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Research Article

A STUDY ON HOMOLOGY AND HETEROLOGY BETWEEN PANASIA-II & PAK-98 LINEAGES OF FOOT AND MOUTH DISEASE VIRUS SEROTYPE-O

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Abstract

To understand the dynamics of epidemics, it is crucial to evaluate prior exposure to infectious diseases as a possible defense against newly developing strains. The cross-protection prediction is unresolved matter that holds great importance, particularly in the field of Foot-and-Mouth Disease Virus (FMDV). The new methods for controlling FMD involve the implementation of vaccine, limited movement, containment and elimination of cattle that are either affected or vulnerable to the disease. Therefore, it is necessary to use a serological analysis strategy that applies association coefficients (r1-values) to identify effective vaccines. The primary objective of this study was aimed to explain the genetic homologous and heterologous association among two ancestries of FMDV serotype O found in Pakistan (Pk) and to find a suitable strain from field for selection of vaccinated seed that provide cross-protection against both ancestries. Virus neutralization tests (VNTs) were used to evaluate an antigenic association among the two groups of viruses, indicates a significant degree of antigenic similarity between stains within a particular cluster. Conversely, r1 values ranging from 0.07-0.2 were obtained from cross-combinations among clusters, suggesting antigenic variation amongst virus strains. These results were further supported by in vitro tests, which showed that heterologous combinations of PanAsia-II and PAK-98 isolates showed antigenic variation, except for 25-06-PK-19, SDG-43-FSD-PK, and TANWL-34PK, which showed a 0.3 (r1 value). In contrast, when considering homologous combination of strains from both lineages showed antigenic similarity. Furthermore, it was projected that these combinations would provide protective titers during in-vivo. As a conclusion, it is recommended that all strains, except 25-06-PK-19, SDG-43-FSD-PK, and TANWL-34PK considered as potential vaccine candidates for effectively managing outbreaks of FMDV serotype-O, due to their ability to confer broad-spectrum immunity.

Keywords: Foot-and-Mouth disease (FMD), pandemic, vaccine, epidemic, Serotype-O, Pakistan.
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1. INTRODUCTION

The remarkable genetic variety of RNA viruses, which are representative of diseases such as influenza virus and Foot and Mouth Disease Virus (FMDV), has led to vast research on these viruses. This intrinsic characteristic exhibit noteworthy consequences, particularly when it comes to developing attenuated or inactivated vaccinations that are efficient on closely related field strains (Mattion et al., 2004). This was shown throughout the Pandemic of influenza A (H1N1) in 2009 (Garten et al., 2009). During 2019 to 2022, 532 FMD outbreaks were confirmed, 489 of which were serotype O. This represents 92% of

FMD cases globally. Outbreaks increased 6-fold in 2021 as compared to 2019 and 2020. Multiple states and Union Territories reported outbreaks in 2021. Serotype O caused 92% of FMD outbreaks in 2021, suggesting an increase in outbreaks. Additionally, serotype O caused 98%, 83%, and 93% of FMD outbreaks in 2019, 2020, and 2022, respectively (Dahiya et al. 2023). Selecting new vaccine seed strains is necessary due to antigenic virus resistance to conventional vaccinations. A vaccine strain with cell adapted FMDV and few amino acid and antigenic changes may be possible. When vaccines are unavailable, field isolates are improved to make them. This phase alone begins in-vivo and in-vitro vaccine efficacy testing (Smith et al., 2004, Paton et al., 2005, OIE, 2008). This voyage requires a lot of resources and time, limiting the number of isolates that can be realistically evaluated and increasing the risk of selecting poor vaccine strains. A computational model that accurately predicts strains with high cross-protection potential could speed up vaccine development, reduce expenses, and reduce ethical considerations related to animal testing (Reeve et al., 2010).

