Inoculation was directed via both intramuscular as well as subcutaneous routes. The 1st blood sample was collected on day 0 before priming. The 2nd, 3rd, and 4th sampling was collected after 30, 60 and 90 days respectively. All vacutainers were placed into dry ice bags and were moved instantly to Veterinary research and disease investigation center Balogram Swat, Pakistan and kept at 4 for 24 hours. The serum was separated via centrifugation. The serum samples were properly labeled and stored in Ultra Cold Freezer at -20 for additional processing.

The Complement Fixation Test (CFT)

The CFT was performed to measure the anti-FMD O antibody titer from serum samples from vaccinated cattle as per recommendations of[11] with little modification.

Enzyme-Linked Immunosorbent Assay (ELISA)

Post challenged groups of Achai, Jersey and Holstein Friesian breeds, IgY antibodies were detected at day 30, 60 and 90 via using ELISA kit, (Meilian Biological Technology Co., Ltd.) by follow the manufacturer's instruction. Briefly, at day 30, 60 and 90 post challenged of vaccine to Achai, Jersey and Holstein Friesian breeds, their blood were collected and preceded for getting plasma and serum samples. Next, samples were examined in triplicate; every ELISA plate contained their own standards. Dilution for IgY 1:20,000 as a detection of antibody. Afterward, the samples were incubated with tetramethylbenzidine for 30 min (Sigma), reaction was stopped via 2 M H2SO4 (55 μL/well). Primary wavelength for read was 450 nm by using an ELX 800 universal micro plate reader (BIO-RAD Model 680) and absorbance was measured at 490 nm. To compute antibody concentration, the mean of the duplicate values for each sample was calculated. The mean value of the blanks was subtracted from the measured antibody concentration to account for non-

Table 1 - PCR and RT-qPCR primers list with references.

Primers	Target gene	Bp	Reference or source
F-5,-GGTGGTGCTAAGCGTGTTAT-3,	GAPDH	264	K01458
R-5,-ACCTCTGTCATCTCTCCACA-3,			
F-5,- AGCTGACGGTGGACCTATTATT-3,	IFN-γ	259	Y07922
R-5,- GGCTTTGCGCTGGATTC-3,			
F-5, -TCTGGGACCACTGTATGCTCT-3,	IL-2	256	AF000631
R-5, -ACACCAGTGGGAAACAGTATCA-3,			
F-5,-AGAGCTCGGTGACCTCAGAC-3	IL-4	140	DQ852343
R-5,-CTTGCATGGCGGTCTTTAG-3			
F-5,-GAACTCCCTGGGGGAAAAC-3	IL-10	145	AF0680058
R-5,-GGCTTTGTAGACGCCTTCCT-3			
F-5, -CTCAGCAGTTGGTCATCTCC-3,	IL-12	588	19; 169(1-2): 82-92.
R-5, -CACTGCCTTCCTGACACTCC-3,			
F-5,-CTCCGATCCCTTATTCTCCTC-3,	IL-17	292	AJ493595

specific binding, after read blank adjusted data were transferred to an Excel file. The concentration of the standard and their absorbance value for each plate was created; each sample concentration of the antibody was expressed as micrograms /milliliter.

Cytokines expression by Quantitative real-time polymerase chain reaction (RT-qPCR)

RNA was isolated from all samples of challenged groups with control by using the TRIzol Plus RNA Purification kit (Invitrogen) following the manufacturer's instructions, and then eluted 100 ul RNase-free water to determine concentration, transparency and concentration of RNA were measured spectrophotometrically at an optical density of 260 nm, via NanoDrop spectrophotometer ND-2000, (Thermofisher, MA) and verified the purity of RNA via the ratio of absorbance at 260 nm / 280 nm[17]. To reverse transcribed into cDNA using M-MLV reverse transcriptase (Promega). The cDNA was analyzed by Quantitative real time PCR (RT-qPCR). The oligonucleotide primers used for analyses, GAPDH and specific target genes primers are shown in supplement (Table 1). Briefly, RT-qPCR Light cycler 96 (Roche, Switzerland) was used. Total 10 µl mix comprised 4.5 µl of SYBR Green Supermix (Bio-Rad, Hercules) 0.5 µl of each primer, forward and reverse, 1 µl of cDNA and 3.5 distilled water. Standard curve was created by using log10 diluted standard RNA, and individual's levels of transcript were standardized to those of GAPDH analyzed by the Q-gene program. For normalize RNA levels, the mean threshold cycle (Ct) values for products amplification were calculated by pooling values from all samples in that experiment. Every samples relative level was calculated by pfaffls method[18].

