Received: 15 November 2025 | Revised: 25 December 2025 | Accepted: 3 January 2026

https://doi.org/10.56946/jzs.v4i1.835
Volume 4 (1): 1-9

Journal of Zoology and Systematics

Research Article

Evaluation of the Serological Response after Foot-and-Mouth Disease Vaccination in Pakistan

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Abstract

Among viral diseases, foot and mouth disease (FMD) is a highly contagious, economically important disease of cloven-hoofed animals caused by a virus of the genus *Aphthovirus*, family *Picornaviridae*. A total of 12 calves of about one year old with proper tags were selected for the study. These animals were divided into four groups i.e A, B, C and D (each having 3 animals) and Group C as a control group having 3 animals (including two positive C1 and one negative control animals C2). Group D having 3 animals were named a challenged group as these animals after priming dose were exposed to live virus. Blood samples from animals of all groups were collected on day zero. On day zero, 3 groups (A, B & D) were vaccinated with 3ml Foot and Mouth Disease Research Laboratory (FMDRL) Trivalent Foot and Mouth Disease Vaccine (Oil-based) simultaneously. On the 28th day of Priming dose, Group A was given a booster dose, while Group D, along with a positive control C1, were challenged with live virus. Group B was reared with a priming dose only. Group D, along with the positive control, were examined for the next 10 days for any pathognomonic signs related to FMD. In clinically positive case the signs developed and the animals were tested for the FMD virus. Antibody titer of all the groups was checked through enzyme linked immune sorbent assay (ELISA) on the 28th day and then after every 30 days for 12 months. The protection provided by the "O", "A", and "Asia-1" serotypes in the vaccine on the priming dose was 10, 6, and 6 months, respectively. Similarly, the protective titer provided by the "O", "A", and Asia-1 serotypes in the vaccine on the boosting dose was 13, 13, and 12 months, respectively. The antibody titers provided by the animals of group D (with infection) were 10, 8, and 8 months for O, A, and Asia-1. From the present study, it is concluded and recommended that the FMDRL vaccine is collectively effective and provides a protective titer against all serotypes for at least 6 months on a single dose. However, further studies may be conducted to elucidate the protection provided by the vaccine.

Keywords: FMD, Vaccine, ELISA, Antibody Titer.

1. Introduction

Livestock contributes 61.9% to agricultural Gross Domestic Product (GDP) and 14% to national GDP in Pakistan, supporting over eight million rural families (Economic Survey, 2021–22). Despite its value, the sector faces challenges such as low productivity, poor breeding, limited feed, weak healthcare, and a lack of organized markets [1]. Another research on the dairy and livestock sector in Pakistan has identified that poor genetics, inefficient management, and imbalanced nutrition are major barriers to productivity, limiting milk yield and reproductive performance in cattle and buffaloes. Many farmers struggle with low-yield local breeds and inadequate feeding practices, which contribute to extended calving intervals, low milk output, and reduced herd performance. These issues are compounded by weak veterinary services and the lack of organized surveillance and precision herd management, further constraining improvements in production efficiency and economic outcomes [2]. Among these challenges, Foot and mouth disease (FMD) is a contagious viral infection of cloven-hoofed animals, threatening global livestock economies. While 70 countries are FMD-free , Pakistan and many others remain endemic, with cattle, buffaloes, goats, sheep, and pigs most affected [3]. Vaccination is the primary control tool in endemic regions like Pakistan, yet its effectiveness depends heavily on vaccine quality, potency, and accurate monitoring through serological and diagnostic strategies [4]. Among viral diseases, Foot and Mouth Disease is a highly contagious disease of cloven-hoofed animals caused by a virus of the genus *Aphthovirus*, family *Picornaviridae* [5]. Its genome contains a single-stranded positive-sense RNA molecule of approximately 8 kb [4]. There are seven serotypes of Foot and Mouth Disease virus O, A, C, Asia-1, Southern African territories type (SAT) 1, SAT 2, and SAT 3 [5]. Three serotypes (O, A, and Asia-1) are endemic in Pakistan [6].