Foot-and-mouth disease (FMD) is a highly infectious disease that primarily affects cloven-hoofed animals, including cows, pigs, sheep, goats, and deer. The virus causes an increase in body temperature, difficulty in walking, and the formation of small fluid-filled sacs on various parts of the body such as the mouth, tongue, snout, teats, and feet (Alexandersen et al. 2003; Moraes et al. 2007). The global agricultural livestock business suffers huge financial losses by this disease (Donaldson et al., 2002). African, Asian, and South American regions are endemic for the disease. FMD outbreaks often require restricted animal movement and trade restrictions. The FMDV is classified under the genus Aphthovirus in the Picornaviridae family. It consists of a genome made up of single-stranded, positive-sense RNA molecule of 8200 nucleotides. These agents have icosahedral, non-enveloped single-stranded RNA virions. Viral particles are 30 nanometers diameter (Quinn et al., 2011). The virus consists of seven serotypes: A, O, C, Asia1, and South African Territories 1, 2, and 3 (SAT 12 & 3) (Hwang et al. 2021). An optimal FMD vaccine or vaccination strain should possess antigenic similarity to the pathogenic viruses and induce significant immune responses. The FMDV PanAsia-2 vaccine strains of serotype O provides full immunity against a wide range of diseases (Hwang et al., 2021). However, vaccination strains of serotype A FMDV do not exhibit extensive cross-reactivity in serological matching tests with field isolates because to the antigenic diversity of serotype A FMDV, unlike serotype O (Mahapatra et al., 2016).

The genetic material of the virus contains a sequence of nucleotides that is responsible for producing a long chain of amino acids called a polyprotein. This polyprotein is then broken down into smaller functional proteins by enzymes that are produced by the virus itself. This procedure includes 4 structural and 8 non-structural proteins (Borca et al., 2012). Many studies show that structural protein VP1 is crucial to this process and associated to FMDV antigenic variability. FMDV has a particular vulnerability to acidic environments and demonstrates an accelerated replication rate at the sites of infection. From a serological perspective, FMDV manifests as an intricate assemblage of seven distinct serotypes. Infection caused by a particular serotype confers immunity against subsequent infection by the same serotype yet does not confer protection against other serotypes. The fact of notice is that this serological model lacks conclusive validation as a result of the limited number of intertypic cross-protection studies conducted in animals. In the realm of virology, it is customary to employ serological classification techniques, wherein sera that are specific to well-defined type strains are utilized. These assays include VNT's and a range of

antisera-based tests that rely on the antigenic properties of FMDV (Crowther, 1986).

In vitro, VN titers evaluate how conserved viral neutralization sites are to allow cross-reactivity (Rweyemamu et al., 1978). Although the presence of VN alone does not serve as the sole determinant of protection, it is crucial to acknowledge that VNTs continue to hold significant value as an essential tool for evaluating cross-reactivity. This is primarily due to their ability to provide absolute serological information (Parida et al., 2006). The quantification of antigenic associations in VNTs is achieved using of "r1-values," which serve as a measure denoting the ratio between heterologous and homologous titers. A ratio of r1 that closely approximates 1 suggests the presence of antigenic resemblance among viruses. Typically, r1 values ranging from 0.4 to 1.0 are indicative of significant for cross-protection. Conversely, values below 0.2 for a particular isolate suggest the need for the development of novel vaccine strains. A limit of 0.3 is frequently advocated in the field (Barnett et al., 2001).

2. Materials and Methods

2.1. Propagation of viral strains

Roller bottles and roux flask were utilized, which contained cell monolayers in the logarithmic growth phase at an 80-90% confluence level. A volume of 10 mm of serum-free media was combined with about 2-3 mm of viral samples obtained from the PanAsia II and PAK-98 clusters were put into specific flasks. Subsequently, the flasks were placed into an incubator for 2 hours to promote the adherence of cells. After this time frame, the media was swapped with new media that did not contain serum. Significantly, these isolates were placed into separate flasks to enable separate propagation of different strains. Following this, the cell culture flasks containing both cells and the virus were placed in incubator for 16-18 hours at 37°C. The examination of cytopathic effects was conducted on the subsequent day and it was anticipated that type-O viruses would induce full cell death accompanied by evident cytopathic effects.

2.2. Titration

The determination of the biological titer, known as Tissue Culture Dose 50 (TCID50), for each strain of the FMDV was conducted using the procedure described by Reed and Muench (1938). The present investigation utilized a total of 7 FMDV serotype-O obtained from the PanAsia-II and PAK-98 clusters (Table 1). The majority of the viral isolates completed 1-2 cycles of multiplication in the Baby Hamster Kidney cell line (BHK-21), with a total of 4 transit for multiplication. Some viruses used to be disseminated with the objective of achieving a virus titer beyond 10 TCID50. The supernatant of tissue-culture was collected after the observation of cytopathic effects above 85%. The viruses were then preserved in 50% glycerol at -20°C for urgent use or -80°C for future use virus stock.

