Review

Foot and Mouth Disease Vaccine Strain Selection– Vaccine Manufacturers' Perspectives

Madhanmohan Muthukrishnan[1], Mana Mahapatra[2], Mark M. Rweyemamu[3], Villuppanoor Alwar Srinivasan[4*] and Satya Parida[3,5,6*]

[1]Vaccine Research Centre, Viral Vaccines, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, 600051 India; [2]The Pirbright Institute, Ash Road, Pirbright, Surrey GU24 0NF, UK; [3]SACIDS Africa Centre of Excellence for Infectious Diseases, SACIDS Foundation for One Health, Sokoine University of Agriculture, P.O. Box 3297, Morogoro 25523, Tanzania; [4]Former Executive Director and Research Director, Indian Immunologicals Limited, 33, Telecom Nagar, Gachibowli, Hyderabad- 500032 India; [5]Royal Veterinary College, Hawkshead Lane North Mymms Hatfield Herts AL9 7TA; [6]Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, 00153 Rome, Italy.

Madhanmohan Muthukrishnan and Mana Mahapatra contributed equally to this work.

Abstract | Foot-and-mouth disease (FMD) control and eradication are vital for economic growth and sustainable livestock farming. FMD in endemic countries is usually controlled by regular mass vaccination of the susceptible population. Moreover, FMD has been successfully controlled or eradicated through compulsory vaccination and strict zoo-sanitary measures in Europe and most South American countries. Suitable vaccine strain selection is an important component in the FMD control strategy and is necessary for the FMD control programmes in endemic regions, for the maintenance of vaccine antigen reserves in FMD-free countries. Successful identification of the appropriate vaccine strain and its incorporation into the vaccine will have a direct impact on disease control. The decision to change or incorporate a new strains in vaccine formulations is a complex process, primarily based on experimental, epidemiological, and field observations. Vaccine manufacturers will be hesitant to incorporate a new vaccine strain unless it is essential as it involves time and expenditure in bringing out the vaccine containing a new strains. This paper reviews vaccine manufacturers' expectations for selecting the FMD vaccine strain and the current and recent approaches to FMD vaccine strain selection.

Received | May 24, 2026; **Accepted** | July 13, 2026; **Published** | August 10, 2026
***Correspondence** | Villuppanoor Alwar Srinivasan and Satya Parida; **Emails:** satya.parida@fao.org; satyaparida1964@gmail.com, srinivasanva1948@gmail.com
Citation | Muthukrishnan, M., M. Mahapatra, M.M. Rweyemamu, V.A. Srinivasan and S. Parida. 2026. Foot and mouth disease vaccine strain selection– vaccine manufacturers' perspectives. *Hosts and Viruses*, 13: 105-126.
DOI | https://dx.doi.org/10.17582/journal.hv/2026/13.105.126
Keywords: Foot-and-mouth disease, Vaccine virus strain selection, 2D-MNT, LPB-ELISA, FMD vaccine manufacturers

Copyright: 2026 by the authors. Licensee ResearchersLinks Ltd, England, UK.
This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Introduction

Foot-and-mouth disease (FMD) is a highly contagious and economically important viral disease of cloven-hoofed domestic and wild animals, including cattle, buffalo, sheep, goats, and pigs. The disease is characterized by vesicles in and around the mouth, tongue, snout, feet, and udders, and is associated with fever, lameness, loss of condition, and a drop in milk production in dairy cattle and buffalo. FMD also causes sudden death in young animals due to myocarditis (Thomson and Bastos, 2005). Currently, the disease is endemic in many African and Asian countries (WRL-FMD Annual Report, 2023), while North America, Australasia, Europe, and much of South America are free from FMD.

FMD is caused by a picornavirus, FMD virus (FMDV), which is a single-stranded positive-sense RNA virus belonging to the genus Aphthovirus in the familyPicornaviridae. FMDV exists as seven antigenically distinct serotypes,i.e., serotype O and A (Valle´e and Carre´ 1922), serotype C (Waldmann and Trautwein 1926), Southern African Territories serotypes (SAT 1-3; Brooksby, 1958), and Asia 1 (Brooksby and Rogers 1957; Dhanda *et al*., 1957).

FMD control and eradication are vital for economic growth and sustainable livestock farming. FMD in endemic countries is mainly controlled by regular mass vaccination of the susceptible population. Nevertheless, integrated farming, wider host range, unrestricted movement of animals and animal products, high infectivity rates, the emergence of new virus variants, , and the interface between domestic and wild animals are significant factors that determine the control and eradication of FMD in endemic countries.

However, FMD has been successfully controlled or eradicated through compulsory vaccination and strict zoo-sanitary and import control measures in Europe and most South American countries (Paton *et al*., 2021). Currently, in FMD-free countries, the disease is controlled mainly by slaughtering infected and in-contact animals, along with restrictions on animal movement and animal products.

Currently, FMD vaccines are manufactured by infecting baby hamster kidney (BHK-21) cells with FMDV in biosafety level 3 (BSL-3) containment facilities, followed by chemical inactivation with binary ethyleneimine (BEI) and purification by ultrafiltration (Doel, 2003). During vaccine formulation, the concentrated FMD antigen is blended with a suitable adjuvant, either mineral oil or aluminum hydroxide gel and saponin. The FMD vaccine strains included in the vaccine depend on the country's epidemiological status. Similarly, the epidemiological status of the country also determines the number of FMDV strains to be included in the vaccine; it may be mono-, bi-, tri-, tetra-, or pentavalent, depending on the number of serotypes and/or strains circulating in the country/region. Most commonly used FMD vaccine strainsin different geographical locations are O1-Manisa, O1-Campos, O-3039, O-TUR/5/2009, O-BFS/1860, O-SKR/7/2010, O-TAW/98, A22 Iraq, A-Iran-05, A-G-VII, A-TUR/2006, A-Malayasia-97, A-Eritrea, A-SAU 95, A24 Cruzeiro, Asia1-Shamir, C3 Oberbayern, SAT-1 Rho/78, SAT2 Eritrea 3218, SAT2 ZIM, SAT2 SAU and SAT3 ZIM 2/83 (WRL FMD Quarterly Report, 2025). At the moment, trivalent FMD vaccines containing the O/IND/R2/75, A/IND/40/2000, and Asia1/IND/63/72 vaccine strains are used in India.

Currently, there are twenty-seven recognized FMD vaccine manufacturers (Table 1) producing FMD vaccines across the world (https://www.foot-and-mouth.org/fmd-vaccine-producers), of which nearly half of the FMD vaccine manufactures (n=13) are located in Asia, followed by the Middle-East and Turkey (n=5), South America (n=4), Europe (n=4), and Africa (n=3). Approximately 700 million doses of bivalent or trivalent FMD vaccines are used annually in South American countries (Naranjo and Cosovi, 2013). More than 2 billion doses of monovalent, bivalent, or trivalent FMD vaccines are used in China, and about 50 million doses are used in the rest of Southeast Asia (SEA). In India, around 500 million doses of trivalent FMD vaccines are used in the FMD control program. Further, around 20 million doses of FMD vaccine are used in Middle Eastern countries, while 15 million doses each are used in Turkey, and African countries (Knight-Jones and Rushton, 2013). Globally, around 3.3 billion doses of FMD vaccines are produced every year, although the yearly requirement is much higher.

Most FMD vaccine manufacturersare located in FMD-endemic regions. Further, conventional inactivated FMD vaccines must be manufactured in expensive biocontainment facilities using live FMD

Table 1: *List of FMD vaccine manufactures.*

S. No	Region	Name of the manufacture	Location of production site	Website
1	South America	Biogénesis Bagó	Argentina/ Brazil	http://www.biogenesisbago.com
		Ourofino	Brazil	https://www.ourofinosaudeanimal.com/produtos/bovinos/biologicos/ourovac-aftosa/
		Boehringer-Ingelheim (BI)	Brazil	https://www.boehringer-ingelheim.com/animal-health/live-stock-products/aftopor-aftovaxpur-aftovax-aftobov-oleosa-aftovax-pur-doe
		MSD	Brazil	https://www.msd-animal-health.com/
2	Europe	Boehringer-Ingelheim (BI)	France/UK/	https://www.boehringer-ingelheim.com/animal-health/live-stock-products/aftopor-aftovaxpur-aftovax-aftobov-oleosa-aftovax-pur-doe
		MSD	Germany	https://www.msd-animal-health.com/
		FGBI-ARRIAH	Russia	http://www.arriah.ru/en/main/production/price-vaccines/117
		ShchelkovoBiocombinat	Russia	http://www.biocombinat.ru/en/catalog/32/3123/
3	Asia	Indian Immunologi-calsLimitted	India	https://www.indimmune.com/business-unit/animal-health/vaccines/livestock-vaccines
		Biovet Private Ltd	India	http://biovet.in/
		Brilliant Bio Pharma	India	https://brilliantbiopharma.com
		Veterinary Research Insti-tute (VRI)	Pakistan	
		BWAT Bio. Science and Technology	China	http://www.bvbio.com/
		China Agricultural Vet. Bio. Science and Technology	China	http://www.cavetbio.com/index.html
		Hile Biotechnology	China	http://www.hile-bio.com/
		Lanzhou Biological Phar-maceutical of China Ani-mal Husbandry Industry	China	http://www.cahic.com/
		QYH Biotech	China	https://bioqyh.cnadc.com.cn/qyjjen/index.jhtml
		Shenlian Biotech	China	http://www.shenlianbiotech.com.cn/
		The Spirit Jinyu Biological Pharmaceutical Co., Ltd.	China	http://www.jinyubaoling.com.cn/
		Xinjiang Tecon Animal Husbandry Bio-technology	China	http://www.tcsw.com.cn/html/html/pc/index.html
		Department of Livestock Development (DLD)	Thailand	http://en.dld.go.th/index.php/en/focus-menu/animal-health-menu/vaccine-production-and-usage/animal-vaccines-for-ruminant
4	Middle East and Turkey	MEVAC	Egypt	https://www.me-vac.com/product/ruminants-vaccines
		Veterinary Serum and Vac-cine Institute (VSVRI)	Egypt	http://vsvri.com/ProductsAnimal.html#1
		Razi Vaccine and Serum Research Institute	Iran	https://www.rvsri.ac.ir/portal/home/?STAND-ARD/235246/25752/25755/Veterinary-Vaccines-_Animals
		ŞAP Institute	Turkey	https://vetkontrol.tarimorman.gov.tr/sap
		Vetal	Turkey	http://www.en.vetal.com.tr/
5	Africa	Botswana Vaccine Institute (BVI)	Botswana	https://bvi-bw.com/our-products/
		KEVEVAPI	Kenya	https://kevevapi.or.ke/shop/
		NVI	Ethiopia	http://www.nvi.com.et/products/vaccines-against/rumi-nant-and-equine-diseases/fmd-2/

vaccine strains. The typical volumes of culture fluids handled in the vaccine production process range from 1,000 to 10,000 liters. The related risks of virus release from manufacturing facilities or incomplete inactivation during the vaccine formulation process, and the high cost of a biocontainment vaccine production facility, are plausible reasons why many countries lack a vaccine production facility or restrict FMD vaccine production to local endemic strains.

This paper reviews vaccine manufacturers' expectations for selecting the FMD vaccine strain and discusses the currently used and recent approaches to FMD vaccine strain selection.