Among the domesticated species, cattle, buffalo, sheep, goats, and pigs are most susceptible to Foot and Mouth Disease (FAO, 1984). It is documented that lesions are present, signs such as lameness, unwillingness to eat (which aligns with anorexia), and discomfort can vary by species; there is no cross-immunity among these serotypes due to differences in their antigenicity [7]. "The hallmark feature of clinical FMD is the appearance of characteristic fluid-filled vesicles in areas of non-haired skin, often concurrent with fever and viremia." It also notes that, even when vesicular while vesicles may appear subtle in some animals [8]. Rapid and highly sensitive diagnostic tools are required for efficient control of this disease.

Presently, FMD in the laboratory is diagnosed via ELISA and molecular approaches, i.e., Reverse Transcription Polymerase Chain Reaction (RT-PCR). Serotype-specific RT-PCR analysis not only confirms ELISA serotyping results but can also be used for the screening of ELISA negative samples [9]. These techniques make it possible to identify the virus quickly and precisely, which facilitates the adoption of containment strategies and quick decision-making [10]. In females, the mammary gland is affected. The virus takes shelter and survives in the cells of the pharyngeal epithelium. The timely diagnosis and awareness campaign would help to educate the human population about this disease and get their feedback in case of any outbreak or sporadic case. This investigations would increase disease awareness and epidemiological information [11]. The spread of the virus depends on several factors such as newly purchased animals, entry of infected or carrier animal in the flock, seasonal effects, nutritional deficiency, and rate of movement of wildlife, as well as ruminants within the flock [12] . Besides animal–animal transmission via respiratory aerosols, the virus is disseminated easily via mechanical routes, i.e., fomites, shoes, clothes, vehicles, and veterinary surgical instruments. Furthermore, the uncontrolled transboundary movements of animals have also aggravated its spread [13].

Timely reporting of the outbreak and diagnosis of the persistent and carrier cases led to the effective control of the disease in the sense that its transmission is minimized. These can be achieved when animals are vaccinated with field strains regularly and when rapid, sensitive, and specific diagnostic techniques are available. A liquid-phase blocking sandwich ELISA: was developed for the detection of antibodies against Foot and Mouth Disease virus (FMDV) [14]. The ELISA titers showed a good correlation with those recorded by virus neutralization (VN) tests. The authors suggested that this ELISA could replace the traditional virus neutralization (VN) for assessing antibodies in sera from convalescent and vaccinated animals and for the routine screening of sera from animals intended for export [15].

The present study was conducted to find out the duration of protection provided by the newly developed vaccine against O, A, and Asia-1 serotypes. Moreover, it was also focused on which serotype of the vaccinal strain provides longer immunity.

2. Materials and Methods

2.1 Experimental Animals

A total of twelve (12) healthy calves, approximately one year old, were selected for the experiment. Each calf was individually tagged for identification. All animals were confirmed to be unvaccinated against Foot and Mouth Disease (FMD) before the commencement of the study.

2.2 Experimental design

The animals were randomly divided into four groups (A, B, C, and D), with three claves in each group. The details of each group were shown in the table 1. Blood samples were collected from all groups on day zero before vaccination or infection.

2.3 Vaccination and infection procedure

On day zero, Group A and B were vaccinated with 3 mL of FMDRL Trivalent Foot and Mouth Disease Vaccine (oil-based) via the intramuscular route. Group D after vaccination with priming dose was experimentally infected subcutaneously with live Foot and Mouth Disease virus (Serotypes O, A, and Asia-1) isolated from the field ($TCID_{50}$ = 1 × 10^8). Group C served as control group consisting of negative control C2, receiving no vaccine or infection and C1 as Positive control exposed only to live virus. On the 28th day post-priming, Group A received a booster dose of the same vaccine, whereas Group B received only the priming dose.

Table 1. Show grouping of animals with vaccine detail.

Group	Subgroup	No. of Animals	Treatment Description	Control Type
A	—	3	Priming with booster dose	—
B	—	3	Single priming dose	—
C	**C1**	2	Infected with live virus	Positive Control
	C2	1	Unvaccinated and unexposed	Negative Control
D	**------**	3	Vaccinated but exposed to live virus	Challenge group