Statistical analysis

The test result for each animal was recorded in a Microsoft Excel spreadsheet. Descriptive statistical analysis and multiple logistic regressions were being carried out using State software version 9 (State Corp., College Station, Texas, USA). The true prevalence was calculated using a Win Episcope 2.0 (University of Edinburgh, UK). Data for vaccine potency was analyzed by statistical package for social science (SPSS) version 20.0. One-way ANOVA was done to calculate the antibody response of each inoculation. Additionally, Chi-square assessment was done to observe the bond among qualitative variables. P-value < 0.05 was set as a level of significance.

RESULTS

The point prevalence of FMD was significantly higher in exotic breeds as depicted in table 2. Amongst the exotic breed's prevalence of FMD was statistically higher in Holstein Friesian than Jersey (33.33%). The lowest FMD prevalence was recorded in Achai cattle breed in the present study area.

The O strain prevalence was found significantly higher than Asia-1 and A in all three breeds of cattle (Table 3).

A significant difference of potency in the sense of antibody titer level was recorded amongst different cattle breeds (Table 4). A higher titer was recorded in Achai cattle breed followed by Holstein Friesian and Jersey. Also, a statistically significant variation of cumulative geo means titer was recorded against Desivac vaccine trialed in three different cattle breeds each group having 4 animals. A stronger titer was recorded in Jersey followed by Holstein Friesian and Achai. No variation of CGMT was recorded among control group.

Table 2 - Prevalence of FMD in different cattle breeds in Khyber Pakhtunkhwa, Pakistan.

Breeds	Sample Size	Positive	Prevalence (%)	P-Value
Holstein Friesian	45	19	42.22%	0.027
Jersey	45	15	33.33%	
Achai	45	07	15.5%	

Table 3 - Prevalence of different (FMD) Disease of virus strain circulates in different cattle breeds in, Khyber Pakhtunkhwa, Pakistan.

Breeds	Asia 1 Prevalence	O Prevalence	A Prevalence	P-Value
Holstein Friesian	9 (20.00%)[b]	15 (33.33%)[a]	5 (11.11%)[c]	0.041
Jersey	5 (11.11%)[c]	12 (26.66%)[a]	8 (17.77%)[b]	
Achai	05 (11.11%)[b]	6 (13.33%)[a]	5 (11.11%)[b]	

Table 4 - Comparison of different vaccine potency in different cattle breeds.

Vaccine type	Overall mean Titre of different Vaccines			P-Value
	Achai Breed	Jersey	Holstein Friesian	
UVAS Made	9.0[a]	7.41[c]	7.74[b]	0.043
Desivac	5.39[c]	9.13[a]	8.70[b]	0.037
Control	1.12	1.24	1.37	0.729

Comparison of anti-FMDV antibody titer of various groups

The difference among the three different groups of Achai, Jersey and Holstein Friesian administered with FMDV vaccines was significant that is p≤0.05. Group A (UVAS made primed with Gel Oil-based enhanced with Gel Oil Grounded Vaccine via subcutaneous-intramuscular paths respectively) group B (water-based Vaccine via intramuscular route respectively) group C (left as a control). Group A, B, and C resulted in cumulative geometric mean titer CGMT±SD values of 15.31±1.20, 12.20±1.691 and 1.22±0.71 respectively (Figure 1).

Comparison of two vaccines

The antibody titers obtained were subject to SPSS Version 20.0.0 for the calculation of ANOVA. The results revealed that there was no imperative variation P≥0.05 among the antibody mixtures of FMD trivalent inoculation of UVAS and water-based (Desivac) vaccine at 90th day of immunization screening CGMT±SD ratios of 12.05±.68 and 7.71±5.05 correspondingly (Figure 2- 3).

Comparison of gel oil and water-based

There was a significant difference seen between the adjuvants of vaccine i-e P≤0.05. The animals immunized through priming and boosting dosage of gel oil grounded preparation displayed pointedly increased, the anti-FMD antibody titer as compared to the animals vaccinated with water. Moreover, the CGMT±SD of gel with oil and with water were 15.31±1.20, 8.07±4.04 correspondingly (Figure 3).

Comparison of the route of administration

There was significant difference i-e P≤0.05 between the groups vaccinated with various routes of administration.

The animals immunized with priming dosage with the subcutaneous route and by means of boosting dose intramuscular displayed meaningfully increased anti-FMD antibody titer at the 90th day of immunization associated with those vaccinated intramuscularly for both priming's as well as boosting displayed CGMT±SD 15.31±1.20 and 10.51±2.19 respectively (Figure 4).