Concept of subtypes and their relevance to vaccine strain selection

An FMDV subtype is an antigenic grouping of closely related strains within a serotype (Forman, 1975). Research groups from Germany (Waldmann and Trautwein, 1926) and England (Bedson *et al.*, 1927) independently established that a lesser difference existed between some strains within the serotype (Brooksby, 1982). The complement–fixation test (CFT) was also developed independently at the Pirbright Laboratory in the United Kingdom (Galloway *et al.*, 1948). Since then, CFT has been the most widely employed test for serological typing and subtyping of foot-and-mouth disease virus (Brooksby, 1952; Davie, 1964; Graves, 1960a; Forman, 1974). Pereira (1977) reported multiple subtypes, including 32 serotype A subtypes, 11 serotype O subtypes, 5 serotype C subtypes, 7 serotype SAT 1 subtypes, 3 serotype SAT 2 subtypes, 4 serotype SAT 3 subtypes, and 3 serotype ASIA 1 subtypes.

Several incidents in which field strains shattered vaccine immunity prompted recognition of the need for new vaccines targeting specific subtypes. Identification of virus type and subtype is essential before recommendations on vaccine choice can be made. Arrowsmith (1975) reported that the main reason for subtyping was to advise on vaccine strains and therefore agreed with the principle of relating field strains to reference or vaccine strains. Pereira (1977) reaffirmed the two main purposes of subtyping, namely (i) to assist in epidemiological investigations and (ii) to identify suitable vaccine strains. Several studies emphasized the concept that field strains should be matched to the list of reference vaccine strains for the region (Pereira, 1977; Rweyemamu *et al.*, 1977; Paton *et al.*, 2005; Mahapatra and Parida, 2018).

However, the ever-increasing difficulty of comparing field strains with other subtypes within a serotype is becoming very time-consuming and expensive, and it requires a biocontainment laboratory facility and skilled personnel. Pereira (1977) recognized three main areas in which problems of subtyping could occur; (i) inherent variability of the tests used to compare FMDV strains and the requirement that to be statistically significant each test need to be repeated many times, (ii) the problem of dominance of some subtypes circulating in the region, and lastly, (iii) the subtypes that did not form discrete groups but tended to antigenicaIly merge into one another. Further, many laboratories have been involved in numbering subtypes using their own systems rather than a universal one, which has led to some confusion. Currently, subtype classification has been largely discontinued (Pereira, 1977).

Molecular epidemiology of foot and mouth disease and its significance to vaccine strain selection

The determination of the nucleotide sequence of the viral RNA allowed unequivocal characterization of genetic relationships between strains and gave precise evidence for epidemiological studies (Beck and Strohmaier, 1987; Samuel *et al.*, 1988). Comparing nucleotide sequences among virus strains enabled the construction of phylogenetic trees to visualize genetic relationships. An FMD molecular epidemiology study from isolated viral RNA was reported in the late 1980s (Beck and Strohmaier, 1987; Knowles and Sharma, 1990). Later, with the advent of the polymerase chainreaction (PCR), larger genome regions could be analyzed. Reverse Transcription- Polymerase Chain Reaction (RT-PCR) methodologies capable of amplifying the complete VP1-coding regions of the FMDV O, A, C, and Asia 1 serotypes (Knowles and Samuel, 1995) and Southern African Territories (SAT) serotypes (Bastos, 1998; Knowles and Samuel, 2003) were developed. Mason *et al.* (2003) used complete FMDV genome sequences for molecular epidemiology studies. Nevertheless, it is not always easy to relate genomic sequence data directly to a virus's antigenic properties, as it is hard to distinguish neutral changes from those that affect antigenicity. In addition, sometimes genetically similar viruses can differ antigenically.

The details of FMDV serotypes O, A, C, Asia 1,

SAT1, SAT2, and SAT3 topotypes, lineages, and sub-lineages are presented in Table 2. Currently, type O (eleven topotypes, twelve lineages and eight sub-lineage), type A (three topotypes, twenty one lineages and eleven sub-lineages), type C (three topotypes), type Asia 1 (one topotype and nine lineages), type SAT1 (13 topotypes), type SAT2 (14 topotypes) and SAT3 (five topotypes) have been described (https://www.foot-and-mouth.org/FMDV-nomenclature-working-group/prototype-strains).

Table 2: *Details of topotypes, lineages and sub-lineages of FMDV serotypes.*

Sero-type	Topotype	Lineage	Sub Lineage
O	Cathay	-	-
	East Africa 1 (EA-1)	-	-
	East Africa 2 (EA-2)	-	-
	East Africa 3 (EA-3)	-	-
	East Africa 4 (EA-4)	-	-
	West Africa (WA)	-	-
	Europe-South America (EU-RO-SA)	O_1	-
		O_2	-
		O_3	-
	Indonesia-1 (ISA-1)	-	-
	Indonesia-2 (ISA-2)	-	-
	Middle East-South Asia (ME-SA)	PanAsia	-
		PanAsia-2	
		PanAsia-2	TER-08
		PanAsia-2	FAR-09
		PanAsia-2	BAL-09
		PanAsia-2	SAN-09
		PanAsia-2	ANT-10
		PanAsia-2	PUN-10
		PanAsia-2	QOM-15
		PanAsia-2	KAT-15
		Ind-2001a	-
		Ind-2001b	-
		Ind-2001c	-
		Ind-2001d	-
		Ind-2001e	-
	Southeast Asia (SEA)	Cam-94	-
		Mya-98	-
A	Asia	A_{22}	-
		Iran-87	-
		Iran-96	-
		Iran-99	-
		Iran-05	-
		Iran-05	AFG-07
		Iran-05	ARD-07
		Iran-05	EZM-07
		Iran-05	BAR-08
		Iran-05	FAR-09
		Iran-05	KSS-09
		Iran-05	ESF-10
		Iran-05	HER-10
		Iran-05	SIS-10
		Iran-05	QAZ-11
		Iran-05	SIS-12
		A_{15}	-
		Thai-87	-
		Sea-97	-
		Sea-97	-
		G-VII	-
	Africa	G-I	-
		G-II	-
		G-III	-
		G-IV	-
		G-V	-
		G-VI	-
		G-VII	-
	Europe South America (EU-RO-SA)	A_{12}	-
		A_5	-
		A_{24}	-
		A-81	-
C	Africa	-	-
	Asia	-	-
	Europe South America (EU-RO-SA)	-	-
Asia 1	Asia	G-I	-
		G-II	-
		G-III	-
		G-IV	-
		G-V	-
		G-VI	-
		G-VIII	-
		Sindh-08	-
		G-IX	-
SAT 1	I (Northwest Zimbabwe NWZ)	-	-
	II (Southeast Zimbabwe SEZ)	-	-
	III (Western Zimbabwe WZ)	-	-
	IV (East Africa 1 EA-1)	-	-

Table continues on next column.....

Table continues on next page.....

Sero-type	Topotype	Lineage	Sub Lineage
	V	-	-
	VI	-	-
	VII (East Africa 2 EA-2)	-	-
	VIII (East Africa 3 EA-3)	-	-
	IX	-	-
	X	-	-
	XI	-	-
	XII	-	-
	XIII	-	-
SAT-2	I	-	-
	II	-	-
	III	-	-
	IV	-	-
	V	-	-
	VI	-	-
	VII	-	-
	VIII	-	-
	IX	-	-
	X	-	-
	XI	-	-
	XII	-	-
	XIII	-	-
	XIV	-	-
SAT-3	I(Southeast Zimbabwe SEZ)	-	-
	II(Western Zimbabwe WZ)	-	-
	III (Northwest Zimbabwe NWZ)	-	-
	IV	-	-
	V(East Africa)	-	-

The vaccine strains used in a particular region depend mainly on the serotypes and genotypes circulating there. The World Reference Laboratory for FMD (WRL-FMD) usually recommends vaccine strains for inclusion in the antigen reserves (Table 3) for different regional pools (Table 4) (WRL-FMD Annual report 2023). Recently, the European commission for the control of foot-and-mouth disease (EUFMD), in collaboration with WRL-FMD, developed a tool called "Prioritization of Antigen Management with International Surveillance Tool (PRAGMATIST)" to assist risk managers in making decisions about the FMD vaccines that they purchase/maintain, based on current risks to countries covered by the bank. The tool is currently an Excel spreadsheet based on two distinct considerations:

(i) Antigen risk score, which defines which strains pose the greatest threat of incursion and (ii) Coverage score: Effectiveness of available vaccines to protect against each virus strain (http://www.fao.org/eufmd/global-situation/pragmatist/en/)(Ludi *et al.*, 2022).

Table 3: *WRL FMD vaccine recommendations for FMD free countries (antigen banks).*

Serotypes	Vaccine strains
O	O-TUR/5/2009 (HIGH) O-3039 (HIGH) O-Manisa (HIGH) O-Campos (HIGH) O-BFS/1860 (LOW) O-SKR/7/2010(LOW) O-TAW/98 (LOW)
A	A-TUR/2006 (HIGH) A22 Iraq (HIGH) A-Iran-05 (HIGH) A-Malaysia 97 (HIGH) A-Eritrea (MEDIUM) A-SAU (LOW) A24- Cruzeiro (LOW)
Asia 1	Asia 1-Shamir (HIGH)
C	C1Oberbayern (LOW)
SAT1	SAT1-Rho/78 (MEDIUM)
SAT2	SAT2-Eritrea 3218 (HIGH) SAT2-SAU (HIGH) SAT2-ZIM (MEDIUM)
SAT3	SAT3 ZIM 2/83 (LOW)

The details of WRL- FMD recommended FMD vaccine strains and their year of origin are presented in Table 5. The most commonly used FMD vaccine strains are O1 Campos (1958), O1Kaufbeuren (1966), O1 BFS/1860 (1967), and O1 Manisa (1969). O/IND/R2/75 (1975), A24 Cruzeiro (1955), A22 Iraq (1964), Asia1-Shamir (1989), C1 Oberbayern (1960), C3 Indaial (1971), SAT-1 Rho 12/78 (1978), SAT 2 ZIM (1983), and SAT3 ZIM (1983) represent early isolates collected during 1955 to 1989. It is interesting to note that the O1/Campos vaccine strain (1958), widely used in South America, was also successfully used to control the outbreaks in Taiwan in 1997 and South Korea in 2016 (Bergman *et al.*, 2021). Similarly, high-potency vaccines based on the O1 Manisa vaccine strain (1969) is still able to provide protection against heterologous serotype O strains (Fishbourne *et al.*, 2017; Horsington*et al.*, 2017). This indicates the broad antigenic coverage conferred by the serotype O vaccine strains. Furthermore, Brehm *et al.* (2008) reported intra-serotypic protection against very diverse serotype A strains (A Iran96, A Egypt 06, and A Iran99) with high A22 Iraq antigen payload vaccines despite low levels of antigenic "match".