2.3. Design of Study

2.3.1. Isolates

Already molecularly characterized isolates of the FMDV serotype-O were obtained from the microbiology (MB) portion of Quality Operation Laboratories (QOL) UVAS, Lahore. These isolates were then propagated using previously described method.

2.3.2. Inoculation in Animals

Fifteen experimental animals were utilized in order to produce antisera through inoculation of periodic virus. Three separate groups, each consisting of five animals were set up. The PanAsia II isolates were placed in Group 6-8 includes 25-06/pk2019, SDG-43-FSDPK and SDG-17-BAKPK. The PAK-98 cluster was placed in Group 1-5 includes: NTLH-47PK, THNWL-45PK, HLY-44PK, QOL 07 and 10 in that order. The administration of booster doses of viral samples occurred on days 0, 7, 14, and 21, while blood samples were obtained using sterile containers. Additional dosages of viral samples were provided on every seven days intervals and

subsequent specimens of blood were obtained using sterile containers.

2.3.3. Serum Sampling and Serology:

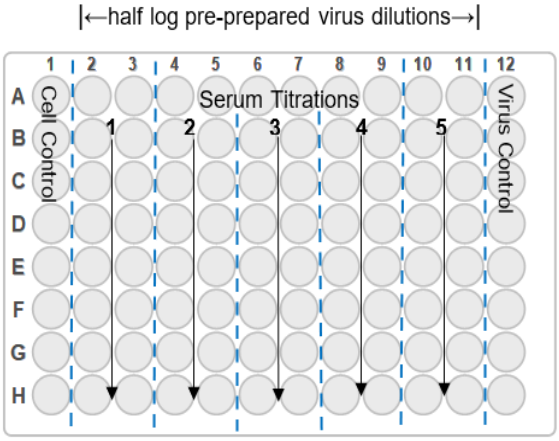
The collection of specimens of serum was conducted by means of venipuncture, utilizing either the jugular or caudal (tail) vein, depending on the species and the availability of appropriate handling equipment. The samples were transferred in ice to the QOL. The serum was isolated and preserved at -20°C prior to experiment. The antibody test included the use of ELISA and VNT, as earlier outlined in the study conducted by (Rweyemamu et al., 1978). The determination of homologous and heterologous titers involved assessing randomly among selected isolates associated to the PAK-98 and PanAsia-II lineages.

2.4. Antibody Detection using ELISA test

ELISA was performed in order to determine the titers of neutralizing antibodies and inhibition value 70% indicates the preservative titer. A coated plate with antigens were labeled with the identification of anti-serum that was produced against isolates of virus found in the field. Samples of serum were diluted to 1/10, 1/30, and 1/90 times using a buffer dilution, were examined in conjunction with positive and negative controls. The measurements were obtained at 450nm wavelength with an ELISA reader.

2.5. Virus Neutralization Test (VNT)

All viral serum isolates were tested for virus neutralization using a VNT. The test used one-dimensional VNT (1D-VNT) (OIE, 2008), and two-dimensional (2D-VNT) (Rweyemamu et al., 1976). The 2D-VNT was modified for serological test of vaccine to employ 5 virus doses instead of 9. The vaccination antiserum was vertically mixed 2-fold in a 96-well plate and 5 virus doses were horizontally added. The VN plate layout is shown in fig. 1 below. This enabled sera titration against all 5 viral dosages. TCID50 quantity for the 5 virus dosages were obtained individually on another plate. A 100 TCID50 virus dosage serum titer was projected using linear regression. The 1D-VNT technique was proposed for the neutralization of anti-FMDV sera, with a pre-determined virus dose of 100 TCID50. A minimum of two replications were conducted for each test, ensuring that all duplicates were required to have a dilution ratio within a 2-fold range of each other. The modified method



exhibits greater efficacy and minimizes the reliance on previous inoculation was implemented.

