



# **WOAH/FAO Foot-and-Mouth Disease Reference Laboratory Network**

## **Annual Report 2023**

### **Editors:**

Donald King, Antonello Di Nardo

and Mark Henstock

The Pirbright Institute, UK

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*Sudan and South Sudan: Final boundary between the Republic of Sudan and the Republic of South Sudan has not yet been determined.*

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## Contents

<b>1</b>	<b>WOAH/FAO FMD Reference Laboratory Network</b>	<b>3</b>
1.1	<i>Principle Goals.....</i>	3
1.2	<i>Reporting Period.....</i>	4
1.3	<i>Collated input from.....</i>	4
<b>2</b>	<b>Global distribution and impact of foot-and-mouth disease</b>	<b>6</b>
2.1	<i>Introduction .....</i>	6
2.2	<i>Overview of the activities of the WOA/FAO FMD Laboratory Network during 2023 .....</i>	10
2.3	<i>Regional distribution of different FMD viral lineages .....</i>	13
2.4	<i>Vaccine matching and recommendations .....</i>	17
<b>3</b>	<b>Overview of Network surveillance activities in each of the regional endemic pools</b>	<b>18</b>
3.1	<i>Pool 1 Regional synopsis .....</i>	18
3.2	<i>Pool 2 Regional synopsis .....</i>	19
3.3	<i>Pool 3 Regional synopsis .....</i>	21
3.4	<i>Pool 4 Regional synopsis .....</i>	23
3.5	<i>Pool 5 Regional synopsis .....</i>	25
3.6	<i>Pool 6 Regional synopsis .....</i>	27
3.7	<i>Pool 7 Regional synopsis .....</i>	28
<b>4</b>	<b>Improving the quality of laboratory tests from FMD reference laboratories</b>	<b>30</b>
4.1	<i>Proficiency testing schemes (PTS) organised by the Network Partners.....</i>	30
4.2	<i>Supply of reagents.....</i>	32
4.3	<i>Training courses organised by Network partners.....</i>	37
4.4	<i>Collaborative projects.....</i>	41
4.5	<i>In vivo potency studies undertaken during 2021 .....</i>	49
	<b>Appendix 1 Details of clinical samples from field cases from countries in FMDV endemic regions tested during 2023</b>	<b>52</b>
	<b>Appendix 2 Vaccine matching studies undertaken by Network partners during 2023</b>	<b>54</b>
	<b>Appendix 3 Nucleotide sequence analysis</b>	<b>59</b>
	<b>Appendix 4 Selected phylogenetic trees for 2023</b>	<b>61</b>
	<b>Appendix 5 The 18<sup>th</sup> Annual Meeting of the WOA/FAO FMD Reference Laboratory Network</b>	<b>69</b>

# 1 WOAHO/FAO FMD Reference Laboratory Network

## 1.1 Principle Goals

The Network of WOAHO/FAO FMD Reference Laboratories has been established with two principal goals:

1) To understand global virus distribution patterns and use these data to inform vaccine recommendations

and

2) To harmonise and improve the quality of laboratory testing carried out by international and national reference laboratories.

These activities require sharing and joint evaluation of surveillance information from laboratory diagnosis, serotyping, genetic characterisation and vaccine matching tests and harmonisation of standards for diagnostic procedures.

This report is divided into two parts providing an update on progress towards each of these goals.