Table 4: *WRL FMD vaccine recommendations for the different regional pools.*

Regional pools	Country	Circulating FMD viral topotypes and lineages	WRL FMD Vaccine recommendations
Pool 1	South-east Asia with spill over into Eastern Asia	Serotype O: • SEA/Mya-98 • ME-SA/PanAsia • ME-SA/Ind2001d • ME-SA/Ind2001e • CATHAY Serotype A: • ASIA/Sea-97 Serotype Asia-1	Internationally produced vaccines: • O: Campos, Manisa, Primosky& 3039 • A: Arg2001, A24 Cruzeiro, Iraq/64, Malaysia/97, Zabaikalsky& A22-IRQ. • Asia 1: Shamir Locally produced vaccines (at RRL SEA): • O: 189/87 (Udornthani/87) • A: Lopburi/12, Sakolnakorn/97 • Asia1: Petchaburi/85 Locally produced vaccines (at FGBI ARRIAH): • O: Ind-2001d, Mya-98, PanAsia, PanAsia-2 • A; G-VII, Iran-05, Sea-97 • Asia1: Shamir, Sindh-08 Locally used vaccine strains (by Chinese manufactures): • O/Mya-98 (O/Mya98/BY/2010 and Re-O/Mya98), O/HK99 • Re-A/Sea-97 (Re-A/WH/09) • Asia1/GV (Asia1/JSL/06)
Pool 2	Southern Asia	Serotype O: • ME-SA/Ind-2001 • ME-SA/PanAsia-2 Serotype A: • ASIA/IND (genotype VII also known as genotype 18) Serotype Asia-1:	Internationally produced vaccines: • O/ME-SA/PanAsia-2 (or suitable alternative). • O/TUR/09 vaccine • O-3039 Locally produced vaccines (by Indian suppliers): • O/IND/R2/1975 • A/IND/40/2000 Asia1/IND/63/1972
Pool 3	Western Asia with spill over into North Africa	Serotype O: • ME-SA/PanAsia-2 (ANT-10 and QOM-15) • ME-SA/Ind-2001 • EA-3 Serotype A: • ASIA/Iran-05 (SIS-10, SIS-12, SIS-13 and FAR-11)] • ASIA/G-VII Serotype Asia-1: Sindh-08	• Internationally produced vaccines • O/ME-SA/PanAsia-2 (or suitable alternative) • O/Manisa • A Iran-05 (or A TUR 06) • A22/Iraq • Asia-1 Shamir o A/G-VII Locally produced vaccines (at FGBI ARRIAH): • O/PanAsia-2 • A/ASIA/G-VII • A/ASIA/Iran-05 (A/Krasnodarsky/RUS/2013) • Asia-1/Sindh-08 Locally produced vaccines: • O/PanAsia-2/QOM-15 • A05 variant • A/Asia/G-VII • Asia 1/TUR 15 (Sindh-08) • Asia1/Shamir
Pool 4	Eastern Africa with spill over into North Africa	Serotype O: • EA-2 • EA-3 • EA-4 • ME-SA/Sharqia-72 • ME-SA/Ind2001 Serotype A • AFRICA/I • AFRICA/IV • AFRICA/VII • ASIA/Iran-05BAR-08 Serotype SAT 1 :I, IX Serotype SAT 2: IV, VII, XIII Serotype SAT 3	Internationally produced vaccines: • O/Manisa • O/PanAsia-2 (or equivalent) • A/Eritrea • SAT2/Eritrea Locally produced vaccines from KEVIVAPI (Kenya): • O: K 77/78 - EA1 • A: K5/80 - G1 • SAT1: T155/71 - NWZ • SAT2: K52/84 - IV Locally produced vaccines from Ethiopia: • Serotype O (EA-3) • Serotype A (G-IV) • Serotype SAT 2 (VII)

Table continues on next page.....

Regional pools	Country	Circulating FMD viral topotypes and lineages	WRL FMD Vaccine recommendations
Pool 5	Western Africa	Serotype O: • WA and EA-3 Serotype A: • AFRICA/G-IV & G-VI Serotype SAT 1 • Topotype X Serotype SAT 2: • Topotype VII	Internationally produced vaccines: • O/Manisa • O/Maghreb • O/PanAsia-2 (or equivalent) • O: 3039 • A: Eritrea • SAT 2: Eritrea & Zimbabwe Locally produced vaccines: • O: NIG 04/14 • O: WA and EA-3 topotypes • A: NIG 07/13 • A: West Africa (G-IV lineage) • SAT 1: Topotype X • SAT 2: NIG 03/12 • SAT 2: Topotype VII • O, A, SAT 1 & SAT 2 (Boru-Vacc, Nigeria)
Pool 6	Southern Africa	Serotype SAT 1: Topotypes I, II and III Serotype SAT 2: Topotypes I, II and III Serotype SAT 3: Topotypes I, II and III)	Internationally produced vaccines: • O: O Manisa • SAT 1: SAT105 • SAT 2: SAT251 • SAT 3: SAT306 Locally produced vaccines • O: O Manisa • SAT 1: SAT105, SAT109 and SAR/9/81 • SAT 2: SAT251, SAT2035, SAR/3/04 and KNP/1/10 • SAT 3: SAT306, SAT309 and KNP/10/90
Pool 7	South America		Locally produced vaccines: O: O1 Campos A: A24 Cruzeiro, A/Arg/2001 C: C3 Indaial

Table 5: *Details of WRL FMD recommended FMD vaccine strains and their year of origin.*

S. No	Serotypes	Vaccine strains	Year of origin
1	O	O1-Campos	1958
2	O	O1-Kaufbeuren	1966
3	O	O1-Manisa	1969
4	O	O-3039	*
5	O	O-BFS/1860	1967
6	O	O- IND/R2/75	1975
7	O	O-Israel 85 (4625)	1985
8	O	O-Magheb 99	1999
9	O	O-PanAsia 2	2009
10	O	O-SKR 2010	2010
11	O	O-SKR/7/2010	2010
12	O	O-Taiwan	1999
13	O	O-TAW/98	1998
14	O	O-TUR/5/2009	2009
15	A	A22 Iraq	1964
16	A	A24 Cruzeiro	1955
17	A	A-Arg/2001	2001
18	A	A-Eritrea	1998
19	A	A-Iran 96	1996
20	A	A-Iran-05	2005
21	A	A-Malaysia 97	1997
22	A	A-SAU 95	1995
23	A	A-SAU/23/86	1986
24	A	A-TUR/2006	2006
25	Asia-1	Asia1-Shamir	1989
26	SAT 1	SAT-105 Rho 12/78	1978
27	SAT 2	SAT2 Eritrea 3218	*
28	SAT 2	SAT2-SAU	2000
29	SAT 2	SAT2-ZIM	1983
30	SAT 3	Sat3 Zim 2/83	1983
31	C	C1 Oberbayern	1960
32	C	C3 Indaial	1971

Table continues on next column.....

* *The year of origin is not available for these vaccine strains.*

South America and Europe eradicated or nearly eradicated FMD with well-established vaccine strains formulated into high-quality vaccines; however, no

significant variants emerged in vaccinated populations. The use of well-known strains with broad antigenic coverage has many advantages over adapting and characterizing newly emerging strains for inclusion in the vaccine (Bergman *et al*., 2021). Many genetic variations among FMDV strains have been reported over the past 10 years, but these variations have not necessitated the development of any new vaccine strain for the control of FMD outbreaks in most countries so far.

The genetic relationship between vaccine strains and field viruses is evaluated by the percentage homology of the VP1 sequences. However, these genetic differences are not as evident by antigenic comparisons (Bergman *et al*., 2021). In a recent study, the South American type O vaccine O1Campos, belonging to the Europe –South America (EURO-SA) topotype, was tested invitro against single virus isolates representing three topotypes (Southeast Asia (SEA), Middle East–South Asia (ME-SA), and Cathay), and exhibited acceptable in vitro cross-protection (Galdo novo *et al*., 2017). Similarly, the Indian type O vaccine O/IND/R2/75, belonging to the ME-SA topotype, has been reported to be broadly cross-reactive (Mahapatra *et al*., 2015) even after a lapse of fifty years. This Indian type O vaccine was also found to be a good match with 79% of the viruses used in the study, including viruses representing topotypes East Africa-2, East Africa-3, and MYA-98 strains (Mahapatra *et al*., 2017).

FMD epidemiology is characterized by waves of infection driven by newly emerging FMDV variants that supplant older lineages (Knowles *et al*., 2009). However, it is evident that many of these new antigenic variants do not always persist (Upadhyaya *et al*., 2014; Mahapatra *et al*., 2016; Bergman *et al*., 2021). Nevertheless, incorporating homologous emerging strains/ sequences into the vaccine does not necessarily always lead to outbreak control. In some cases, these variants could be temporarily added to traditional vaccines to address local situations. So, the emergence of new genetic variants does not always require a change in vaccine strain, and, wherever possible, established vaccine strains with broad antigenic coverage can be used. Additionally higher antigenic payloads will help in conferring adequate immunity to resist infection with the variant FMDV strains.

For example, in India, to date, genetic characterization of FMDV serotype O strains reveals circulation of Middle East-South Asia (ME-SA) topotype, and within this topotype lineage O/ME-SA/Ind2001 and O/ME-SA/PanAsia are the two predominant genetic groups circulating in the Indian sub-continent (Subramaniam *et al*., 2015, 2022). The O/ME-SA/Ind2001 lineage, since its first report in 2001, has diversified into at least 5 sub-lineages (Ind2001a, b, c, d, and e). Further, in a recent study, the emergence of a novel genetic cluster (O/ME-SA/2018 lineage) was reported in India (Dahiya *et al*., 2021, 2023). Nevertheless, the antigenic characterization of these field isolates revealed that the circulating predominant genetic lineages and the emerging novel lineage showed better antigenic match with the current Indian serotype O vaccine strain O/IND/R2/75. These data suggest that genetic variants may appear and disappear, and hencegenetic studies may not always provide insight into the potential protection conferred by a particular vaccine strain.

FMD vaccine manufacturers expectations

Development of a new vaccine strain is warranted only when an antigenically significant variant virus emerges and persists in regions or countries where regular vaccination programmes are employed and frequent vaccine failures are observed in the field. Such situations support the obvious concept of developing a new vaccine strain from a local field isolate, so that the field viruses and vaccine strains are as closely matched as possible (Doel, 2018). Vaccine manufacturers are compelled to change vaccine strains when disease outbreaks recur in vaccinated herds and farmers lose confidence in the vaccine's ability to control the disease.

The following attributes are very important for FMD vaccine manufacturers while developing a new vaccine strain (V.A. Srinivasan, Personal communication):

1. Candidate vaccine virus strain adapting to BHK-21 suspension cells with minimum passage levels.
2. Virus titers in BHK-21 suspension cells are satisfactory.
3. Antigen(146S) yields are satisfactory.
4. 146S antigen yields before and after BEI treatment should show minimal variation, indicating the stability of the new candidate virus antigen.
5. For the aluminum hydroxide (alum) vaccine, the supernatant after adsorption should be checked for 146S content. Adsorption of more than 95% of the antigen indicates that the new virus strain

adsorbssatisfactorily.
6. The candidate vaccine virus strain induces a strong protective immune response, as measured by an invitro serum neutralization test (SNT) and invivo challenge experiments in target animals.

These properties can only be achieved against the background of a comprehensive vaccine strain development programme (Doel, 2003). The vaccine strain selection must be carried out very carefully, as the strain must be fit for purpose and possess all the qualities required of a vaccine strain, i.e., yield, stability, immunogenicity, and efficacy.

FMD vaccine manufacturers often encounter the following issues when developing a new FMD vaccine strain (Doel, 2018).