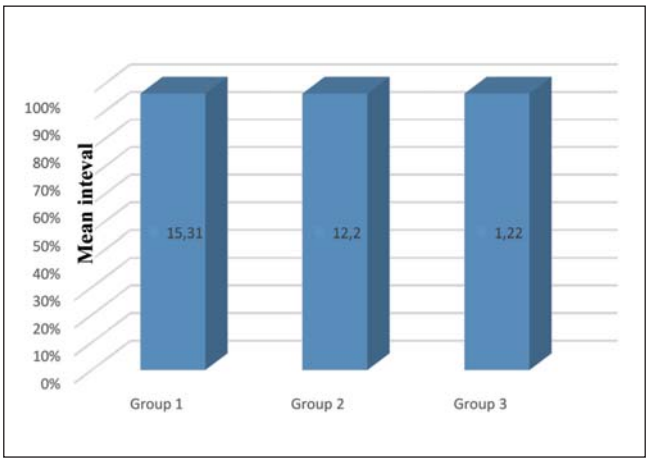

Figure 1 - Comparison of mean anti-FMD virus antibodies. The difference among the three different groups of Achai, Jersey and Holstein Friesian administered with FMDV vaccines was significant that is p≤0.05.

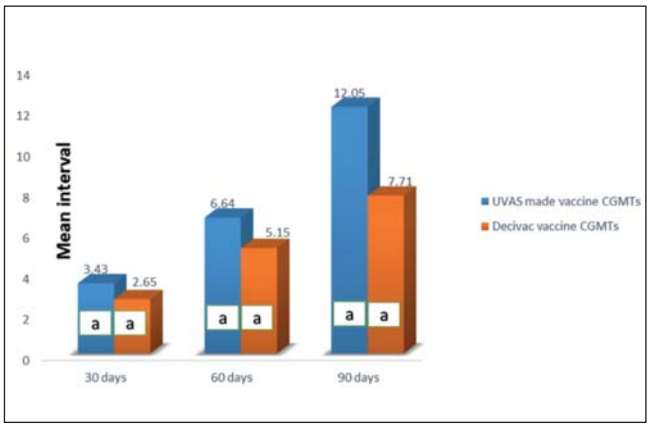

Figure 2 - Comparison of days' post-vaccination. There was non-significant difference P≥0.05 between the antibody titers of FMD trivalent vaccine of UVAS and water based (Desivac) vaccine at 90th day of inoculation showing CGMT ±SD values of 12.05±.68 and 7.71±5.05 respectively.

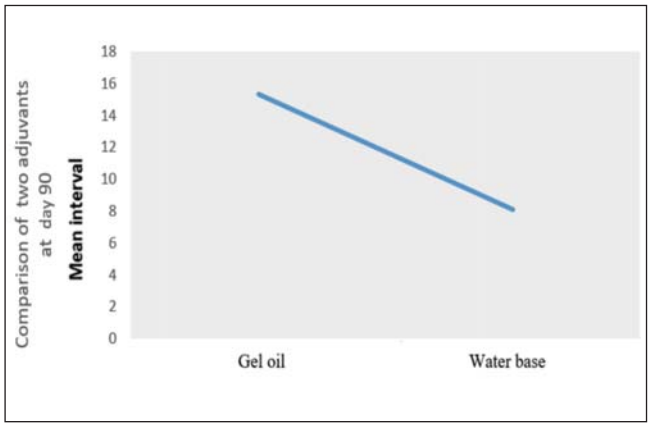

Figure 3 - Comparison of Gel Oil base and water base. Animals inoculated with priming and boosting dose of gel oil based vaccine showed significantly high anti FMD antibody titer than animals inoculated with water for both priming and boosting. CGMT±SD of gel with oil and water with water were 15.31±1.20, 8.07±4.04 respectively.

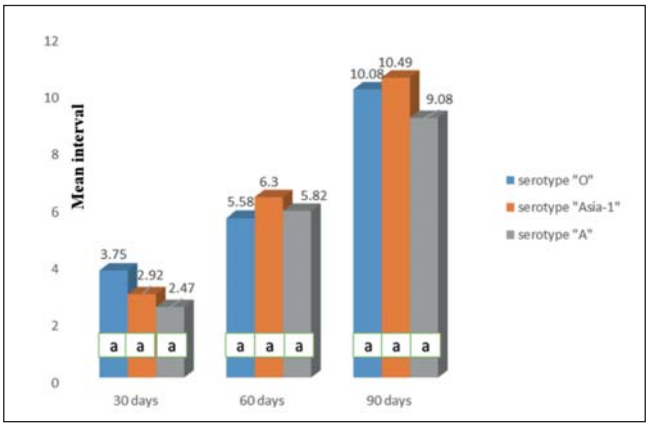

Figure 5 - Comparison among different serotypes of FMD. There was non-significant difference i-e P≥0.05 among the anti FMD antibody titers produced in response of different three serotypes of FMD. The serotypes are serotype "O", "serotype Asia 1" and Serotype "A" showing CGMT±SD values 10.08±5.46, 10.49±5.49 and 9.08±4.73 respectively.