Fig 1. Vaccination antiserum vertically mixed 2-fold in a 96-well plate

3. Results:

3.1. Virus Isolates, TCID50, ELISA, and VNTs the PanAsia-II and PAK-98 clusters

The present study encompassed the identification of 3 virus isolates from the clade PanAsia-II and 5 virus isolates from the clade PAK-98, all of which have been categorized as Type "O" (Table 1). The procedure involved the isolation of viruses, followed by culturing using the BHK-21 cell line revival method. Subsequently, 7 virus isolates were subjected to molecular analysis. These isolates included 3 PanAsia-II cluster and 5 PAK-98 cluster (Table 1). The TCID50/100µl of the virus dose was determined and a total of 40 animals were tested for virus exposure. Additionally, booster vaccinations dosage was given at 7, 14, and 21 days to induce the formation of sera against every virus isolate. The determination of antibody titers was conducted using

the ELISA method. In order to assess the antigenic linkages between virus isolates from the PanAsia-II and PAK-98 clusters, VNTs were performed with careful consideration of detail.

The serological test ELISA was performed in order to determine the titers of neutralizing antibodies and the preservative titer with an inhibition value of 70%. The measurements were obtained at 450nm wavelength with an ELISA reader indicated

Table 1. PanAsia-II & PAK-98 viruses with accession number (Gene bank).

Serotype	Clade	Strain	Origin country	Accession No. (GenBank)
O	PanAsia-II	SDG-43-FSD-PK	Pk	MN116044
O	PanAsia-II	SDG-17-BAKPK	Pk	MN116045
O	PanAsia-II	25-06-PK-19	Pk	MN103290
O	PAK-98	HLY-44-PK	Pk	MK934707
O	PAK-98	GCLHR-35PK	Pk	MK934704
O	PAK-98	BHKSKH-39PK	Pk	MK934703
O	PAK-98	TANWL-34PK	Pk	MK934702
O	PAK-98	MNLH-81PK	Pk	MK934701

3.1.1. Virus Multiplication and Determination of TCID50

A total of 8 isolates of FMDV serotype-O were utilized obtained from PanAsia-II and PAK-98 clusters. Most of these viruses completed a couple of cycles of replication in the BHK-21. In specific cases, the viruses went through previous replication to

Table 2. TCID50 values of PanAsia-II viruses

Virus ID	25-06-pk-19	SDG-17-BAKPK	SDG-43-FSDPK
TCID50/100ul	$10^{4.3}$	$10^{4.6}$	$10^{4.8}$

Table 3. TCID50 values of PAK-98 viruses.

Virus ID	HLY-44PK	GCLHR-35PK	THNWL-45PK	BHKSKH-39PK	NTLH-47PK
TCID50/100ul	$10^{4.8}$	$10^{4.7}$	$10^{4.3}$	$10^{4.6}$	$10^{4.3}$

Table 4: Data obtained with VNT

ID Serum Sample	1:10 fold	1:30 fold	1:90 fold
BHKSKH-39PK	0.50	0.67	0.81
HLY-44-PK	0.27	0.37	0.62
GCLHR-35PK	0.23	0.44	0.67
TANWL-34PK	0.09	0.35	0.56
MNLH-81PK	0.09	0.48	0.81
Control -ve	1.72	1.73	1.63
Control +ve	0.083	0.21	0.03

get a titer of virus above 10 TCID50. The acquisition of supernatant was conducted upon the detection of a cytopathic effect over 85%. Following that, the viruses were preserved in 50% glycerol at -20°C for urgent use or -80°C for future use of a virus stock. The values of TCID50 for all 8 virus strains are listed in Tables 2 and 3.

3.2. Serological Assays

3.2.1. ELISA

that all 8 isolates of serum showed a titer inhibition 70% at 1/30-fold dilution.

$$\text{Inhibition percentage} = 100 - \frac{\text{serum OD}}{\text{Reference OD}} * 100$$

Reference OD = mean of negative control

Vaccination is an important strategy in the management of diseases. The necessity to determine the virus or antigen content inside a vaccine with accurate IA, such as the gold-standard VNT, arises from the significant (>0.05 p value) change seen in VP1 protein of FMDV. This methodology facilitates the assessment of recently identified variants and provides that the vaccine utilized in the production of FMD are compatible with these newly emerged strains. Consequently, this strategy provides protection for infected animals by offering them protection against the disease. The findings indicate that there were antigenic differences observed in different isolates of PanAsia-II with PAK-98, except of 25-06-PK-19, SDG-43-FSD-PK and TANWL-34K, which showed 0.3 (r1-value), when coupled with SDG-17-BAK-PK. These mixtures are improbable for providing resistance when faced with

challenges. On the other hand, it is worth noting that all strains derived from both clusters exhibited a comparable antigenic profile when tested in same isolates, thus showing potential for producing protective antibody levels during in vivo tests. Overall, it can be concluded that the majority of strains exhibit broad-spectrum protection against FMDV type O, except for 25-06-PK-19, SDG-43-FSD-PK and SDG-17-BAK-PK and these 3 strains specifically confer protective titers against all PanAsia-II lineage strains. Consequently, the identified strains show potential as vaccine candidates for the effective control of outbreaks associated with FMDV type O.