1. Lengthy time scale, logistics, and high cost with particular reference to full regulatory testing and licensing.
2. Failure to adapt to suspension culture, some isolates only grow in monolayers.
3. Failure to grow to commercially viable yields.
4. Screening hampered by insufficient quality, quantity, and number of field isolates.
5. Rare properties such as 146S sensitivity to inactivating agent (e.g., SAT 2 Kenya 227/66) and propensity to aggregate, which compromise process recoveries.
6. Slow growth at scale-up, thus reducing the annual production capacity.
7. Disqualification of an isolate because of a high risk of rejection by regulatory authorities (For example, use of a bovine isolate from a country recognized as 'high risk' in terms of bovine spongiform encephalopathy (BSE).

For example, in India, until the year 2003, FMD quadrivalent vaccines incorporating the following vaccine strains, i.e., O/TNN/24/84 or O/IND/R2/75, A/IND/17/82 or A/IND/7/77, Asia 1/WBN/117/85 or Asia 1/IND63/72, and C/IND/51/79 or C/BOM/64 were manufactured by the different FMD vaccine manufacturers. During October 2003, the Government of India (GoI), Indian Council of Agricultural Research (ICAR), and Project Directorate on FMD (PD-FMD) (Current name ICAR-National Institute on Foot and Mouth Disease) harmonized the FMD vaccine strains to FMD trivalent vaccines incorporating the following vaccine strains: O/IND/R2/75, A/IND/17/82, and Asia 1/IND/63/72. Moreover, the FMD type C vaccine strain has been withdrawn from the FMD vaccine since October 2003. National Institute on Foot and Mouth Disease (NIFMD) has been vested with the responsibility of identifying and supplying appropriate vaccine strains to FMD vaccine manufacturers in India. It is interesting to note that the vaccine strains used in FMD vaccines in India originated between 1972 and 1982. Further, the FMD type A/IND/17/82 vaccine strain was replaced by A/IND/40/2000 during September 2008 (Srinivasan, 2021; Madhanmohan and Srinivasan, 2012). So, the new type A vaccine strain was developed after a nearly 25-year gap. NIFMD has identified a new FMDV type A strain (A/IND/27/2011) for incorporation in the vaccine (Mohapatra *et al*., 2018).

In conclusion, it is important to recognize that vaccine strain development, when required, may not be straightforward. It requires time and resources to conduct the tests required to produce a commercially viable product and to satisfy regulatory authorities. Vaccine manufacturers will be hesitant to incorporate a new vaccine strain unless it is absolutely essential. So, frequent changes of FMD vaccine strains are not always feasible for FMD vaccine manufacturers.

Current methods of FMD vaccine strain selection

Selection of suitable vaccine strains is an important component of FMD control and is key to FMD control programmes in endemic regions, as well as to the establishment and maintenance of vaccine antigen reserves for use in the event of new FMD incursions in FMD-free countries. The decision to change or include new strains in vaccine formulations is a multifaceted process, based on experimental, epidemiological, and field observations (WOAH, 2022). Field isolates should be selected based on epidemiological information,i.e., at different stages of an outbreak, from different geographical locations, and/or from different hosts (Alonso *et al*., 1987). The emergence of a new virus strain may be characterized by rapid dissemination with many outbreaks, especially in vaccinated herds, and isolates from such epidemics are a priority for vaccine matching. Moreover, isolates with prevalence within the outbreak are the best candidates for the vaccine-matching test (WOAH, 2022). The vaccine strains to be selected for vaccine matching tests depend on the serotype and/or strain of the field virus, its origin, and any details about its

characteristics.

The selection of a suitable FMD vaccine strain is based on invivo and invitro vaccine matching tests. The in vitro matching tests measure the cross-reactivity of a bovine post-vaccine serum (BVS) with the field strain in question, and the results are usually expressed as antigenic relationship(r1) values. An antigenic relationship value or 'r1-value' is defined as: the reactivity of BVS against the heterologous (field) strain divided by the reactivity of BVS against the homologous (vaccine) strain (WOAH, 2022). Using cattle to raise immune sera for selecting vaccine strains may, at times, become expensive and ethically unacceptable if many field strains are identified as candidate vaccine strains. In this case, the use of immune guinea pig serum raised against the purified 146S antigen will help shortlist the best candidate vaccine strain.

The serological relationship between a field isolate and a vaccine virus ('r1' value) can be established by (i) virus neutralization test (VNT), (ii) liquid phase blocking enzyme-linked immunosorbent assay (LPBE-ELISA), or (iii) complement fixation tests (CFT). *In-vitro* neutralization may be more relevant to predict in-vivo protection by the vaccine than other measures of virus-antibody interactions. VNT can be performed using the checkerboard titration method or another layout, depending on the laboratory's facilities and the scientists'and technicians'experience. LPBE-ELISA depends on the availability of capture and detector antibodies suitable for the field strains in question and can be carried out with inactivated virus. For either VNT or ELISA, post-vaccination sera are usually collected from at least five cattle 21–30 days after primary vaccination and/or 21–30 days after booster vaccination. Sera are used individually or pooled, after excluding low responders (Mattion *et al*., 2009).

In-vitro vaccine matching test

Two-dimensional (checkerboard) neutralization test (2D-VNT): This is the recommended gold-standard test (WOAH, 2022) for FMDV vaccine matching and measures in vitro cross-protection. Bovine vaccinated serum raised against the vaccine strain is used in this test. The titres of this serum against 100 TCID50 of the homologous vaccine virus and the same dose of a field isolate are compared to estimate the immunological coverage of the vaccine strain in relation to the field virus (Rweyemamu *et al*., 1978). Antibody titres of the vaccine serum against the vaccine virus and the field isolate for each virus dose used are calculated using the Spearman–Kärber method (Karber, 1931). Bovine vaccinated serum titre is estimated by mixing serum and a definite dose of each virus and measuring it by regression.

In the 2D-VNT an r1-value ≥0.3 indicates that the field isolate is sufficiently similar to the vaccine strain and use of a vaccine based on this strain is likely to confer protection against infection with the field isolate while values less than 0.3 indicates the vaccine strain and the field isolate to be dissimilar and, development of a new vaccine strain should be considered for optimal coverage in future outbreaks. Tests should always be repeated more than once,preferably three times,to have full confidence in the result (Rweyemamu, 1984).

Expectancy of protection (EPP) determined by a one-dimensional neutralization test

Vaccine matching based on EPP values is widely used in South American countries where correlation tables are available for each vaccine strain used in the region. BVS is mixed with a known titer of vaccine virus, usually 100 TCID50, to determine the antibody titer. BVS titres against each virus are measured using the previously described 2D-MNT (WOAH, 2022) method, andtitres are expressed per ml. The relationship between vaccine strain and field isolate is expressed by the EPP value.Individual EPP values are determined for each neutralization titre using predefined correlation tables, and the mean EPP is then calculated for each group of sera (vaccinated and booster-vaccinated). To interpret the results, it is necessary to have a correlation defined between invitrotiters and invivo challenge protection against 10,000 Bovine tongue infective doses (BTID50) of vaccine virus. A mean EPP value of 75% in booster-vaccinated animals indicates that the vaccine strain is appropriate for conferring protection against the field virus (WOAH, 2022).

Liquid-phase blocking (LPBE) - ELISA

The liquid-phase blocking ELISA is recommended for vaccine matching by calculating the r1 or EPP value (WOAH, 2022). LPBE- ELISA detects any residual antigen remaining after an overnight reaction between dilutions of serum and a pretitrated virus dose (Kitching *et al*., 1988). In the LPBE, an r1-value

of ≥0.4 is considered indicative of a good vaccine match; an r1-value less than 0.4 indicates significant differences from the vaccine strain, suggesting the vaccine is unlikely to protect (Ferris and Donaldson, 1992). In LPBE, a mean EPP of 75% in booster-vaccinated animals indicates that the vaccine strain is appropriate for conferring protection against the field virus (WOAH, 2022).

Sequence-based vaccine strain selection

Sequence-based vaccine strain selection is a recent development and may be a promising area for further research, as all capsid amino acid residues responsible for variability and escape from vaccine protection are defined by sequence data (Mahapatra and Parida, 2018). An earlier study in the seasonal influenza virus showed that the "r1-value" is affected by antigenic variation, which in turn is the consequence of genetic evolution leading to amino acid substitution (Li *et al*., 2016). Earlier studies on FMDV suggested that models based on serological and capsid sequence data might help in the rapid selection of suitable vaccine strains (Reeve *et al*., 2016; Mahapatra and Parida, 2018).

The current procedure is to sequence the genomes of FMD viruses and to study the deduced amino acid sequence differences in antibody-binding sites on the virus surface, thereby predicting antigenic relatedness and cross-protection between FMD viruses (Paton *et al*., 2019). However, correlation of antigenic relatedness and cross-protection is complicated by the lack of cross-protection in in vivo challenge studies (Paton *et al*., 2019).Further, variable dominance of antigenic sites may complicate efforts to predict cross-protection from capsid gene sequences (Mahapatra *et al*., 2012), requiring more complex models that can account for changing immunodominance and identify the direct effects of identified epitope changes (Paton *et al*., 2019).

Reeve *et al*. (2010) developed an in silico predictive model (a linear mixed-effects model) of viral cross-reactivity between serotypes SAT1 and SAT2 using capsid amino acid sequence data, neutralizing antibody titres, and capsid structural information. The results of the predicted epitopes in SAT1 and SAT2 viruses by this approach were consistent with antigenic sites previously determined by monoclonal antibody resistant-mutant studies (Reeve *et al*., 2010, 2016; Maree *et al*., 2011), and provide a quick and alternative method for antigenic site identification and vaccine strain selection. Further, similar studies have been reported for serotypes O (Reeve *et al*., 2016) and A (Bari *et al*., 2015; Rahman *et al*., 2015). Further, Bayesian models were developed to identify antigenic relationships and their predictors, accounting for variation in pairwise cross-neutralization titres using only viral sequences and structural data (Davies *et al*., 2017).

However, the sequence–based vaccine strain selection approach is currently at an initial developmental stage, and to date, no FMD vaccine strain has been selected based on this approach and compared with the existing in-vitro vaccine matching methods, i.e., the micro neutralization test (MNT) or liquid phase blocking ELISA (LPB-ELISA) and *in-vivo* cross-protection challenge test. Therefore, further studies are warranted to evaluate this approach for selecting FMD vaccine strains in the future.

In-vivo vaccine matching

In an invivo vaccine-matching test, vaccinated animals are challenged with a virulent field strain. This will take both potency and cross-reactivity into account. However, this method requires the use of live FMDV and requires appropriate biosecurity procedures and practices to be followed. Moreover, this procedure is relatively slow, expensive, and requires specific expertise.