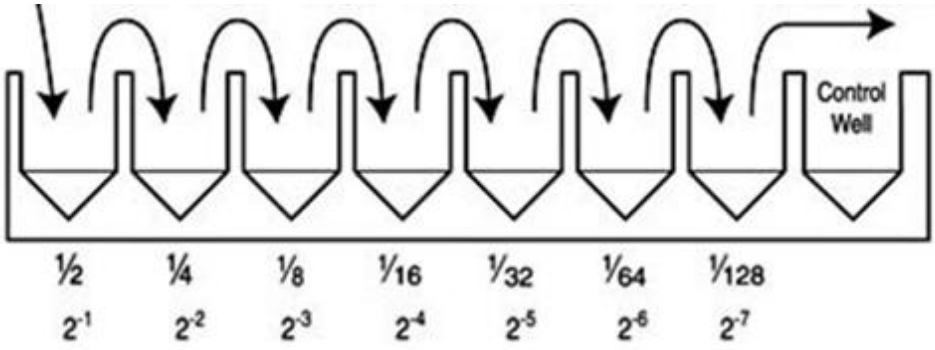

Figure 1. 100µL volume of 1/16 test serum dilution shown in plate layout.

2.4 Clinical observation

Following infection, animals in Group D were monitored daily for 10 consecutive days for any pathognomonic signs of FMD (including fever, salivation, vesicular lesions on the mouth and feet, and lameness). Once clinical signs developed, samples were collected to confirm the presence of FMD virus.

2.5 Sample collection and serological analysis

Blood samples were collected from all groups: On day 0 (pre-vaccination/infection), On day 28 post-priming, And subsequently every 30 days for a period of 13 months. The antibody titers against FMD virus serotypes (O, A, and Asia-1) were determined using the Bile Salt-Dependent Lipase (BDSL) ELISA Kit following the manufacturer's standard protocol.

2.6 Titration assay

Two-fold dilution: First 1/16 dilution of the control sera and a 1/8 dilution of the test sera were prepared in a Microplate. For the control serum dilution, 15µL of undiluted control bovine serum was added to 225µL diluent buffer A, while for the test serum dilution, 20µL of the serum was added to 140µL of diluent buffer A. These volumes were sufficient for a single Microplate. For each run and serotype, the Positive control (C++) (ELISA Kit provided control) was additionally titrated in the same manner as the test serum.

A volume of 50ul of diluent buffer A was added to all columns (3-12) for each polypropylene U-bottom micro plate. According to the plate layout, 50µL volume of the pre-diluted control sera was added to the appropriate wells. A volume of 50µL diluent buffer A was added to the antigen control (Ca) wells. Then, according to the plate layout out a volume of 50µL pre-diluted test serum and the C++ control was added to the appropriate wells of rows 3-12. The contents in the well were mixed carefully by filling and emptying the pipette tip several times, taking care not to introduce air bubbles. This results in a 100µL volume of 1/16 test serum dilution shown in Plate Layout Figure 1. A volume of 50µL was transferred from row A to B, B to C, and onward up to row H, shown in Table 1, by using new pipette tips [16].

2.7 Data analysis

The antibody titers obtained from ELISA were plotted with the help of Matplotlibto compare the immune responses among the vaccinated, infected, and control groups throughout the study.

3. Results

The blood samples were collected from the selected animals at the Livestock Research and Development Station, Surezai, Peshawar, for the period of one year. The animals kept isolated at semen production unit (SPU) Surezai were visited regularly and observed keenly for the development of clinical signs and any other abnormality on a predesigned proforma. The Parameters observed/recorded were temperature, anorexia, macules, papules, vesicles, lameness, salivation, weight loss, and diarrhea. The animals with the priming dose were safe after challenge.

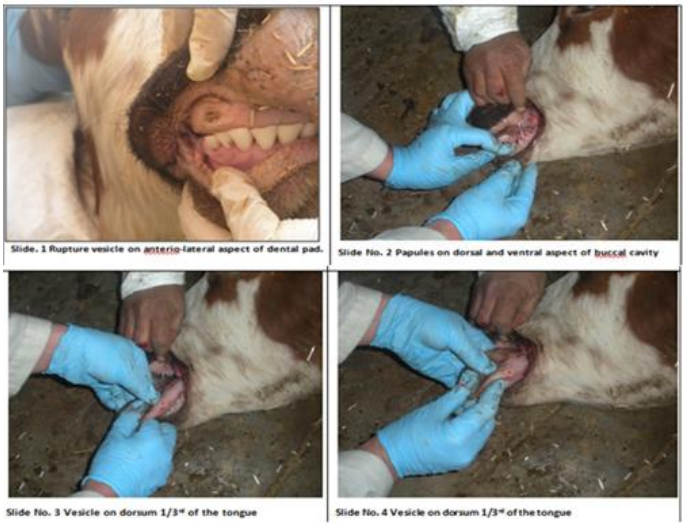

Figure 2. The clinical manifestation after experimental infection of the calf.