SDG-17-BAK-PK			
PAK-98 lineage	Homologous titre	Heterologous titre	r1-value
25-06-PK-19	2.70	2.34	0.44
GCLHR-35PK	2.92	2.18	0.18
BHKSKH-39PK	2.63	1.72	0.12
HLY-44-PK	2.72	2.07	0.22
MNLH-81PK	2.81	2.12	0.20
SDG-43-FSD-PK	2.54	2.02	0.30
TANWL-34PK	2.41	1.91	0.32
25-06-PK-19			
GCLHR-35PK	2.92	2.18	0.18
BHKSKH-39PK	2.64	2.08	0.27
HLY-44-PK	2.71	1.92	0.16
MNLH-81PK	2.81	2.04	0.17
SDG-17-BAK-PK	2.75	2.33	0.38
SDG-43-FSD-PK	2.44	2.10	0.45
TANWL-34PK	2.66	1.94	0.14
SDG-43-FSD-PK			
25-06-PK-19	2.38	2.01	0.43
GCLHR-35PK	2.41	1.84	0.27
BHKSKH-39PK	2.70	2.01	0.20
HLY-44-PK	2.51	1.94	0.27
MNLH-81PK	2.78	1.96	0.15
SDG-17-BAK-PK	2.63	2.10	0.30
TANWL-34PK	2.63	2.21	0.38

4. Discussion

Vaccination continues to play a crucial role in combating the FMDV in several developing nations such as Pakistan. Immunological assays (IA) of the utmost quality, such as the VNT assume a crucial role in evaluating the emergence of novel strains. Ensuring the efficient protection of susceptible animals necessitates the alignment of FMD vaccine production with the genetic composition of newly emerging strains. Previous studies have emphasized the significance of this alignment, specifically in relation to Pakistani FMD serotype-O isolates documented from 2002 to 2006. These isolates exhibited genetic similarity with Pak-98, Iran-2001, PanAsia, or PanAsia-II 2006 lineages. The primary approach for vaccine matching procedures mostly depends on in vitro techniques, such as VNT, ELISA, and polyclonal antibodies complement fixation (Paton et al., 2005).

This study aimed to investigate the cross-reactivity of strains belonging to two prominent clades, namely PanAsia-II and PAK-98 which have been associated with major outbreaks in multiple cities in Pakistan between 2012 and 2019. The efficacy of imported FMD vaccinations has been hindered due to variations in genetic makeup, particularly in the coding region of VP-1 sequences, which differ from the strains responsible for ongoing outbreaks in Pakistan. Therefore, this work rigorously used established procedures to determine both homologous and heterologous titers using association coefficients (r1 values),

which are essential for the identification of appropriate seed strains of vaccine (Reeve et al., 2010, Ahmed et al., 2018; Jamal et al. 2021). Several studies have been undertaken to track the occurrence of different FMDV serotypes-O lineages in Pakistan. The FMD epidemics in the KPK region of Pakistan have been attributed to viral strains such as O/IRN/2001, O/IRN/8/2005, O/PAK/2005, and O/PAK/2008, which are classified under the PAK 98 lineage. Moreover, it was shown that sub-lineages within the O type PanAsia-II lineage had discernible differences largely in the sequences of the coding region of VP-1 between 2012 and 2015. The PanAsia-II lineage was first detected in Nepal in 2003, and later identified in Bhutan in 2003-2004. Following this, a similar occurrence was observed in Afghanistan in 2004 and later in Pakistan during the years 2006-2007 (Schumann et al., 2008; Klein et al., 2008). The genetic variability of RNA viruses, such as the FMDV and influenza virus, has been comprehensively described. One of the immediate outcomes resulting from genetic variety is that attenuated or inactivated vaccines, which are developed from these adopting viruses, mostly provide immunity against other closely related strains. This tendency was prominently observed in the influenza type-A pandemic in 2009. In regard to this particular attribute exhibited by RNA viruses, it is of utmost importance to evaluate the potential for cross-reactivity and, consequently, the probable cross-protection among sera derived from vaccine and field virus strains. In situations where current vaccines are unable to provide sufficient immunity against newly emerged viruses with different antigenic properties, the identification and adoption of new vaccine seed strains may become necessary. In order to establish the antigenic associations between isolates, this study applied linear combined effect models. These models took into consideration the variations in pair-wise cross-neutralization titers, which were entirely based on viral structural and sequences data (Reeve et al., 2010).