Brehm *et al*. (2008) conducted several heterologous protection studies using FMDV serotype A viruses, where the in vitro r1-value suggested that the vaccine virus was not a good match (Brehm *et al*., 2008). In this study, cattle were vaccinated with a serotype A vaccine and challenged with another heterologous strain of the same serotype. In 6 of 8 cases, a protective dose (PD50) above 6 was obtained, indicating that the response exceeded the threshold for the emergency vaccine bank. This study demonstrated that high-potency emergency vaccines could provide cross-protection between different subtypes of serotype A strains, even though invitro matching tests suggested the vaccine was not a good match (Brehm *et al*., 2008). Similar invivo studies in South America involving serotype O vaccines reported acceptable protection following challenge, whereas the invitromatching values (r1-values) were below the cut-off (Maradei *et al*., 2011). In contrast, Nagendrakumar *et al*. (2011) conducted a cross-protection study vaccinating

animals with the O1Manisa vaccine and challenging them with O1Campos virus (Nagendrakumar *et al.* (2011). Although the r1-value suggested the vaccine was a good match, there was a poor correlation between serology and *in vivo* cross-protection results. This study also reported that intra-serotypic cross-protection required very high-potency vaccines. Similarly in a heterologous challenge experiment vaccinated with a highly potent (> 6 PD50) FMD O1Manisa vaccine were protected upon challenge with an O/SEA/MYA-98 virus (O/SKR/2010), although the in-vitro r1-value was at the lower end of an acceptable threshold (~ 0.3) (Horsington *et al.*, 2017) and partially protected cattle when challenged with the O/ME-SA/Ind-2001 lineage field virus where the r1-value was very low (Fishbourne *et al.*, 2017). Similarly, a further study in pigs reported incomplete protection when pigs vaccinated with a highly potent (>6 PD50) FMD O1Manisa vaccine were challenged with the O/SEA/MYA-98 virus (O/VIT/2010). Further, the efficacies of O1 Manisa + O 3039 bivalent vaccine and O 3039 monovalent vaccine were studied for cross-protection against heterologous challenge with the O Jincheon strain (FMDV O/Jincheon/SKR/2014), and the efficacy of the O1 Manisa + O 3039 bivalent vaccine was much better than that of the O 3039 monovalent vaccine, even though the serological relationship (r1value) between O Jincheon and O 3039 matched according to the WOAH Terrestrial Manual (Kim *et al.*, 2019). In a heterologous challenge experiment, animals vaccinated with a highly potent (>6 PD50) FMD A/MAY/97 vaccine were protected upon challenge with a representative field virus from the A/ASIA/G-VII lineage, although the invitro r1value was very low (Dekkar *et al.*, 2020)., In another study animals vaccinated either with a highly potent (>6 PD50) FMD A/MAY/97 vaccine or the A22Iraq 64 vaccine were protected upon challenge with a variant strain of FMDV A/Asia/G-IX/SEA-97 lineage, although the invitro r1-value was below the threshold (Nagendrakumar *et al.*, 2020). These studies indicate a lack of certainty regarding the correlation between invitro and invivo results, species variation, and, therefore, the design of such experiments warrants further investigation (Mahapatra and Parida, 2018).

Pathway to introduce a new FMD vaccine strain for field application

A pathway to introduce a new FMD vaccine strain for field application is presented in Figure 1.Initially, in order to identify an appropriate candidate vaccine strain, 4 to 5 field isolates are selected for raising rabbit convalescent serum (RCS) (Rweymamu *et al.*, 1984a; Rweymamu and Ouldridge, 1982; Ndiritu *et al.*, 1983; Srinivasan *et al.*, 1983; Belwal *et al.*, 1986, 1989; Azad *et al.*, 1995; Yuvaraj *et al.*, 2013). This helps narrow the number of isolates for the final selection of the candidate vaccine strain. One or two isolates are selected based on the *in vitro* study using RCS, and then immune guinea pig serum (IGPS) is raised against purified 146S antigen. This process will allow selection of one candidate vaccine strain for production of bovine vaccinal serum (BVS) (Rweymamu*et al.*, 1984a; Rweymamu and Ouldridge, 1982; Ndiritu *et al.*, 1983; Srinivasan *et al.*, 1983; Belwal *et al.*, 1986, 1989; Azad *et al.*, 1995; Yuvaraj *et al.*, 2013). Preparation of BVS for the field isolates is time-consuming, cumbersome, and expensive. To avoid this, initial screening may be performed using rabbit convalescent serum (RCS) and immune guinea pig serum (IGPS) raised against the field isolates.

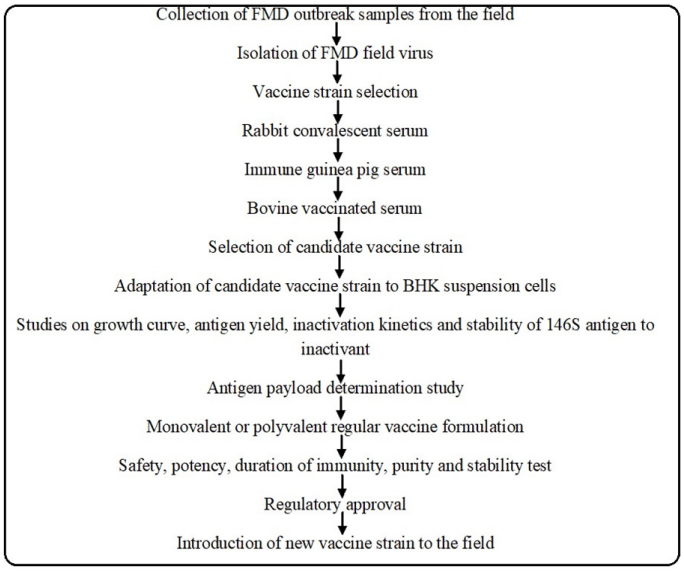

Figure 1: *Flow chart–Pathway to introduce new vaccine strain in vaccine for field application.*

Rabbit convalescent serum may be prepared as follows. Briefly, rabbits (n=5/each isolate) were inoculated with one ml of BHK-21 monolayer-adapted field (live) virus by intravenous route. Blood samples are collected from all the animals on the 28th day post-inoculation (dpi). The serum samples are inactivated at 56°C for 30 minutes and stored at -20°C until further use (Rweymamu*et al.*, 1984a; Rweymamu and Ouldridge, 1982; Ndiritu *et al.*, 1983; Srinivasan *et al.*, 1983; Belwal *et al.*, 1986, 1989; Azad *et al.*, 1995; Yuvaraj *et al.*, 2013). Similarly, immune guinea pig

serum is also prepared using the selected field isolates. Guinea pigs were immunized with inactivated and purified 146S antigen blended with Freund's complete adjuvant by the intramuscular route. On 21 days post vaccination, guinea pigs are given a booster dose of the 146S antigen formulated with Freund's incomplete adjuvant (Ferris and Donaldson, 1984), followed by exsanguination on 35 days post vaccination. The serum samples are inactivated at 56°C for 30 minutes and stored at -20°C until further use. Finally, an *in vitro* vaccine matching test using the two-dimensional micro neutralization test (2D-MNT) (Rweyemamu *et al*., 1978) is performed with RCS and IGPS.

Then, BVS is prepared using the selected candidate vaccine strain. Briefly, a minimum of 5 FMD seronegative calves per vaccine candidate areused to prepare BVS. The calves are vaccinated with the monovalent candidate vaccine, followed by a booster dose at 21 days post-vaccination (dpv), and blood samples are collected at 35 dpv. The serum samples are inactivated at 56°C for 30 minutes and stored at -20°C until further use (Rweymamu*et al*., 1984a; Rweymamu and Ouldridge, 1982; Ndiritu *et al*., 1983; Srinivasan *et al*., 1983; Belwal *et al*., 1986, 1989; Azad *et al*., 1995; Yuvaraj *et al*., 2013; Nagendrakumar *et al*., 2011; Mahapatra *et al*., 2015). Further, *in vitro* vaccine matching tests using the two-dimensional micro neutralization test (2D-MNT) (Rweyemamu *et al*., 1978) are carried out using BVS. The most appropriate vaccine candidate strain may be selected using this approach.

The selected vaccine candidate strain is then adapted to the BHK-21 monolayer cell line, clone 13, as described earlier (MacPherson and Stoker 1962; Mowat and Chapman 1962). Further, the BHK-21 monolayer-adapted vaccine candidate strain is pasagged in BHK suspension cells (Capstick *et al*., 1962; Telling and Elsworth, 1965). Master seed virus (MSV) may be prepared and stored at -70°C or in the vapor phase of liquid nitrogen (-196°C). Working seed viruses (WSV) may be expanded in one or a few more passages from the master seed stock and used to infect the final production scale cell culture. MSVs must be proven to be pure and free from extraneous agents (WOAH, 2022).

The *in vitro* growth kinetics of the suspension cell-adapted vaccine strain can be determined using multistep growth curve analysis. Briefly, the BHK-21 suspension cells were infected at a multiplicity of infection (MOI) of 0.01 with the vaccine strain and incubated at 37 °C. The virus is harvested at various time points up to 24 hr and, and the titre at each time point is estimated by the endpoint dilution method. The titer of the virus is expressed as mean tissue culture infective dose 50 per ml (TCID50/ml) from duplicate experiments (Karber, 1931).

The antigen yield of the selected vaccine candidate strain may be quantified using a sucrose density gradient method. Measurement of 146S particle concentrations as originally described by Fayet *et al*. (1971) and refined by others (Barteling and Meloen, 1974; Doel *et al*., 1981; Doel and Mowat, 1985) remains a precise and invaluable procedure in FMD vaccine production and is the single most useful parameter for the formulation of effective FMD vaccines.

FMD virus inactivation is an important step in the production of FMD vaccines. Previously, the FMD virus was inactivated with formaldehyde, which was later replaced by aziridine compounds (Brown *et al*., 1963). Binary ethyleneimine (BEI) is widely used nowadays as an inactivating agent (Bahnemann, 1973; Aarthi *et al*., 2004) by most FMD vaccine producers. Safety testis a mandatory test to ensure the absence of live virus in the final vaccine (B.P. Vet; I.P. Vet; WOAH, 2022).

Using BEI in a two-tank (vessel) system in accordance with good manufacturing practice (GMP), it is possible to meet the Pharmacopeia requirement of less than 1 infectious particle per 1000 liters of FMD antigen preparation. This criterion is inadequate to ensure successful inactivation; hence, it is appropriate to follow the stricter criteria suggested by Aarthi *et al*. (2004), wherein a single dose of 1.6 mM BEI can inactivate FMD viruses satisfactorily within 24 h. Inactivation kinetics and tissue culture amplification tests are commonly used by FMD vaccine producers to assess inactivation efficacy. Inactivation kinetics provide an indication of the expected time of inactivation and the order of inactivation. Validation of the inactivation process is an essential part of quality assurance. Stability of the 146S antigen to the inactivant is an important factor in selecting the new vaccine strain.

The antigen payload of the candidate vaccine strain may

be determined using the homologous cattle challenge test (Nagendrakumar *et al*., 2011; Madhanmohan *et al*., 2012). Briefly, experimental vaccines with different antigen payloads of the candidate vaccine strain formulated either with oil-in-water (O/W) or water-in-oil-in-water (W/O/W) adjuvant and a minimum of six FMD seronegative cattle calves per antigen payload are vaccinated by the appropriate route. Two FMD seronegative cattle calves are used as unvaccinated controls. Blood samples are collected on day 0 and 28 days post vaccination. Serum samples are subjected to serum neutralization test (WOAH, 2022). Further, vaccinated and unvaccinated control animals are challenged intra-dermolingually on days 21-28 post-vaccination with 10,000 bovine infective dose 50 (BID50) of the homologous challenge virus (Nagendrakumar*et al*., 2011; WOAH, 2022). The protection percentage is determined for each antigen payload. The optimal antigen payload may be determined by the percentage of protection. Alternatively, Probit regression analysis may be used to determine the optimum antigen payload for varying degrees of protection (50%, 80% or 100%) (Nagendrakumar *et al*., 2011).