One animal of group C1 was anorexic, depressed, and had an average temperature of 105.5°F with salivation. The

animals were showing inflammation in the buccal cavity with vesicles on the exterior aspect of the dental pad of the right side, one vesicle on the interior surface of the lower lip, the posterior dorsum 1/3 of the tongue, and small papules on the dorsal lips on the anteroposterior aspects. Whereas no lameness was observed in these animals and no lesions were detected on the hooves Figure 2. Samples (i-e, swab and vesicular fluid) were taken from these clinically infected animals in viral transport media and shifted to FMDRL, Veterinary Research Institute (VRI), and Peshawar for performing ELISA.

3.1 Month-wise detail report for each serotype in all groups

3.1.1 Serotype O antibody titer priming

The antibody titer response against Serotype O following priming dose was recorded over 12 months period in a group "B" animal (Figure 3). Initial titers in December and January were relatively low, averaging around 40, representing that the protective titer is yet not developed. A sharp increase was observed from February to May, where titers peaked at approximately 250–260, indicating a strong primary immune response. From June onwards, a gradual decline was recorded, with titers decreasing to around 150 units by June–August, and further dropping to 60 in September and 30 by October–November. This downward trend reflects the natural waning of antibody levels following the initial priming phase.

3.1.2 Serotype O antibody titer in group a with boosting dose

The antibody titer response against Serotype "O" was monitored over a one-year period in group "A" as shown in (Figure 4). The antibody titer was approximately 130 in December and January, which represents the baseline level. A significant increase was observed from February to May, where titers reached 250–260, indicating a strong booster response. From June onward, titer values declined steadily, returning to baseline levels (130) and remaining constant until November. This pattern reflects a typical post-boosting rise and subsequent waning of antibody levels over time.

3.1.3 Serotype O antibody titer after infection

The antibody titer response against Serotype "O" was evaluated over a one-year period in infected animals of group D, as shown in (Figure 5). A rapid and strong immune response was observed soon after infection. Initial titers in December were relatively low 40, followed by a sharp increase in January, reaching 250–260. Elevated titers were maintained from January through July, indicating a prolonged and stable immune response induced by natural infection.

A gradual decline in antibody levels began in August, decreasing to 130, and further decreased to 60 from September to November. This reduction suggests the expected waning of circulating antibodies several months after infection, although measurable titers persisted up to a year.

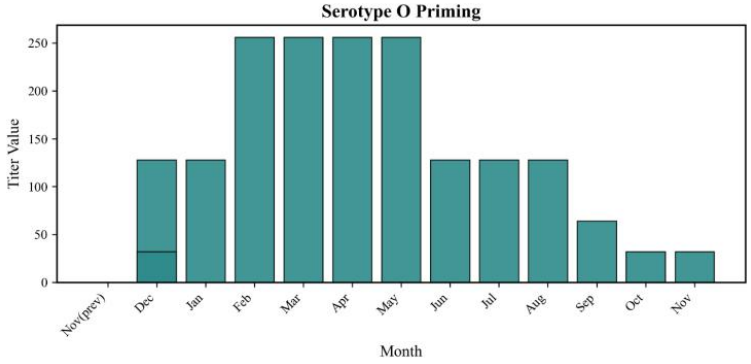

Figure 3. Antibody titer developed against serotype "O" in Animals of group "B".

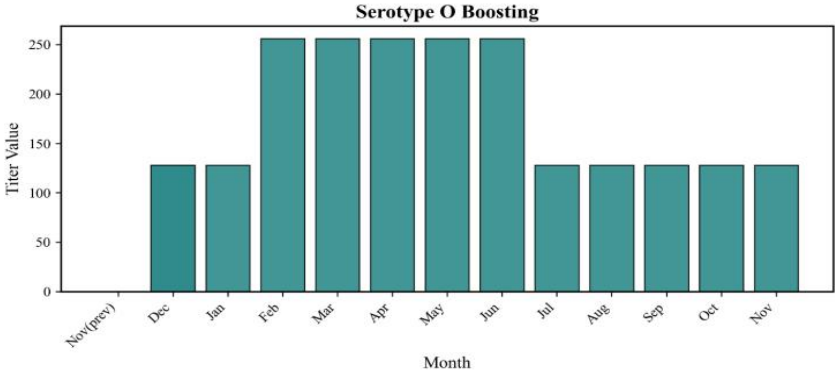

Figure 4. Antibody titer developed against serotype "O" in Animals of group A after booster dose.