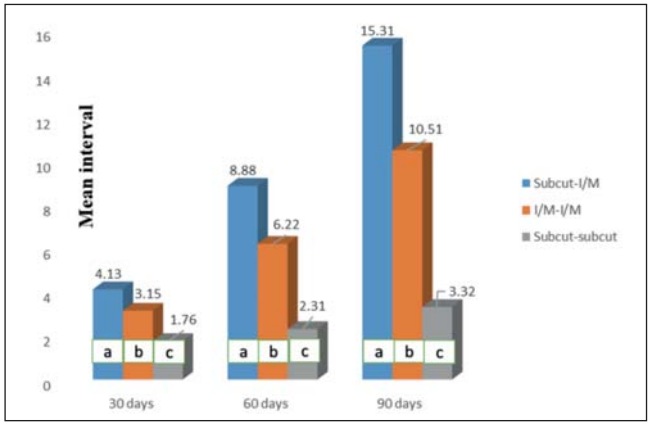

Figure 4 - Comparison of different vaccine administration. There was significant difference i-e P≤0.05 between the groups vaccinated with various routes of administration. The animals inoculated with priming dose through s/c followed by boosting dose I/m showed significantly high anti FMD antibody titer at 90th day of inoculation compared with those inoculated intramuscularly for both priming and boosting showing CGMT±SD 15.31±1.20 and 10.51±2.19 respectively.

Moreover, the animals immunized by means of oil-based vaccines for both priming as well as boosting via S/c showed marked significant decline in the anti-FMD antibody titer.

Comparison of different serotypes

There was a non-significant difference i-e P≥0.05 between the anti-FMD antibody titers of different three serotypes of FMD. The serotypes are serotype "O", "serotype Asia 1" and Serotype "A" showing CGMT±SD values 10.08±5.46, 10.49±5.49 and 9.08±4.73 respectively (Figure 5).

Cytokine Gene Expression and IgY titer

Immune response amongst challanged groups was determined via expression levels of cytokines, cytokine IL-17 and IFNγ, expression was significanltly ($P < 0.05$) upregulated at day 30, 60 and 90 that showed theirs initial immune response as compared with unchallenged group. Furthermore, IL-2, IL-4, 1L-10 and IL-12 were unregulated in challenged groups at day 30 period but showed significantly upregulated at day 60 and 90 as compared with control (Figure 6 and 7). In adding, humoral adaptive immune response specified that greater levels of antibodies against FMD were identified in the plasma of challenged breeds as compared with control (Figure 7) that showed their suggestive evidence and indication that IL-17 are mainly involved in initial mediating immune response. Among different routes the IgY titer significantly higher in challenged groups at day 30, 60 and 90 that supported the results of cytokines. In addition, gel oil grounded preparation presented increased the IgY titer as compared to the animals vaccinated with water. Moreover, ELISA test supported the results of cytokines.

DISCUSSION

In this study, 3 different serotypes 'O' ASIA 1' and 'A' of (FMD) Disease virus were carried under the study to find out the response of vaccine against (FMD) disease. The results of the research proved no significant variation (p≥0.05) between the (FMD) antibody titers displayed in reply of 3 diverse serotypes of FMD. The serotypes are serotype "O", "serotype Asia 1" and Serotype "A" showing CGMT±SD ranges 10.08±5.471, 10.49±5.49 and 9.08±4.73 respectively. The findings of the current study are in agreement with the results of[19,20] that used different serotypes of the FMD viruses for safety evaluation and antibody titers of the vaccines. The different serotypes were combined in vaccines were also reported in their study. In present study the titers of antibody of every (FMD) Disease serotypes were observed similarly in all studies cases. It can be concluded that there was no significant difference among the effect of antibody titer of vaccine. The study of[21] tested FMDV quadrivalent PEG concentrated vaccine with double oil emulsion that was tested in goats. The serum neutralization titers were 2.76, 2.94, 3.0 and 3.22 for serotypes O, A, C and Asia 1 after 90-day post-vaccination, while, AGS vaccine, gave titers 1.02, 1.68, 1.02 and 1.27 for O, A, C and Asia 1, respectively. During current