The regular vaccine batch is prepared with an optimized antigen payload, either with an oil-in-water (O/W) adjuvant for ruminants or a water-in-oil-in-water (W/O/W) adjuvant for pigs and ruminants. The vaccine may be prepared in either monovalent or polyvalent formulation and subjected to safety, duration of immunogenicity, and potency tests in the target species,i.e., cattle (WOAH, 2022).

Safety test

A trial batch of vaccine needs to be tested in target species for safety by each recommended route of administration, and a minimum of 8 animals of each target species must be included in the safety test. Double-dose (e.g., two injections) and repeat-single-dose (after 14 days) tests using formulated vaccines containing the maximum permitted payload and number of antigens are recommended. The animals receive a total of three injections. The vaccinated animals are monitored for any untoward reactions (local) and systemic reactions not less than 14 days post each vaccination.Any undue reaction attributable to the vaccine should be assessed and may prevent its acceptance by the National Veterinary Authority (WOAH, 2022).

Potency test

The acceptable potency test for evaluating a new candidate vaccine strain will be the PD50 method, as described in the British Pharmacopoeia (BP) 2025, the European Pharmacopoeia (EP) 2022, and the Indian Pharmacopoeia (IP) 2022. Moreover, a validated indirect potency test can also be used. Alternative methods (such as VNT or LPB-ELISA) could be used if suitably validated (WOAH, 2022). Commonly used potency tests include the PD50 test and the protection against generalized foot infection (PGP) test (WOAH, 2022). The vaccine used in endemic countries should contain at least 3 PD50 per dose for cattle and protect at least 75% of vaccinated animals in a PGP test.Further, the National Veterinary Authority may decide to use indirect tests (such as VNT or LPB-ELISA), provided there is a correlation between antibody levels and protection against challenge with 10,000 BID50. Moreover, efficacy tests in other target species, such as sheep and goats (Madhanmohan *et al*., 2012) and water buffalo (*Bubalus bubalis*) (Madhanmohan *et al*., 2014), are reported earlier. Currently, the vaccine is being tested in cattle, and the data can be used for other susceptible species (WOAH, 2022).

Duration of immunity

Vaccine manufacturers need to provide duration-of-immunity data to regulators as part of the vaccine registration process. The duration of vaccine immunity in cattle can be demonstrated using either an animal challenge test or a serology method. Efficacy is usually tested at the end of the claimed period of protection (WOAH, 2022).

Purity

The purity of the vaccine may be determined by testing for antibodies against non-structural proteins (NSPs) (WOAH, 2022). WOAH (2022) described the recommended test method to test the purity of the FMD vaccine. As part of the registration procedure, vaccine manufacturers can test their vaccines using the recommended method (WOAH, 2022). If the tested vaccine dose does not elicit NSP antibodies in vaccinated animals, the vaccine manufacturers can claim in their product literature that their vaccine does not elicit NSP antibodies.

Stability

Vaccine manufacturers need to submit stability data for the vaccine as part of the licensing procedure.

Real-time stability tests and accelerated stability tests may be used to determine the vaccine's stability.

Finally, the development documents may be submitted to the relevant National Veterinary Authority for appropriate approval to use the selected vaccine strain in the field (WOAH, 2022).

Conclusions and Recommendations

In summary, selecting a suitable FMD vaccine strain is an important component of FMD control and is necessary for FMD control programmes in endemic regions, as well as for the establishment and maintenance of vaccine antigen reserves to be used in the event of new FMD incursions in FMD-free countries. There are invivo and invitro methods to determine the most appropriate vaccine to combat the disease. Successful identification of the appropriate vaccine strain and its incorporation into the vaccine will have a direct impact on disease control and on the vaccine manufacturers' confidence. The decision to change or include new strains in vaccine formulations is a multifaceted process, primarily based on experimental, epidemiological, and field observations. Developing the FMD vaccine strain may not be easy. It requires time and finances to conduct the tests required for a commercially viable product and to obtain regulatory approvals. Vaccine manufacturers will be hesitant to incorporate a new vaccine strain unless it is absolutely essential. The frequent changes of the FMD vaccine strain will be unacceptable to the FMD vaccine manufacturers. The use of well-known strains with broad antigenic coverage and a higher antigen payload has many advantages over attempts to change the vaccine strains based on limited molecular epidemiological studies.

Author's Contribution

All authors contriuted equally to the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The author have declared no conflict of interest.

References

Aarthi, D., Ananda Rao, K., Robinson, R. and Srinivasan, V.A., 2004. Validation of binary ethyleneimine (BEI) used as an inactivant for foot and mouth disease tissue culture vaccine. Biologicals, 32(3): 153-156. https://doi.org/10.1016/j.biologicals.2004.09.001

Alonso, F.A., Casas Olascoaga, R.C., Astudillo, V.M., Sondahl, M.S., Gomes, I. and Vianna Filho, Y.L., 1987. Updating of foot-and-mouth disease virus strains of epidemiological importance in South America. Biol. Centr. Panam. Fiebre Aftosa, 53: 11–18.

Arrowsmith, A.E., 1975. Variation among strains of type A foot-and-mouth disease virus in the Eastern Mediterranean region 1964-1972. J. Hyg. (Lond)., 75(3): 387-397. https://doi.org/10.1017/S0022172400024451

Azad, H.M., Srinivasan, V.A. and Crowther, J.R., 1995. Serological study of type A Indian foot-and-mouth disease virus isolates. Acta Virol., 39(4):193–196.

Bahnemann, H.G., 1973. The inactivation of foot and mouth disease virus by ethyleneimine and propyleneimine. Zbl. Vet. Med. B., 20(5): 356-360. https://doi.org/10.1111/j.1439-0450.1973.tb01136.x

Bari, F.D., Parida, S., Asfor, A.S., Haydon, D.T., Reeve, R., Paton, D.J. and Mahapatra, M., 2015. Prediction and characterization of novel epitopes of serotype A foot-and-mouth disease viruses circulating in East Africa using site-directed mutagenesis. J. Gen.Virol., 96 (Pt5): 1033-1041. https://doi.org/10.1099/vir.0.000051

Barteling, S.J. and Meloen, R.H., 1974. A simple method for the quantification of 140S particles of foot-and-mouth disease virus (FMDV). Arch. Gesamte.Virusforsch., 45(4): 362-364. https://doi.org/10.1007/BF01242879

Bastos, A.D., 1998. Detection and characterization of foot-and-mouth disease virus in sub-Saharan Africa. Onderstepoort. J. Vet. Res., 65(1): 37-47.

Beck, E. and Strohmaier, K., 1987. Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. J. Virol., 61(5): 1621-1629. https://doi.org/10.1128/jvi.61.5.1621-1629.1987

Bedson, S.P., Maitland, H.B. and Burbury, Y.M., 1927. Foot and-mouth disease research committee, 2nd report, HMSO, London, UK.

Belwal, L.M., Palanisamy, R., Nagaiah, K., Kalanidhi, A.P., Jagannatha, H.M., Ramanna, B.C. and Srinivasan, V.A., 1986. Serological study of foot and mouth disease virus type A in India. Rev. Sci. Tech., 5(3): 723-730. https://doi.org/10.20506/rst.5.3.264

Belwal, L.M., Srinivasan, V.A. and Kant, R., 1989. Strain differentiation of foot and mouth disease virus type Asia 1 isolates of Indian origin. Rev. Sci. Tech., 8(3): 771-778. https://doi.org/10.20506/rst.8.3.423

Bergmann, I.E., Malirat, V., Andrea Pedemonte, A. and Maradei, E., 2021. Challenges in foot-and-mouth disease virus strain selection as an input to attain broad vaccine intraserotype cross-protection. Expert. Rev. Vaccines., 20(1): 13-22. https://doi.org/10.1080/14760584.2021.1877137

Brehm, K.E., Kumar, N., Thulke, H.H. and Haas, B., 2008. High potency vaccines induce protection against heterologous challenge with foot-and-mouth disease virus. Vaccine, 26(13): 1681-1687. https://doi.org/10.1016/j.vaccine.2008.01.038

Brooksby, J.B., 1958. The virus of foot-and-mouth disease. Adv. Virus. Res., 5: 1–37. https://doi.org/10.1016/S0065-3527(08)60670-3

Brooksby, J.B., 1952. The technique of complement fixation in foot-and-mouth disease research. Agric. Res. Council Rep. Ser. No. 12.

Brooksby, J.B. and Rogers, J., 1957. Methods used in typing the virus of Foot-and mouth disease at Pirbright, 1950–55. In: Methods of typing and cultivation of foot-and-mouth disease virus: Project No. 208. Paris: European Productivity Agency of the Organization of European Cooperation (OEEC).

Brooksby, J.B., 1982. Portraits of viruses: Foot-and-mouth disease virus. Intervirology, 18(1-2): 1–23. https://doi.org/10.1159/000149299

Brown, F., Hyslop, N.S., Crick, J. and Morrow, A.W., 1963. The use of acetylethyleneimine in the production of inactivated foot-and-mouth disease vaccines. J. Hyg (Lond)., 61(3): 337-344. https://doi.org/10.1017/S0022172400039620

Capstick, P.B., Telling, R.C., Chapman, W.G. and Stewart, D.L., 1962. Growth of a cloned strain of hamster kidney cells in suspended cultures and their susceptibility to the virus of foot-and-mouth disease. Nature, 195: 1163-1164. https://doi.org/10.1038/1951163a0

Dahiya, S.S., Subramaniam, S., Biswal, J.K., Das, B., Prusty, B.R., Ali, S.Z., Khulape, S.A., Mohapatra, J.K. and Singh, R.K., 2021. Genetic characterization of foot-and-mouth disease virus serotype O isolates collected during 2014–2018 revealed dominance of O/ME–SA/Ind2001e and the emergence of a novel lineage in India. Transbound. Emerg. Dis., 68(6): 3498–3508. https://doi.org/10.1111/tbed.13954

Dahiya, S.S., Subramaniam, S., Mohapatra, J.K., Rout, M., Biswal, J.K., Giri, P., Nayak, V. and 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. https://doi.org/10.3390/v15071529

Davie, J., 1964. A complement fixation technique for the quantitative measurement of antigenic differences between strains of the virus of foot-and-mouth disease. J. Hyg (Lond)., 62(4): 401-411. https://doi.org/10.1017/S0022172400040146

Davies, V., Reeve, R., Harvey, W.T., Maree, F.F. and Husmeier, D., 2017. A sparse hierarchical Bayesian model for detecting relevant antigenic sites in virus evolution. Comput. Stat., 32: 803–843. https://doi.org/10.1007/s00180-017-0730-6

Dekker, A., Sanz-Bernardo, B., Singanallur, N.B., Ludi, A.B., Horsington, J., Eblé, P.L., King, D.P. and Vosloo, W., 2020. Cross-protection induced by a A/MAY/97 emergency vaccine against intra-serotype heterologous challenge with a foot-and-mouth disease virus from the A/ASIA/G-VII Lineage. Vaccines (Basel), 8(1): 24. https://doi.org/10.3390/vaccines8010024

Dhanda, M.R., Gopalakrishnan, V.R. and Dhillon, H.S., 1957. Note on the occurrence of atypical strains of foot-and-mouth disease virus in india. Indian J. Vet. Sci. Anim. Hus., 26-27: 79-84.