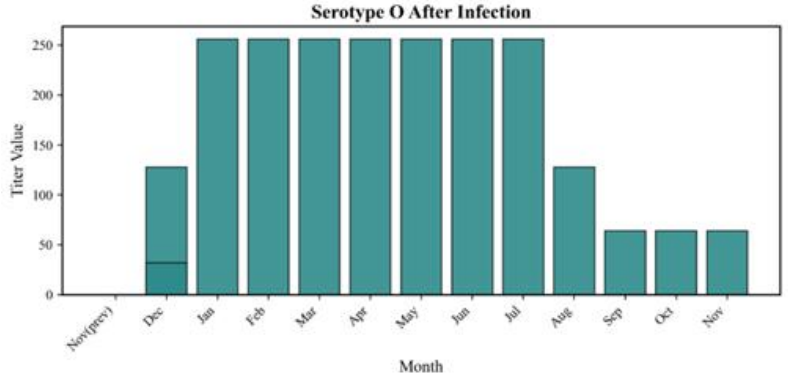

Figure 5. Antibody titer developed against serotype "O" infection in Animals of group D.

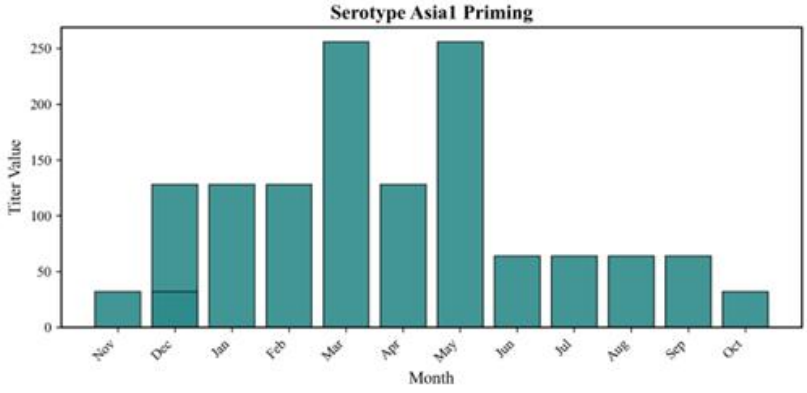

Figure 6. Antibody titer developed against serotype "Asia-1" in Animals of group B.

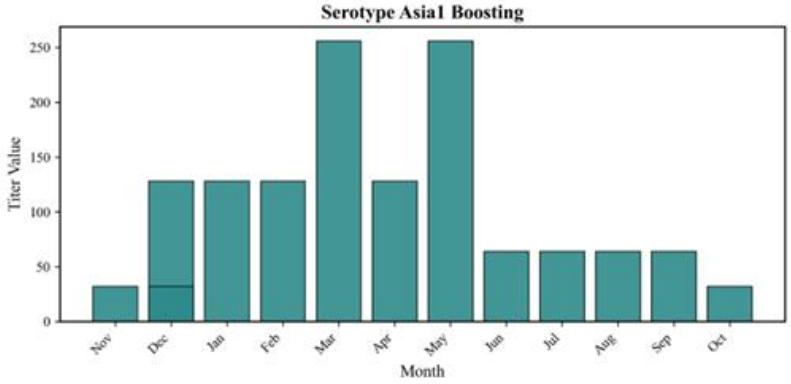

Figure 7. Antibody titer developed against serotype "Asia-1" in Animals of group A.

3.1.4 Serotype "Asia-1 titer" in Animals of group B and group A after Priming dose

The antibody titer response against Serotype Asia-1 was evaluated following primary immunization over a ten-month period as shown in (Figure 6). Initial titers in November and December were low, averaging around 30, representing the pre-immune baseline. A steady increase occurred between January and February, with titers reaching approximately 130. A significant peak was recorded in March and May, where titer values reached 250–260, indicating a strong primary immune response. Following this peak, a marked decline was observed, with antibody levels dropping to around 70 from June to September, and further reduced to 40 in October, demonstrating the gradual waning of immunity over time. booster dose, and group D after the infection.