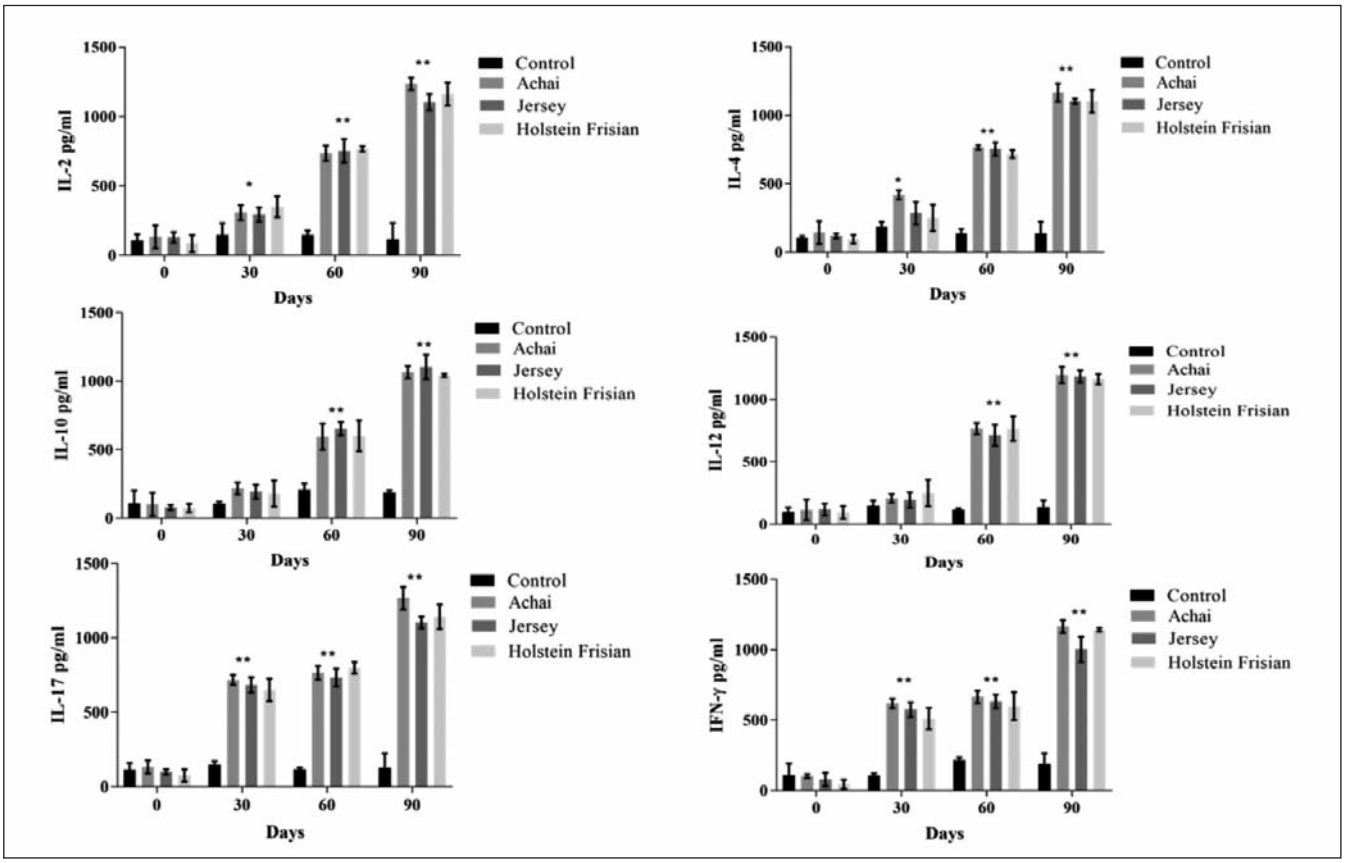

Figure 6 - Expression levels of cytokines, cytokine IL-17 and IFNγ, expression was significanltly ($P < 0.05$) upregulated at day 30, 60 and 90 that showed their initial response as compared with unchallenged group. Furthermore, IL-2, IL-4, IL-10 and IL-12 were unregulated in challenged groups at day 30 period but showed significantly upregulated at day 60 and 90 as compared with control.

experiment various responses of immune were observed significant ($P \geq 0.05$) statistically. The mention difference in the immune response was observed because of the addition of oil adjuvant in vaccination that increased time duration of the potency of immunity as compared to the water-based vaccination. Moreover, the vaccine which was gel based showed rapid antibody titers that are more important during early response of vaccination is required in case of outbreak. The strain-based prevalence against O, Asia 1 and A antibodies against all the three tested strains were found. O strain prevalence was found significantly higher than Asia-1 and A. In Holstein Friesian O was found the highest-circulating FMD viral strain followed by Asia1 and A. In Jersey breed O was found significantly higher followed by A and Asia 1. While in Achai FMD viral strain O was also found to be the highly prevalence FMD viral strain followed by Asia 1 and A respectively. The present study is in line with the previous studies of[22,23] who stated that in Pakistan, the most prevalent serotypes are O, Asia-I and A respectively. Same statement has been reported by the[6], who had reported that the distribution of various (FMD) Disease serotypes virus in different areas of Pakistan showed that O serotype was detected high prevalence serotype followed by Asia 1 and A (F, D) disease serotype C. Furthermore,[24] found highest proportion of positive samples of serotype O, followed by serotype A and serotype Asia-1 respectively.