Doel, T.R. and Mowat, G.N., 1985. An international collaborative study on foot and mouth disease virus assay methods. Quantification of 146S particles. J. Biol. Stand., 13(4): 335-344. https://doi.org/10.1016/S0092-1157(85)80048-2

Doel, T.R., 2003. FMD vaccines. Virus Res., 91(1): 81-99. https://doi.org/10.1016/S0168-1702(02)00261-7

Doel, T.R., 2018. Classical FMD vaccines. FAO/EuFMD Open session 2018. October 29-31, 2018, Italy.

Doel, T.R., Fletton, B.W. and Staple, R.F., 1981. Further developments in the quantification of small RNA viruses by U.V. photometry by sucrose density gradients. Dev. Biol. Standard.,

50: 209-219.

Fayet, M.T., Fargeaud, D., Louisot, P., Stellman, C. and Roumiantzeff, M., 1971. Mesure physico-chimique des particules 140S du virus de la fie`vre aphteuse. Ann. Inst. Pasteur., 121(1): 107-118.

Ferris, N.P. and Donaldson, A.I., 1984. Serological response of guinea pigs to inactivated 146S antigens of foot and mouth disease virus after single or repeated inoculations. Rev. Sci. Tech., 3(3): 563-574. https://doi.org/10.20506/rst.3.3.173

Ferris, N.P. and Donaldson, A.I., 1992. The world reference laboratory for foot and mouth disease: A review of thirty-three years of activity (1958–1991). Rev. Sci. Tech. Off. Int. Epiz., 11(3): 657–684. https://doi.org/10.20506/rst.11.3.626

Fishbourne, E., Ludi, A.B., Wilsden, G., Hamblin, P., Statham, B., Bin-Tarif, A., Brocchi, E., Grazioli, S., Dekker, A., Eblé, P. and King, D.P., 2017. Efficacy of a high potency O_1 Manisa foot-and-mouth disease vaccine in cattle against heterologous challenge with a field virus from the O/ME-SA/Ind-2001 lineage collected in North Africa. Vaccine, 35(20): 2761-2765. https://doi.org/10.1016/j.vaccine.2017.02.047

Forman, A.J., 1974. A study of foot-and-mouth disease virus strains by complement fixation. I. A model for the fixation of complement by antigen/antibody mixtures. J. Hyg. (Lond.), 72(3): 397-405. https://doi.org/10.1017/S0022172400023639

Forman, A.J., 1975. The sub-type classification of strains of foot-and-mouth disease virus. J. Hyg. (Lond), 74(2): 227-232. https://doi.org/10.1017/S0022172400024281

Galdo Novo, S., Malirat, V., Maradei, E.D., Espinoza, A.M., Smitsaart, E., Pedemonte, A.R., Mattion, N. and Bergmann, I.E., 2017. Antigenic and immunogenic spectrum of foot-and-mouth disease vaccine strain O_1 Campos against representative viruses of topotypes that circulated in Asia over the past decade. Vaccine, 35(18): 2303-2307. https://doi.org/10.1016/j.vaccine.2017.03.026

Galloway, I.A., Henderson, W.M. and Brooksby J.B., 1948. Strains of the virus of foot-and-mouth disease recovered from outbreaks in Mexico. Proc. Soc. Exp. Biol. Med., 69(1): 57-63. https://doi.org/10.3181/00379727-69-16617

Graves, J.H., 1960a. The differentiation of subtypes (variants) of foot-and-mouth disease virus by serologic methods. I. Complement fixation test. Am. J. Vet. Res., 21: 687-690.

Horsington, J., Perez, C.B., Maradei, E., Novo, S.G., Gonzales, J.L., Singanallur, N.B., Bonastre, P. and Vosloo, W., 2017. Protective effects of high-potency FMDV O_1 Manisa monovalent vaccine in cattle challenged with FMDV O/SKR/2010 at 7 or 4 days post vaccination. Vaccine, 35(38): 5179-5185. https://doi.org/10.1016/j.vaccine.2017.07.102

Kärber, G., 1931. Beitrag zur kollektiven behandlung pharmakologischer reihenversuche. Archive. Für. Experimentelle. Pathologie. Pharmakol., 162: 480–483. https://doi.org/10.1007/BF01863914

Kim, T., Hong, J.K., Oem, J.K., Lee, K.N., Lee, H.S., Kim, Y.J., Ryoo, S., Ko, Y.J., Park, J.H., Choi, J., Lee, S.H., Jo, H.J., Lee, M.H., Kim, B. and Kim, J., 2019. Cross-protective efficacy of the O1 Manisa + O 3039 bivalent vaccine and the O 3039 monovalent vaccine against heterologous challenge with FMDV O/Jincheon/SKR/2014 in pig. Vaccine, 37(12): 1702-1709. https://doi.org/10.1016/j.vaccine.2018.11.080

Kitching, R.P., Rendle, R. and Ferris, N.P., 1988. Rapid correlation between field isolates and vaccine strains of foot-and-mouth disease virus. Vaccine, 6(5): 403-408. https://doi.org/10.1016/0264-410X(88)90139-9

Knight-Jones, T.J. and Rushton, J., 2013. The economic impacts of foot and mouth disease - what are they, how big are they and where do they occur? Prev. Vet. Med., 112(3-4): 161-173. https://doi.org/10.1016/j.prevetmed.2013.07.013

Knowles, N.J. and Samuel, A.R., 1995. Polymerase chain reaction amplification and cycle sequencing of the 1D gene of foot-and-mouth disease viruses. Session of the research group of the standing technical committee of the European commission for the control of foot-and-mouth disease. FAO, Rome, 19-22 September 1994, Vienna, Austria.

Knowles, N.J. and Samuel, A.R., 2003. Molecular epidemiology of foot-and-mouth disease virus. Virus Res., 91(1): 65-80. https://doi.org/10.1016/S0168-1702(02)00260-5

Knowles, N.J. and Sharma, G.K., 1990. A study of antigenic variants of foot and mouth disease virus type A in India between 1977 and 1985. Rev. Sci. Tech., 9(4): 1157-1168. https://doi.org/10.20506/rst.9.4.527

Knowles, N.J., Nazem Shirazi, M.H., Wadsworth,

J., Swabey, K.G., Stirling, J.M., Statham, R.J., Li, Y., Hutchings, G.H., Ferris, N.P., Parlak, U., Ozyörük, F., Sumption, K.J., King, D.P. and Paton, D.J., 2009. Recent spread of a new strain (A-Iran-05) of foot-and-mouth disease virus type A in the Middle East. Transbound. Emerg. Dis., 56(5): 157-169. https://doi.org/10.1111/j.1865-1682.2009.01074.x

Li, C., Hatta, M., Burke, D.F., Ping, J., Zhang, Y., Ozawa, M., Taft, A.S., Das, S.C., Hanson, A.P., Song, J., Imai, M., Wilker, P.R., Watanabe, T., Watanabe, S., Ito, M., Iwatsuki-Horimoto, K., Russell, C.A., James, S.L., Skepner, E., Maher, E.A., Neumann, G., Klimov, A.I., Kelso, A., McCauley, J., Wang, D., Shu, Y., Odagiri, T., Tashiro, M., Xu, X., Wentworth, D.E., Katz, J.M., Cox, N.J., Smith, D.J. and Kawaoka, Y., 2016. Selection of antigenically advanced variants of seasonal influenza viruses. Nat. Microbiol., 1(6): 16058. https://doi.org/10.1038/nmicrobiol.2016.58

Ludi, A.B., McLaws, M., Armson, B., Clark, J., Di Nardo, A., Parekh, K., Henstock, M., Muellner, P., Muellner, U.J., Rosso, F., Prada, J.M., Horton, D.L., Paton, D.J., Sumption, K. and King, D.P., 2022. Pragmatist: A tool to prioritize foot-and-mouth disease virus antigens held in vaccine banks. Front. Vet. Sci, 9: 1029075. https://doi.org/10.3389/fvets.2022.1029075

Macpherson, I. and Stoker, M., 1962. Polyoma transformation of hamster cell clones--an investigation of genetic factors affecting cell competence. Virology, 16: 147-151. https://doi.org/10.1016/0042-6822(62)90290-8

Madhanmohan, M., Nagendrakumar, S.B., Kumar, R., Anilkumar, J., Manikumar, K., Yuvaraj, S. and Srinivasan, V.A., 2012. Clinical protection, sub-clinical infection and persistence following vaccination with extinction payloads of O1 Manisa foot-and-mouth disease monovalent vaccine and challenge in goats and comparison with sheep. Res. Vet. Sci., 93(2): 1050-1059. https://doi.org/10.1016/j.rvsc.2011.10.006

Madhanmohan, M. and Srinivasan, V.A., 2012. Quality assurance of FMD vaccines in India. GFRA scientific workshop on surveillance, epidemiology, vaccination and control of foot-and-mouth disease. 17 to 19 April 2012, Hazyview, South Africa.

Madhanmohan, M., Yuvaraj, S., Nagendrakumar, S.B., Srinivasan, V.A., Gubbins, S., Paton, D.J. and Parida, S., 2014. Transmission of foot-and-mouth disease virus from experimentally infected Indian buffalo (*Bubalus bubalis*) to in-contact naïve and vaccinated Indian buffalo and cattle. Vaccine, 32(39): 5125-5130. https://doi.org/10.1016/j.vaccine.2014.03.094

Mahapatra, M. and Parida, S., 2018. Foot and mouth disease vaccine strain selection: Current approaches and future perspectives. Expert. Rev. Vaccines., 17(7): 577-591. https://doi.org/10.1080/14760584.2018.1492378

Mahapatra, M., Hamblin, P. and Paton, D.J., 2012. Foot-and-mouth disease virus epitope dominance in the antibody response of vaccinated animals. J. Gen. Virol., 93(Pt3): 488-493. https://doi.org/10.1099/vir.0.037952-0

Mahapatra, M., Statham, B., Li, Y., Hammond, J., Paton, D. and 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. https://doi.org/10.1016/j.vaccine.2016.02.057

Mahapatra, M., Upadhyaya, S., Aviso, S., Babu, A., Hutchings, G. and Parida, S., 2017. Selection of vaccine strains for serotype O foot-and-mouth disease viruses (2007-2012) circulating in Southeast Asia, East Asia and Far East. Vaccine, 35(51): 7147-7153. https://doi.org/10.1016/j.vaccine.2017.10.099

Mahapatra, M., Yuvaraj, S., Madhanmohan, M., Subramaniam, S., Pattnaik, B., Paton, D.J., Srinivasan, V.A. and Parida, S., 2015. Antigenic and genetic comparison of foot-and-mouth disease virus serotype O Indian vaccine strain, O/IND/R2/75 against currently circulating viruses. Vaccine, 33(5): 693-700. https://doi.org/10.1016/j.vaccine.2014.11.058

Maradei, E., Perez Beascoechea, C., Malirat, V., Salgado, G., Seki, C., Pedemonte, A., Bonastre, P., D'Aloia, R., La Torre, J.L., Mattion, N., Rodríguez Toledo, J. and Bergmann IE., 2011. Characterization of foot-and-mouth disease virus from outbreaks in Ecuador during 2009-2010 and cross-protection studies with the vaccine strain in use in the region. Vaccine, 29(46): 8230- 8240. https://doi.org/10.1016/j.vaccine.2011.08.120

Maree, F.F., Blignaut, B., Esterhuysen, J.J., de Beer, T.A.P., Theron, J., O'Neill, H.G. and Rieder, E., 2011. Predicting antigenic sites on the foot-and-mouth disease virus capsid of the South African