3.1.5 Serotype Asia-1 antibody titer boosting

The antibody titer response against Serotype Asia-1 following booster immunization was monitored over a ten-month period in group A, as showing in (Figure 7). During November and December, titer values were relatively low, averaging around 30, indicating residual post-primary immune activity. From

January to February, titers stabilized at approximately 130, reflecting sustained immune memory.

A pronounced booster effect was observed in March and May, with titers peaking at 250–260, signifying a strong secondary immune response upon antigen re-exposure. Subsequently, antibody levels declined gradually from June through September, maintaining moderate levels around 70, before dropping to 40 in October. This pattern demonstrates a typical booster-induced amplification of humoral immunity, followed by a progressive decline in antibody persistence over time.

3.1.6 Serotype Asia-1 antibody titer after infection

The antibody titer response against Serotype Asia-1 following infection was monitored over a ten-month period in group D, as shown in (Figure 8). During November and December, antibody levels were relatively low, averaging around 30, indicating the early post-infection phase. From January to February, titers increased steadily to approximately 130, reflecting the development of an adaptive immune response.

A significant peak in antibody titers was observed in March and May, reaching 250–260, representing a strong serological response to active infection. Subsequently, a noticeable decline occurred, with titers decreasing to 70 between June and September, and further reducing to 40 in October. This pattern demonstrates an initial robust immune activation following infection, followed by a gradual decline in circulating antibodies over time, suggesting the waning of humoral immunity in the absence of continued antigenic stimulation.

3.1.7 Serotype "A" antibody titer priming

The antibody titer response against Serotype A was evaluated following primary immunization over a ten-month period in group B (Figure 9). During November and December, antibody titers remained low, averaging around 30, representing the pre-immune baseline. A gradual rise was observed in January and February, with titers increasing to approximately 130, reflecting the initiation of the primary immune response.

A pronounced peak was recorded in March and April, where titers reached 250–260, indicating a strong activation of humoral immunity. Thereafter, antibody levels declined steadily, decreasing to 130 in May and June, and further dropping to 70 between July and September. By October, titers had reduced to 40, demonstrating a progressive decline in antibody persistence typical of a primary immune response in the absence of booster stimulation.

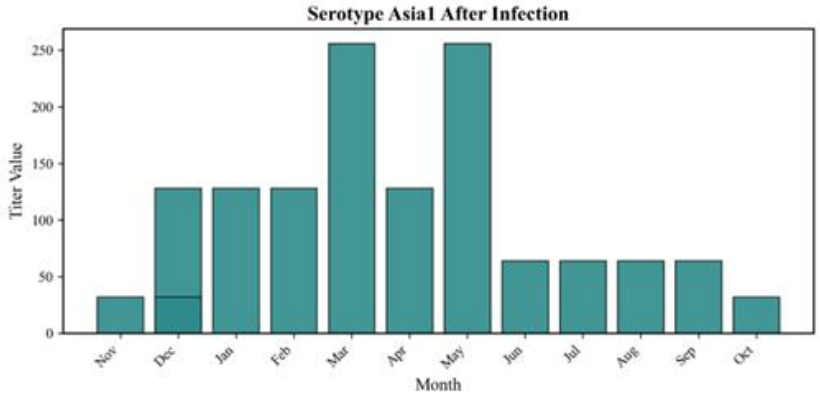

Figure 8. Antibody titer developed against serotype "Asia-1" infection in Animals of group D.

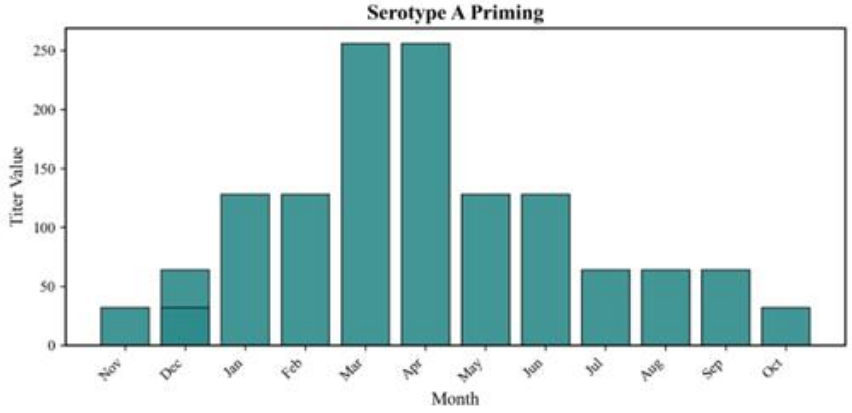

Figure 9. Antibody titer developed against serotype "A" in Animals of group B.