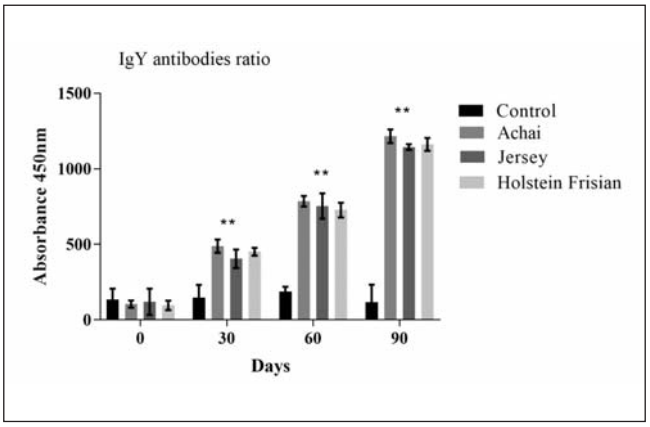

Figure 7 - IgY level at day 30, 60 and 90. Each value is represented in triplicate±SD. significantly difference (P≤ Value 0.05).

The data revealed non-momentous alteration ($p>0.05$) between the three dissimilar groups in which one was be kept as control directed with FMDV inoculations when assessed at day 30 and noteworthy alteration ($p<0.05$) at day 60 and 90 post-immunization. Group 1, 2 and 3 displayed (CGMT±SD) ratios of 15.31±1.20, 12.20±1.69 and 1.22±0.70 respectively. Moreover, the analysis of modification displayed momentous alteration ($p<0.05$) within the antibodies among assemblies on days 60 and 90. In addition on day 60 in assembly 1, inoculated by means of UVAS gel adjuvant shot for priming and oil adjuvant by way of booster dosage, uppermost average range was seen tracked through group 2, inoculated through water grounded Deccivac vaccine. Moreover, the average ratio of antibodies was decreased in Group 3 and was found once the antibody titer was calculated at day 90 alike outline was detected for antibodies.

The outcomes are in line as described by[25] who used inactivated

FMD virus suspension was mixed with aluminum hydroxide gel induced detectable level of anti-FMD virus antibodies in rabbits. They found that antibodies in serum appeared earlier in the rabbits when vaccinated with Aluminum Hydroxide gel (AHG-FMDV) vaccine as compared to those vaccinated with Lanolin (LAN-FMDV) and Montanide Oil Base (OBFMDV) vaccines. The adjuvants such as aluminum hydroxide gel and oils are still used in vaccine production as a base of vaccine formulations. Gel type adjuvant is commonly used in vaccines of veterinary importance. Liquid paraffin was commonly used as mineral oil (oil adjuvant) in animal vaccines. The emulsifying agents like Mannide monooleate (Arlacel-A) and Sorbitan monooleate (Span- 80) were used in 10% concentration in vaccines in the research[26].

The reliability of different inoculations mainly rests on the emulsifier concentration[27,28]. The gel was least toxic for animal tissues, hence suitable for use in bovine, caprine, equine and even in human vaccines, while oil adjuvant vaccine induces a vaccine depot at the site of inoculation hence causes inflammation due to irritation, and recruiting lymphocytes and antigen-presenting cells at the injection site[26] used oil- or AI (OH) 3/ saponin-adjuvanted vaccines in an experiment that demonstrated potency and competency of vaccine inducing antibodies in first week of administration in cattle. Dissimilar results in diverse species of animals were described[28]. Found that water FMD inoculation gave minute duration of resistance and antibody titer quickly fall although oil adjuvant FMD vaccines had lengthier period of immunity.