## 1.2 Reporting Period

1<sup>st</sup> January 2023 - 31<sup>st</sup> December 2023

## 1.3 Collated input from

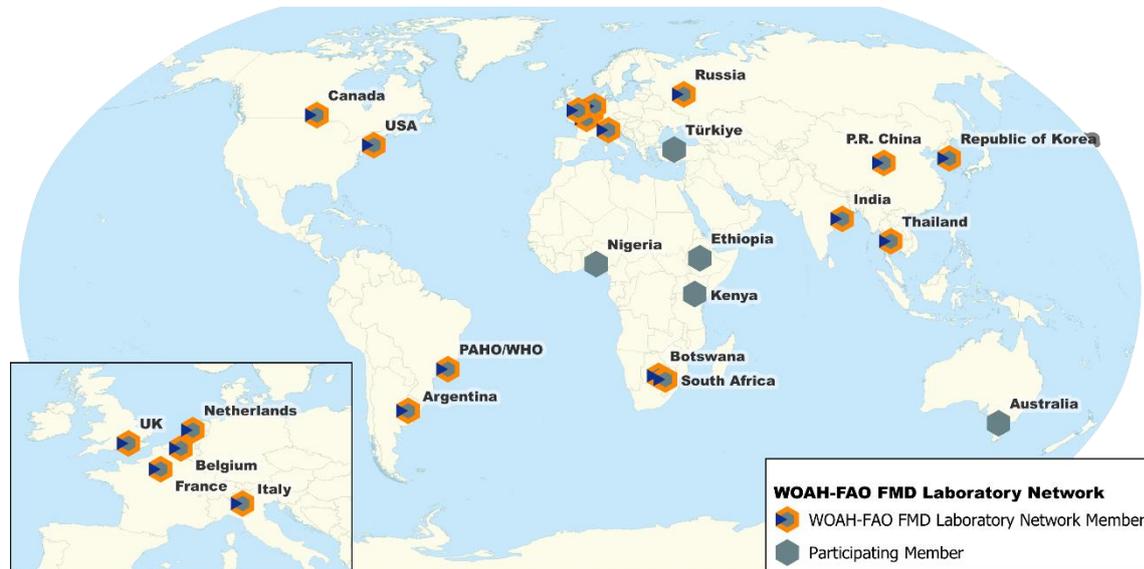


Figure 1-1: Participating laboratories

 WOAHO Reference Laboratory for Foot and Mouth Disease Dirección de Laboratorio Animal, SENASA, Argentina	 WOAHO collaborating centre for validation, quality assessment and quality control of diagnostic assays and vaccine testing for vesicular diseases in Europe, and FAO Reference Centre for Vesicular Diseases Sciensano, Ukkel, Belgium
 WOAHO Reference Laboratory for Foot and Mouth Disease BVI, Gaborone, Botswana	 Centro Panamericano de Fiebre Aftosa y Salud Pública Veterinaria (PANAFTOSA/VPH) and WOAHO Reference Laboratory for FMD Rio de Janeiro, Brazil
 WOAHO / FAO FMD Reference Laboratory National Centre for Foreign Animal Disease National Centres for Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada	 WOAHO and China National FMD Reference Laboratory Lanzhou Veterinary Research Institute (LVRI), CAAS, Gansu, People's Republic of China
 WOAHO / FAO FMD Reference Laboratory French Agency for Food and, Environmental and Occupational Health & Safety (ANSES), Maisons-Alfort, Paris, France	 FAO Reference Centre for FMD ICAR – National Institute on FMD, International Centre for Foot and Mouth Disease, Aragul, Bhubaneswar, India

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WOAH / FAO FMD Reference Laboratory



Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Italy

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FAO FMD vaccine quality control center



Wageningen Bioveterinary Research (WBVR), Lelystad, The Netherlands

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FAO Reference Laboratory for FMD in Africa and WOAHO FMD Reference Laboratory



Transboundary Animal Diseases Programme, ARC-Onderstepoort Veterinary Institute (ARC-OVI), South Africa

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FAO World Reference Laboratory for Foot-and-Mouth Disease and WOAHO FMD Reference Laboratory



The Pirbright Institute Pirbright, Surrey, United Kingdom

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WOAH Reference Laboratory for Foot and Mouth Disease



Animal and Plant Quarantine Agency (QIA), Anyang city, Gyeonggi-do, Republic of Korea

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FAO Reference Centre for FMD for Central Asia and West Eurasia and WOAHO Reference Laboratory for FMD



Federal Governmental Institute, Centre for Animal Health (FGI ARRIAH), Vladimir, Russian Federation