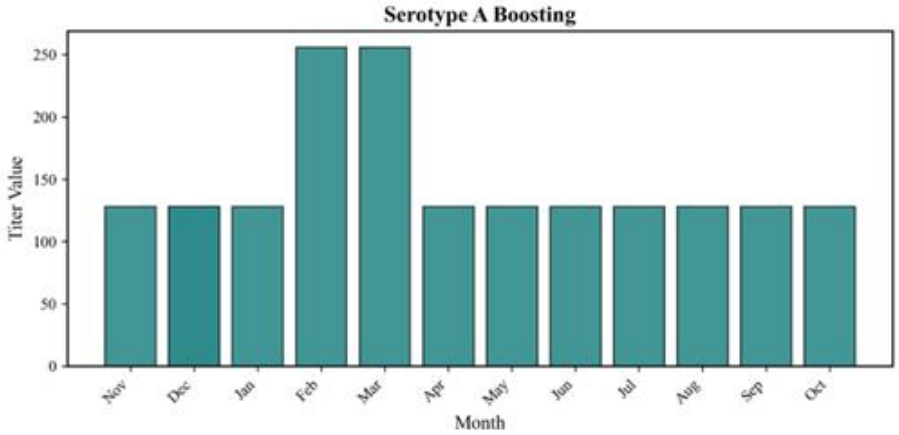

Figure 10. Antibody titer developed against serotype "A" in Animals of group A.

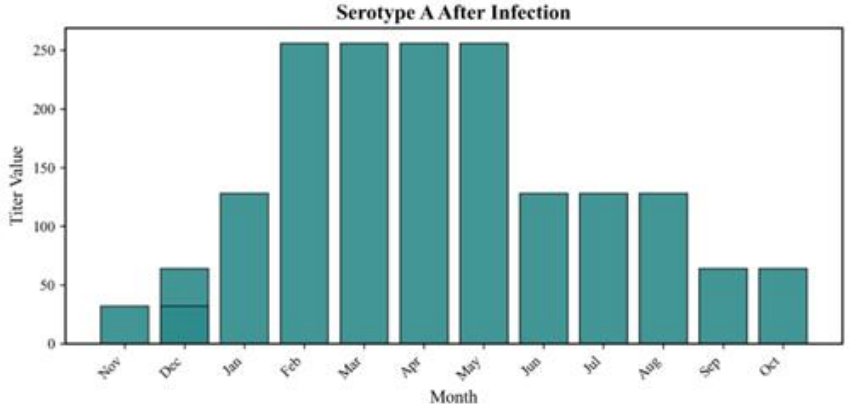

Figure 11. Antibody titer developed against serotype "A" infection in Animals of group D.

3.1.8 Serotype "A" antibody titer after the boosting

The antibody titer response against Serotype A was monitored for ten months after booster immunization in group A shown in (Figure 10). During November to February, titers remained steady around 120–130, representing the baseline level before secondary stimulation. A sharp rise was observed in March and April, reaching 250–260, indicating a strong secondary humoral response. Thereafter, antibody levels declined to baseline values (120–130) and remained constant through May to October, suggesting prolonged antibody persistence and effective immune memory following booster administration.

3.1.9 Serotype A after infection

The antibody titer response against Serotype A was assessed over a ten-month period following infection in group D, as shown in (Figure 11). During November and December, antibody levels were low (30–60), representing the initial exposure phase. A marked rise occurred in January and February, reaching 130, followed by a pronounced peak from March to May, where titers attained 250–260, indicating a strong immune activation due to infection. Subsequently, antibody levels declined gradually, dropping to 130 in June–August and further reducing to 60 by September and October, reflecting the natural waning of antibodies after infection.

4. Discussion

Many countries vaccinate against FMD, and the first consideration when selecting a vaccine is whether it protects against the strains of virus circulating in the country or in neighboring countries. Serological data are valuable when assessing response to vaccination and for epidemiological surveillance of vaccinated and non-vaccinated animals, particularly when vaccine failures suspected.