The difference in antibody titers in the current study was found non-significant ($P>0.05$) between the antibody titers of FMD trivalent inoculation of UVAS and Deccivac water-based at 90th day of vaccination viewing (CGMT±SD) ratios of 12.05±.68 and 7.71±5.05 respectively. There was a momentous difference ($p<0.05$) among the adjuvants of inoculation. The animals immunized with priming dosage of gel grounded vaccine tracked via oil-based boosting displayed significantly increased anti-FMD antibody titer as compared to the animals immunized with oil for mutually priming as well as boosting. The (CGMT±SD) of gel by means of oil and water with water was 15.31±1.20, 8.07±4.04 correspondingly. The water grounded inoculations were proceeding less efficacious than the oil-adjuvant inoculations of cattle and sheep. The advancement in vaccine construction is focused on the assortment of the appropriate adjuvant that can elaborate increased as well as prolonged immunity.

Further, we hypothesize that the various functions and characteristics of the different cytokines and their immune regulatory molecules that secreted by the various subsets of T-helper cells that previous described by different authors[28-15]. Generally, when stimulated via macrophages and/or dendritic cells, T-helper cells typically produce IL-1 and other pro-inflammatory cytokines that stimulate their differentiation into Th1, Th2, and Th17. In present study, at day 30, IL-17 expression was upregulated. Beside, activated Th1 cells primarily produced IFNγ, and sometimes IL-2, that are very important for protection against intracellular pathogens via activation and promote of phagocytes and production of complement-fixation antibodies[14]. In this study, IFN-γ showed significant effect from day 30, while IL-10 and IL-12 showed no significant difference at day 30, whereas at day 60 and 90 found significantly difference. Moreover, IL-4 and IL-2 showed unregulated difference among breeds at day 30, while showed upregulated at day 60 and 90. This may indicate the IL-17 cells play initial role and involvement in the rising of immune responses. Further, the presence of high levels antibodies in challenged breeds and upregulated IL-17 at three-time period also supports the results of cytokines expression. These results showed that IL-17 cells are primarily involved in signaling mechanism underlying the inflammatory responses. This study makes positive linked with previous reported by[15-30,31].

CONCLUSIONS

It is concluded that point prevalence of FMD was significantly higher and exotic breeds i.e., Holstein Friesian and Jersey, O strain prevalence was found significantly higher than Asia1 and A in Holstein Friesian, Jersey and Achai. Holstein Friesian is the most susceptible breed as compared to Jersey and Achai cattle breed. The University of Veterinary and Animal Sciences (UVAS) made an oil base vaccine via subcutaneous-intramuscular route showed best produced higher anti-FMDV antibody titers results against FMD as compared to Decivac water-based vaccine. The present study on seroprevalence of Foot and Mouth Disease in cattle is essential for further epidemiological studies to develop effective disease control strategies, particularly in the areas where animal movement is not restricted. Moreover, these results suggest that any forthcoming plans to moderate vaccine against FMD should be particularly based on their impending to induce Th17 (IL-17) cytokine excretion.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

References

1. Ko, Y.J., Jeoung, H.Y, Lee, H.S, Chang, B.S., Hong, S.M., Heo, E.J et al. (2009). A recombinant protein-based ELISA for detecting antibodies to foot-and-mouth disease virus serotype Asia 1. J. Virol. Meth. 159: 112-118.
2. Economic survey, Economic. Adviser's wing finance division, government Pakistan (2015).
3. Davies, G. Foot and mouth disease. (2002) Res.Vet. Sci. 73: 195-199.
4. Zahur, A. B., H. Irshad, Hussain. M, R. Anjum and M. Q. Khan. (2006). Transboundary Animal diseases in Pakistan. *J. Vet. Med. B. Infect. Dis. Vet.* Public Health. 53: 19- 22.
5. Economic survey, Economic. Adviser's wing finance division, government Pakistan (2011).
6. Jamal, S. M., G. Ferrari, S. Ahmed, P, Normann and G. J. Belsham. (2011). Genetic diversity Of foot- and mouth disease virus serotype O in Pakistan and Afghanistan. *Infect. Genet. E. vol.* 11:1229-1238.
7. Jamal, S. M., S. Ahmed, M. Hussain and Q. Ali. (2010). Status of foot-and-mouth disease in Pakistan. *Arch. Virol.* 155: 1487-1491.
8. Kitching, R.P. and Hughes, G.J. (2002). Clinical variation in foot and mouth disease: sheep and goats. Revue Scient fique Et Technique-Office International des Epizooties. 21: 505-510.
9. Lombard, M., and Fussel. A. (2007). Antigen and Vaccine banks: technical requirements and the role of European antigen bank in the emergency foot and mouth disease vaccination. Revue Scientifique et technique, (International Office of Epizootics). 26: 117-134.
10. Marciani, D.J. (2003). Vaccine adjuvants: role and mechanism of action in vaccine immunogenicity. Dug Dis. Today. 8: 934-943.
11. OIE, A. (2008). Manual diagnostic tests vaccines of terrestrial animals. Office International des, Epizooties Paris France. 1092-1106.
12. St. Paul, M., Brisbin, J, T., Abdul-Careem, M. F., Sharif, S. (2013). Im-