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Regional Reference Laboratory for Foot and Mouth Disease in Southeast Asia (RRLSEA)



Department of Livestock Development, Pakchong, Thailand

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FAO Reference Centre for FMD and other vesicular diseases for the Americas and the Caribbean and WOAHO FMD Reference Laboratory



Foreign Animal Disease Diagnostic Lab, Plum Island Animal Disease Center (PIADC), Greenport, United States of America

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Additional input kindly supplied by:

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Australian Centre for Disease Preparedness (ACDP)



Geelong, Australia

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Foot and Mouth Disease Laboratory



Embakasi, Kenya

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Şap Institute (and WELNET FMD)



Ankara, Türkiye

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Animal Health Institute (AHI), Ministry of Agriculture



Sebeta, Ethiopia

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National Veterinary Research Institute



Vom, Plateau State, Nigeria

## 2 Global distribution and impact of foot-and-mouth disease

Foot-and-mouth disease (FMD) is a highly contagious viral disease that infects a wide variety of domestic and wild cloven-hooved hosts. Its presence impacts upon rural livelihoods and restricts trade opportunities for countries where the disease is endemic and poses a constant threat to those countries that are free of the disease. In endemic countries, the economic costs associated with FMD are estimated to be US\$6.5–21 billion annually, with outbreaks in FMD-free countries and zones potentially causing economic losses of >\$1.5 billion. FMD virus lineages are not randomly dispersed throughout the world but are associated with particular ecological niches. The distribution of these FMD virus lineages is affected by cyclical upsurges in the prevalence of particular strains that may be associated with the evolution of FMD viruses to escape protective immunity in susceptible livestock populations and/or opportunities presented by movements of animals and their products. These features can give rise to pandemic events where FMDV lineages spread widely to affect new regions.

Global surveillance for FMD is necessary to identify the current hazards and to predict heightened risk so that appropriate diagnostic tools and vaccines are available for detection and control. This requires sustained effort directed towards the monitoring of FMD outbreaks and ideally also of FMDV circulation and persistence, along with collection and characterisation of FMD viruses and integration of findings with associated epidemiological intelligence. Such an extensive effort requires a coordinated approach encompassing national and international disease laboratories of the **WOAH/FAO FMD Laboratory Network** ([www.foot-and-mouth.org](http://www.foot-and-mouth.org)) along with partnering laboratories, commercial vaccine and diagnostic providers. The worldwide distribution of the different serotypes and variants of FMD virus (as compiled in 2023) and the associated activities of the Network laboratories are presented in this report.

### 2.1 Introduction

Global surveillance undertaken by the WOA/FAO FMD Laboratory Network aims to monitor the distribution of FMD viruses to predict risk for endemic and FMD-free countries. FMDV is unevenly distributed throughout the world reflecting factors such as livestock density and species mix, patterns of husbandry, animal movement and trade, wildlife reservoirs and incentives and capacities for disease control. The virus exists as seven serotypes and multiple subtypes where cross-immunity is absent or incomplete. The situation is dynamic and complex and affected by viral evolution, waxing and waning of host immunity and changing ecosystems and trading patterns. Despite the opportunities for spread of FMDV into new regions, viruses tend to recur in the same parts of the world, presumably reflecting some degree of either ecological isolation or adaptation. On this basis, the global pool of FMD viruses can be subdivided into seven 'regional pools' in which genetically and antigenically distinctive virus strains tend to occur within a defined region.