Vaccination of the calves with the locally produced trivalent FMDRL Vaccine adjuvanted with Montanide oil induced antibody titers higher than the recommended protective level (90) for three serotypes, including O, A, and Asia-1, as determined by ELISA. The duration of protection provided by the O, A", and Asia-1 serotypes at the priming dose was 10, 6, and 6 months, respectively whereas protection titer provided the following the booster dose was increased to 13, 13, and 12 months, respectively. These findings were consistent with previous reports demonstrating that Montanide ISA 206 achieved early protective antibody titers and longer lasting immunity [17, 18]. More recent studies show that Montanide-adjuvanted FMD vaccines produce stronger and persistent antibody responses than aqueous vaccine formulations, particularly after a booster vaccination,

due to enhanced antigen retention and immune stimulation. The shorter duration of primary protection observed for in serotypes A and Asia-1 aligns with previous evidence indicating that these serotypes often require higher antigen levels or updated seed strains to match the immunity achieved by serotype O. Overall, the present findings confirmed that the FMDRL trivalent Montanide-based vaccine induced effective and durable immunity, especially after booster vaccination , and emphasize the need for optimizing antigen content to improve serotype-specific protection [19, 20]. These finding also supported by Wasfy et al. [21]. Antibody titers reached protective levels between 14–18 weeks following booster vaccination [22]. neutralizing antibody peaks correlate with viral clearance, explaining why clinical symptoms often decline by day 5 post-infection, as also reported by Cui et al. [23].

Studying the immune status in experimentally infected calves, as evaluated by ELISA showed that peak circulating FMD virus type A and O antibodies were recorded at the 16th weeks post-infection. Similar findings were obtained by El-Sayed et al. [24], and Sala et al. [25], recording peaks of FMD antibodies in infected cattle between 15-16 weeks post-infection. Also, Eschbaumer et al. [26] stated that the clinical signs decline with the appearance of circulating FMD-specific antibody at around 4 to 5 days post-infection. Antibody titers remained within the protective level up to 36 weeks for type O and 32 weeks for type A reported by El-Sayed et al. [27]. The present findings reaffirm that the FMDRL trivalent Montanide ISA 206–based vaccine provides early-onset and durable immunity against serotypes O, A, and Asia-1, particularly after booster administration. This outcome supports the integration of oil-adjuvanted vaccines in national control programs and highlights the importance of antigenic matching and routine potency testing to ensure optimal serotype-specific protection [28].

5. Conclusion

Based on the findings of this study, it was concluded that the FMDRL vaccine exhibited strong immunogenicity and produced protective antibody titers against all tested foot and mouth disease virus (FMDV) serotypes for at least six months following a single vaccination dose. The vaccine effectively elicited a broad and sustained immune response in vaccinated animals. However, further studies were recommended to evaluate the duration of immunity beyond six months and to assess the vaccine's protective efficacy under field conditions and against newly emerging FMDV strains. These investigations would provide a more comprehensive understanding of the level of protection conferred by the FMDRL vaccine and support the development of improved control strategies against FMD.

Author contributions

Ahmad Sohail: Methodology, Writing–original draft; Natasha Kashif, Muhammad Tauqeer and Mansoor Ahmad: Laboratory work, data analysis and write up; Sher Bahadar Khan: Writing–review & editing; Muhammad Bilal, Mirza Ali Kahn & Fawad Khan: Field trial and animal Management; Abdul Sajid: Project administration, Conceptualization, Supervision;

Ethical approval

The study was approved by the Institutional Ethics Committee of the College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University, Mardan (Approval No. 2574; Date: 12-08-2023) and conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC).

Conflicts of Interest

The authors report no conflicts of interest.

Acknowledgment

This research work represents a part of my M.Phil. research project. I am deeply grateful to the College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University, Mardan, and the Veterinary Research Institute, Peshawar, for providing me with the invaluable opportunity, facilities, and guidance to complete my research. Their continuous support and encouragement played a vital role in the accomplishment of this work.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

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How to cite this article: Sohail, A., Sajid, A., Kashif, N., Khan, S. B., Tauqeer, M., Ahmad, M., Khan, F., Bilal, M., Khan, M. A. Evaluation of the Serological Response after Foot-and-Mouth Disease Vaccination in Pakistan. Journal of Zoology and Systematics, 4(1), 1–9.