5. Conclusion

Virus neutralization tests (VNTs) were used to evaluate an antigenic association among the two groups of viruses, indicates a significant degree of antigenic similarity between stains within a particular cluster. As a conclusion, it is recommended that all strains, except 25-06-PK-19, SDG-43-FSD-PK, and TANWL-34PK considered as potential vaccine candidates for effectively managing outbreaks of FMDV serotype-O, due to their ability to confer broad-spectrum immunity.

6. REFERENCES

Ahmed, Z., Pauszek, S.J., Ludi, A., LaRocco, M., Khan, E.U.H., Afzal, M., Arshed, M.J., Farooq, U., Arzt, J., Bertram, M. and Brito, B., 2018. Genetic diversity and comparison of diagnostic tests for characterization of foot-and-mouth disease virus strains from Pakistan 2008–2012. Transboundary and emerging diseases, 65(2), pp.534-546.

Alexandersen, S., Zhang, Z., Donaldson, A. I., & Garland, A. J. M. (2003). The pathogenesis and diagnosis of foot-and-mouth disease. Journal of comparative pathology, 129(1), 1-36.

Barnett, P.V., Samuel, A.R. and Statham, R.J., 2001. The suitability of the 'emergency'foot-and-mouth disease antigens held by the International Vaccine Bank within a global context. Vaccine, 19(15-16), pp.2107-2117.

Borca, M.V., Pacheco, J.M., Holinka, L.G., Carrillo, C., Hartwig, E., Garriga, D., Kramer, E., Rodriguez, L. and Piccone, M.E., 2012. Role of arginine-56 within the structural protein VP3 of foot-and-mouth disease virus (FMDV) O1 Campos in virus virulence. Virology, 422(1), pp.37-45.

Crowther, J. R., 1986. Antigenic structure of foot and mouth disease virus.

Revue scientifique et technique (International Office of Epizootics), 5(2), pp.299-314.

Dahiya, S. S., Subramaniam, S., Mohapatra, J. K., Rout, M., Biswal, J. K., Giri, P., ... & Singh, R. P. (2023). Foot-and-mouth disease virus serotype O exhibits phenomenal genetic lineage diversity in India during 2018–2022. Viruses, 15(7), 1529.

Donaldson, A., Lowe, P. and Ward, N., 2002. Virus-crisis-institutional Change: the Foot and Mouth Actor Network and the Governance of Rural Affairs in the UK. Sociologia Ruralis, 42(3), pp.201-214.

Garten, R.J., Davis, C.T., Russell, C.A., Shu, B., Lindstrom, S., Balish, A., Sessions, W.M., Xu, X., Skepner, E., Deyde, V. and Okomo-Adhiambo, M., 2009. Antigenic and genetic characteristics of swine-origin 2009 A (H1N1) influenza viruses circulating in humans. science, 325(5937), pp.197-201.

Hemadri, D., et al. (2002). "Emergence of a new strain of type O foot-and-mouth disease virus: its phylogenetic and evolutionary relationship with the PanAsia pandemic strain." Virus Genes 25(1): 23-34.

Hwang, J. H., Lee, G., Kim, A., Park, J. H., Lee, M. J., Kim, B., & Kim, S. M. (2021). A vaccine strain of the A/Asia/Sea-97 lineage of foot-and-mouth disease virus with a single amino acid substitution in the p1 region that is adapted to suspension culture provides high immunogenicity. Vaccines, 9(4), 308.

Jamal, S. M., Khan, S., Knowles, N. J., Wadsworth, J., Hicks, H. M., Mioulet, V., ... & Belsham, G. J. (2021). Foot-and-mouth disease viruses of the O/ME-SA/Ind-2001e sublineage in Pakistan. Transboundary and Emerging Diseases, 68(6), 3126-3135.