munostimulatory properties of Toll-like receptor ligands in chickens. Vet. Immunol. Immunopathol. 152:191-199.
13. Oh, S. T., Lillehoj, H. S. (2010). The role of host genetic factors and host immunity in necrotic enteritis. Avian Pathol. 45:313-316.
14. Cosmi L., Maggi L., Santarlasci V., Liotta F., Annunziato F. (2014). T helper cells plasticity In inflammation. Cytometry., 85:36- 42.
15. Narsale A, Moya R, Davies JD. (2018). Human CD4 + CD25 + CD127 hi cells and the Th1/Th2 phenotype. Clin. Immunol., 188:103-112.
16. Nawaz, Z., Arshad, M., Rahman, S.U. and Iqbal, Z. (2015) Epidemiological investigation of foot and mouth disease in bovines of Faisalabad. J. Agric. Res. 53:129-135.
17. Wang J., Yi M., Chen X., Muhammad I., Liu F., Li R. (2016). Effects of colistin on Amino acid neurotransmitters and blood-brain barrier in the mouse brain. Neurotoxicol. Teratol., 55, 32-37.
18. Pfaffl MW. (2001). A new mathematical model for relative quantification in real-time RTPCR. Nucleic Acids Res., 29 (9), 45e-45.
19. El-Bagoury, G.F., El-Habbaa, A.S., Gamil, M.A. and Fawzy, H.G. (2014). Evaluation of an inactivated combined oil vaccine prepared for foot and mouth disease and bovine fever viruses. Ben. Vet. Med. J. 27:1:221 231.
20. Selim, A., Abouzeid, N., Ahaour, A. and Sobhy, 368 N. (2010). Comparative study for immune efficacy of two different adjuvants bivalent FMD vaccines in sheep. J. Am. Sci. 6:370 1292-1298.
21. Patil, P., Bayry, J., Ramakrishna, C., Hugar, B., Misra, L. and Natarajan, C. (2002). Immune responses of goats against foot-and-mouth disease quadrivalent vaccine: comparison of double oil emulsion and aluminum hydroxide gel vaccines in eliciting immunity. Vaccine. 366 20: 2781-2789.
22. Zulfiqar, M. (2003). Draft Report for Development of National Disease Control Policy for Foot and Mouth Disease in Pakistan under the FAO Project "Support for Emergency Prevention and Control of Main Trans-Boundary Animal Diseases in Pakistan Rinderpest, FMD, and PPR."
23. Anjum, R., Hussain, M., Zahoor, A.B., Irshad, H. and Farooq, U. (2006). Epidemiological Analyses of Foot and Mouth Disease in Pakistan. Int. J. Agric. Biol., 5, 648-651.
24. Abubakar, M., Irfan, M. and Manzoor, S. (2015). Peste des petits ruminants in Pakistan; past, present and future perspectives. J. Anim. Sci. Technol. 57: 15-32.
25. Muhammad, K., Chaudhry, Z.I., Rabbani, M., Altaf, I., Tariq, M.A. and Anees, M. (2011). In process quality control factors affecting the potency of foot and mouth disease virus vaccine. Pak. J. Zool. 43: 249-254.
26. Dalsgaard, K. (1987). Adjuvants.Vet. Immunol. Immunopathol. 17: 145-152.
27. Chowdhury, S., Rahman, M., Rahman, M. and Rahman, M.M. (1996). Strains of foot-and-mouth disease virus in different districts of Bangladesh. Asian-Aust. J. Anim. Sci. 9: 315-317.
28. Daoud, H.M., Ibrahim, E.E.S., El-Din, W.M.G. and Hassanin, A.I.H. (2013). Preparation of Foot and Mouth Disease trivalent vaccine type A, O, SAT2 with the determination of the Guinea pig protective dose 50 (GPPD50). Vet. World. 6: 844-851.
29. Vignali DA., Collison LW. Workman CJ. (2008). How regulatory T cells work. Nat. Rev. Immunol. 8:523-532.
30. Fasina YO, Hyun S, Lillehoj. (2019). Characterization of intestinal immune response to Clostridium perfringens infection in broiler chickens. Department of Animal Sciences, Greensboro, NC 27411, USA. Poultry Science. 98:188-198.
31. Ana M. Espino, Francheska Rivera., (2010). Quantitation of cytokine mRNA by real-time RT-PCR during a vaccination trial in a rabbit model of fascioliasis. Vet Parasitol.169 (1-2): 82-92.