The seven 'Regional Pools' referred to throughout this report are shown below (Figure 2-1) and represent:

Pool 1	Southeast Asia with spill over into Eastern Asia
Pool 2	Southern Asia
Pool 3	Western Asia with spill over into North Africa
Pool 4	Eastern Africa with spill over into North Africa
Pool 5	Western Africa
Pool 6	Southern Africa
Pool 7	South America

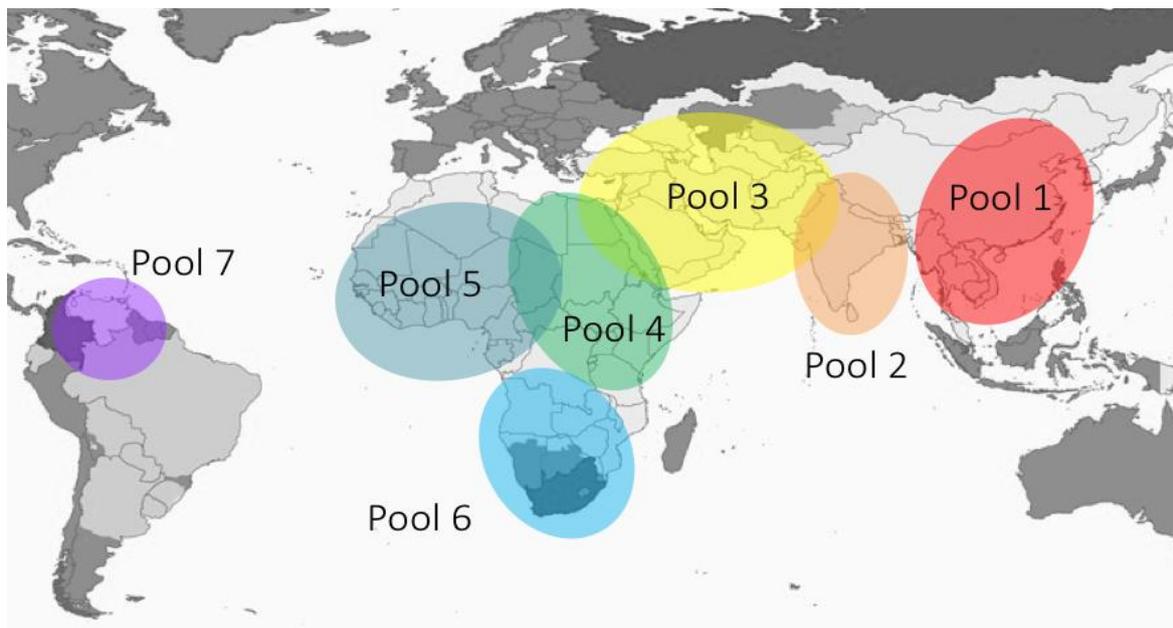


Figure 2-1: Distribution of the seven endemic pools of FMD. Periodically, viruses spread between pools and to free regions, and countries at the interfaces between pools (such as in North Africa and Central Asia) often experience FMD outbreaks from different regional sources. Note on Pools 4-6: In Africa there are currently three FMD virus pools loosely defined as covering East Africa (pool 4), West Africa (pool 5) and Southern Africa (pool 6). A map describing the official WOAH status for these countries can be found at: <https://www.woah.org/en/what-we-do/animal-health-and-welfare/official-disease-status/>

The clustering of FMD viruses into 7 virus pools, with 3 pools covering West Eurasia, South Asia and Southeast Asia, 3 pools covering East, West and Southern Africa and 1 pool covering South America, enables a targeted approach to be applied to the 'Progressive Global Control of FMD' initiative overseen by the WOAH and FAO and for which the Network laboratories will play a pivotal role.

### Overview of the Global situation in 2023

Headline events (Figure2-2) in 2023 include:

- Emergence of the SAT2/XIV topotype in Iraq, Jordan and Türkiye (the first reports of the SAT 2 serotype in these countries) as well as in Bahrain and Oman. Clinical disease in large ruminants was reported to be severe, with higher-than-expected mortality levels in older animals being reported. Analyses of sequences collected from

these cases confirm that they are caused by viruses from the SAT 2/XIV toptotype which are closely related to viruses collected from Ethiopia during 2022.