Territories types using virus neutralization data. J. Gen. Virol., 92(Pt 10): 2297-2309. https://doi.org/10.1099/vir.0.032839-0

Mason, P.W., Pacheco, J.M., Zhao, Q.Z. and Knowles, N.J., 2003. Comparisons of the complete genomes of Asian, African and European isolates of a recent foot-and-mouth disease virus type O pandemic strain (PanAsia). J. Gen. Virol., 84(Pt6): 1583-1593. https://doi.org/10.1099/vir.0.18669-0

Mattion, N., Goris, N., Willems, T., Robiolo, B., Maradei, E., Beascoechea, C.P., Perez, A., Smitsaart, E., Fondevila, N., Palma, E., De Clercq, K. and La Torre, J., 2009. Some guidelines for determining foot-and-mouth disease vaccine strain matching by serology. Vaccine, 27(5): 741-747. https://doi.org/10.1016/j.vaccine.2008.11.026

Mohapatra, J.K., Das, B., Rout, M., Sreenivasa, B.P., Subramaniam, S., Sanyal, A. and Pattnaik, B., 2018. Alternate vaccine strain selection in the wake of emerging foot-and-mouth disease virus serotype A antigenic variants in India. Vaccine, 36(23): 3191-3194. https://doi.org/10.1016/j.vaccine.2018.04.090

Mowat, G.N. and Chapman, W.G., 1962. Growth of foot and mouth disease virus in a fibroblastic cell line derived from hamster kidneys. Nature, 194: 253-/255. https://doi.org/10.1038/194253a0

Nagendrakumar, S.B., Srinivasan, V.A., Madhanmohan, M., Yuvaraj, S., Parida, S., Di Nardo, A., Horsington, J. and Paton, D.J., 2011. Evaluation of cross-protection between O1 Manisa and O1 Campos in cattle vaccinated with foot-and-mouth disease virus vaccine incorporating different payloads of inactivated O1 Manisa antigen. Vaccine, 29(10): 1906-1912. https://doi.org/10.1016/j.vaccine.2010.12.127

Naranjo, J. andCosivi, O., 2013. Elimination of foot-and-mouth disease in South America: lessons and challenges. Philos. Trans. R. Soc. Lond. B. Biol. Sci., 368(1623): 20120381. https://doi.org/10.1098/rstb.2012.0381

Ndiritu, C.G., Ouldridge, E.J., Head, M. and Rweyemamu, M.M., 1983. A serological evaluation of 1979-1982 Kenyan foot-and-mouth disease type SAT 2 viruses. J. Hyg. (Lond.), 91(2): 335-341. https://doi.org/10.1017/S0022172400060356

Paton, D,J., Di Nardo. A., Knowles, N.J., Wadsworth, J., Pituco, E.M., Cosivi, O., Rivera, A.M., Kassimi, L.B., Brocchi, E., de Clercq, K., Carrillo, C., Maree, F.F., Singh, R.K., Vosloo, W., Park, M.K., Sumption, K.J., Ludi, A.B. and King, D.P., 2021. The history of foot-and-mouth disease virus serotype C: The first known extinct serotype? Virus. Evol., 7(1): veab009. https://doi.org/10.1093/ve/veab009

Paton, D.J., Reeve, R., Capozzo, A.V. and Ludi, A., 2019. Estimating the protection afforded by foot-and-mouth disease vaccines in the laboratory. Vaccine, 37(37): 5515-5524. https://doi.org/10.1016/j.vaccine.2019.07.102

Pereira, H.G., 1977. Subtyping of foot-and-mouth disease virus. International Symposium on Foot-and-Mouth Disease. Lyon, 1976. Dev. Biol. Stand., 35: 167-174.

Rahman, T., Mahapatra, M., Laing, E. and Jin, Y., 2015. Evolutionary non-linear modelling for selecting vaccines against antigenically variable viruses. Bioinformatics, 31(6): 834-840. https://doi.org/10.1093/bioinformatics/btu768

Reeve, R., Blignaut, B., Esterhuysen, J.J., Opperman, P., Matthews, L., Fry, E.E., de Beer, T.A., Theron, J., Rieder, E., Vosloo, W., O'Neill, H.G., Haydon, D.T. and Maree, F.F., 2010. Sequence-based prediction for vaccine strain selection and identification of antigenic variability in foot-and-mouth disease virus. PLoS Comput. Biol., 6(12): e1001027. https://doi.org/10.1371/journal.pcbi.1001027

Reeve, R., Borley, D.W., Maree, F.F., Upadhyaya, S., Lukhwareni, A., Esterhuysen, J.J., Harvey, W.T., Blignaut, B., Fry, E.E., Parida, S., Paton, D.J. and Mahapatra, M., 2016. Tracking the antigenic evolution of foot-and-mouth disease virus. PLoS One, 11(7): e0159360. https://doi.org/10.1371/journal.pone.0159360

Rweyemamu, M.M., Pay, T.W.F. and Parker, M.J., 1977. Serological differentiation of foot-and-mouth disease virus strains in relation to selection of suitable vaccine viruses. International symposium on foot-and-mouth disease. Lyon, 1976. Dev. Biol. Stand., 35: 205-214.

Rweyemamu, M.M., Ouldridge, E.J., Head, M. and Ferrari, R., 1984. The effect of antiserum quality on strain specificity assessment of foot and mouth disease virus by the neutralization reaction. J. Biol. Stand., 12(3): 295-303. https://doi.org/10.1016/S0092-1157(84)80009-8

Rweyemamu, M.M. and Ouldridge, E.J., 1982.

Serological analysis of recent type O foot-and-mouth disease virus isolates from Europe. Vet. Rec., 111(8): 63-65. https://doi.org/10.1136/vr.111.8.163

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. J. Hyg. (Lond.), 81(1): 107-123. https://doi.org/10.1017/S002217240005381X

Rweyemamu, M.M., Ouldridge, E.J., Head, M. and Purse, F., 1984a. Evaluation of the antigenic variation within type-A foot and mouth disease virus isolates from Asia. J. Biol. Stand., 12(2): 191-194. https://doi.org/10.1016/S0092-1157(84)80053-0

Samuel, A.R., Knowles, N.J. and Kitching, R.P., 1988. Serological and biochemical analysis of some recent type A foot-and-mouth disease virus isolates from the Middle East. Epidemiol. Infect., 101(3): 577-590. https://doi.org/10.1017/S0950268800029447

Singanallur, N.B., Dekker, A., Eblé, P.L., van Hemert-Kluitenberg, F., Weerdmeester, K., Horsington, J. and Vosloo, W.W., 2020. Emergency foot-and-mouth disease vaccines A malaysia 97 and A_{22} Iraq 64 Offer Good Protection against Heterologous Challenge with A Variant Serotype A ASIA/G-IX/SEA-97 Lineage Virus. Vaccines, 8(1): 80. https://doi.org/10.3390/vaccines8010080

Srinivasan, V.A., 2021. FMD vaccine and vaccination In India: Production, use and quality. FAO-ICAR International conference on scientific developments and technical challenges in the progressive control of Foot-and-Mouth Disease in South Asia. February 13-15, 2012, New Delhi.

Srinivasan, V.A., Ouldridge, E.J., Head, M. and Rweyemamu, M.M., 1983. A serological study of Indian type O foot and mouth disease virus isolates. Rev. Sci. Tech., 2(1): 145-151. https://doi.org/10.20506/rst.2.1.110

Subramaniam, S., Mohapatra, J.K., Sahoo, N.R., Sahoo, A.P., Dahiya, S.S., Rout, M., Biswal, J.K., Ashok, K.S., Mallick, S., Ranjan, R., Jana, C. and Singh, R.P., 2022. Foot-and-mouth disease status in India during the second decade of the twenty-first century (2011-2020). Vet. Res. Commun., 46(4): 1011-1022. https://doi.org/10.1007/s11259-022-10010-z

Subramaniam, S., Mohapatra, J.K., Sharma, G.K., Biswal, J.K., Ranjan, R., Rout, M., Das, B., Dash, B.B., Sanyal, A. and Pattnaik, B., 2015. Evolutionary dynamics of foot-and-mouth disease virus O/ME-SA/Ind2001 lineage. Vet. Microbiol., 178(3-4): 181-189. https://doi.org/10.1016/j.vetmic.2015.05.015

Telling, R.C. and Elsworth, R., 1965. Submerged culture of hamster kidney cells in a stainless steel vessel. Biotechnol. Bioeng., 7: 417-434. https://doi.org/10.1002/bit.260070309

Thomson, G.R. and Bastos, A.D.S., 2005. Foot-and-mouth disease. In: (eds. J.A.W. Coetzer and R.C. Tustin) infectious diseases of livestock, Vol 2, Oxford: Oxford University Press. Tully, D. C., and Fares, pp. 1324–1365.

Traub, E. and Mohalman, H., 1943. Foot-and-mouth disease typing with complement fixation test first communication. Experiment with serum and antigen of guinea pigs. Zentbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig., 150: 289–300.

Upadhyaya, S., Ayelet, G., Paul, G., King, D.P., Paton, D.J. and Mahapatra, M., 2014. Genetic basis of antigenic variation in foot-and-mouth disease serotype A viruses from the Middle East. Vaccine, 32(5): 631-638. https://doi.org/10.1016/j.vaccine.2013.08.102

Valle´e, H. and Carre, H., 1922. Sur la Pluralite´ du Virus Aphteux'Comptes Rendus de L'Acade´mie Des Sciences, French, 174: 1498–500.

Waldmann, O. and Trautwein, K., 1926. Experimentalle Untersuchungen Ueber Die Pluralitet Des Maul-Und Klauenseuche Virus', Berlin TierarztlWochenschr, 42: 569–571.

Wilna, V., Hong, N.T., Geoffrey, F.T., Jacqueline, M.M., Jianning, W., Van Phuc, K., Ngon, Q.V., Phuong le, T.T., Hung, D., Hanh, T.X., Van Hung, V., Anh le, T.Q., Tien, M.T., Quang, le, T.V., Long, N.T. and Nagendrakumar, S.B., 2015. Efficacy of a high potency O1 Manisa monovalent vaccine against heterolgous challenge with a FMDV O Mya98 lineage virus in pigs 4 and 7 days post vaccination. Vaccine, 33(24): 2778-2785. https://doi.org/10.1016/j.vaccine.2015.04.045

WOAH Terrestrial Animal Health Code. 2022. Chapter 3.1.8. Infection with foot and mouth disease virus. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.08_

FMD.pdf accessed 17 September 2023.

WRL-FMD Laboratory Network Report, 2023. Annual report 2023 in Donald King, Antonello Di Nardo and Mark Henstock (eds), The Pirbright Institute, UK.

WRL-FMD Laboratory Network Report, 2025. Quarterly report July-September 2025' in Donald King, Antonello Di Nardo and Mark Henstock (eds), The Pirbright Institute, UK.

Yuvaraj, S., Madhanmohan, M., Nagendrakumar, S.B., Kumar, R., Subramanian, B.M., Mohapatra, J.K., Sanyal, A., Pattnaik, B. and Srinivasan, V.A., 2013. Genetic and antigenic characterization of Indian foot-and-mouth disease virus serotype O isolates collected during the period 2001 to 2012. Infect. Genet. Evol., 13: 109-115. https://doi.org/10.1016/j.meegid.2012.10.004