Klein, J., Hussain, M., Ahmad, M., Afzal, M. and Alexandersen, S., 2008. Epidemiology of foot-and-mouth disease in Landhi Dairy Colony, Pakistan, the world largest Buffalo colony. Virology Journal, 5(1), pp.1-16.

Mahapatra, M., Statham, B., Li, Y., Hammond, J., Paton, D., & Parida, S. (2016). Emergence of antigenic variants within serotype A FMDV in the Middle East with antigenically critical amino acid substitutions. Vaccine, 34(27), 3199-3206.

Mattion, N., König, G., Seki, C., Smitsaart, E., Maradei, E., Robiolo, B., Duffy, S., León, E., Piccone, M., Sadir, A. and Bottini, R., 2004. Reintroduction of foot-and-mouth disease in Argentina: characterisation of the isolates and development of tools for the control and eradication of the disease. Vaccine, 22(31-32), pp.4149-4162.

Moraes, M. P., de Los Santos, T., Koster, M., Turecek, T., Wang, H., Andreyev, V. G., & Grubman, M. J. (2007). Enhanced antiviral activity against foot-and-mouth disease virus by a combination of type I and II porcine interferons. Journal of virology, 81(13), 7124-7135.

Nagendrakumar, S.B., Madhanmohan, M., Rangarajan, P.N. and Srinivasan, V.A., 2009. Genetic analysis of foot-and-mouth disease virus serotype A of Indian origin and detection of positive selection and recombination in leader protease-and capsid-coding regions. Journal of biosciences, 34, pp.85-101.

Oie, A., 2008. Manual of diagnostic tests and vaccines for terrestrial animals. Office international des epizooties, Paris, France, pp.1092-1106.

Parida, S., Oh, Y., Reid, S.M., Cox, S.J., Statham, R.J., Mahapatra, M.,

Anderson, J., Barnett, P.V., Charleston, B. and Paton, D.J., 2006. Interferon-γ production in vitro from whole blood of foot-and-mouth disease virus (FMDV) vaccinated and infected cattle after incubation with inactivated FMDV. Vaccine, 24(7), pp.964-969.

Paton, D.J., Valarcher, J., Bergmann, I., Matlho, O.G., Zakharov, V.M., Palma, E.L. and Thomson, G.R., 2005. Selection of foot and mouth disease vaccine strains-a review. Revue scientifique et technique-Office international des épizooties, 24(3), p.981.

Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S. and Fitzpatrick, E., 2011. Veterinary microbiology and microbial disease. John Wiley & Sons.

Reed, L.J. and Muench, H., 1938. A simple method of estimating fifty per cent endpoints. American journal of epidemiology, 27(3), pp.493-497.

Reeve, R., Blignaut, B., Esterhuysen, J.J., Opperman, P., Matthews, L., Fry, E.E., De Beer, T.A., Theron, J., Rieder, E., Vosloo, W. and O'Neill, H.G., 2010. Sequence-based prediction for vaccine strain selection and identification of antigenic variability in foot-and-mouth disease virus. PLoS computational biology, 6(12), p.e1001027.

Rweyemamu, M.M., Booth, J.C., Head, M. and Pay, T.W., 1978. Microneutralization tests for serological typing and subtyping of foot-and-mouth disease virus strains. Epidemiology & Infection, 81(1), pp.107-123.

Rweyemamu, M.M., Pay, T.W. and Parker, M.J., 1976. Serological differentiation of foot-and-mouth disease virus strains in relation to selection of suitable vaccine viruses. Developments in biological standardization, 35, pp.205-204.

Schumann, K.R., Knowles, N.J., Davies, P.R., Midgley, R.J., Valarcher, J.F., Raoufi, A.Q., McKenna, T.S., Hurtle, W., Burans, J.P., Martin, B.M. and Rodriguez, L.L., 2008. Genetic characterization and molecular epidemiology of foot-and-mouth disease viruses isolated from Afghanistan in 2003–2005. Virus Genes, 36, pp.401-413.

Smith, D.J., Lapedes, A.S., de Jong, J.C., Bestebroer, T.M., Rimmelzwaan, G.F., Osterhaus, A.D. and Fouchier, R.A., 2004. Mapping the antigenic and genetic evolution of influenza virus. Science, 305(5682), pp.371-376.