- The unexpected detection of SAT1/I in Qatar that was most closely related to a Kenyan isolate reinforces the epidemiological connectivity between East Africa and Pool 3. A further outbreak due to SAT 1/I was detected in the Comoros.
- Detection of SAT2/V toptotype viruses as the cause of FMD outbreaks in Algeria (in December 2023).
- Evidence for a new incursion of the O/EA-3 toptotype into Libya which appears to be different to the viruses that caused outbreaks in 2018 and 2022.
- The distribution of the O/ME-SA/Ind-2001e lineage is widespread in Pool 1, where it caused new FMD outbreaks in the Republic of Korea.
- The O/ME-SA/PanAsia-2<sup>ANT-10</sup> has continued to circulate in Pool 3 (East Mediterranean).
- Asia 1 viruses detected in Pool 2 including the first detection of genotype IX in India.

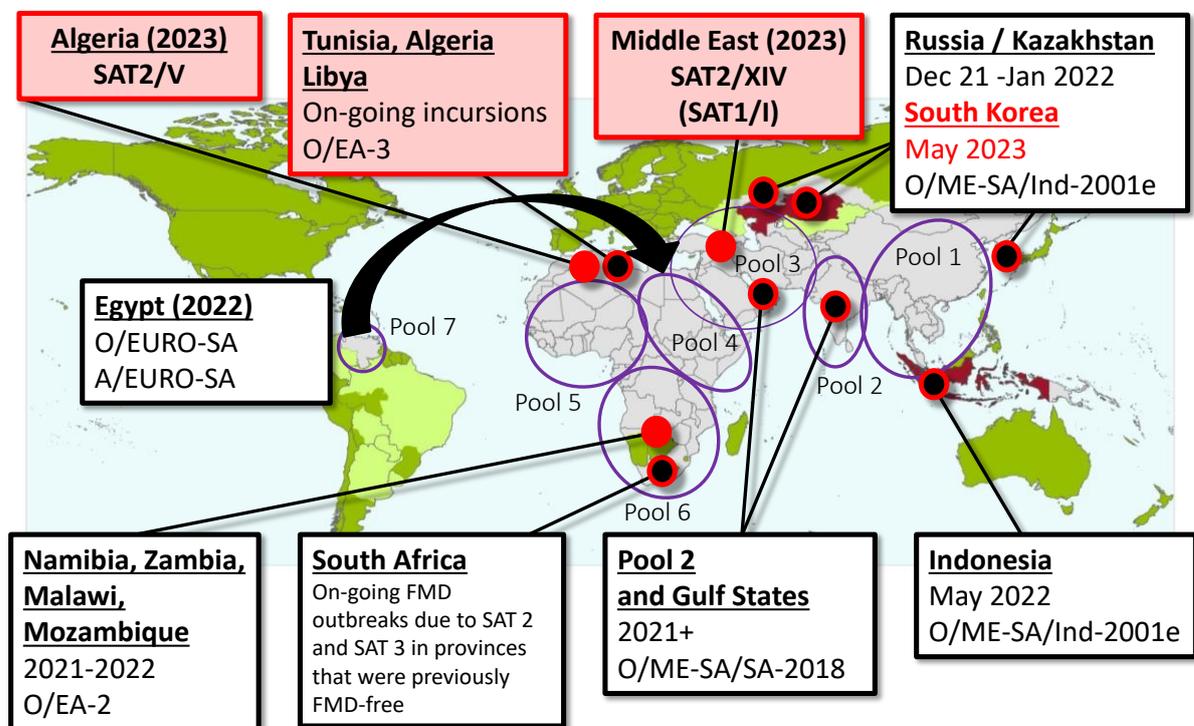


Figure 2-2: Headline FMD events for 2023 (events during 2023 are highlighted in red)

Specific information regarding contemporary FMD outbreaks can be found on the World Animal Health Information System (WAHIS) located on the WOAH website (<https://wahis.woah.org/#/home>), as well as the EMPRES-i+ Global Animal Disease Information System (<https://empres-i.apps.fao.org/>) provided by FAO. Further supplementary data and updates are provided in the WRLFMD/EuFMD Quarterly Report for FMD (<https://www.wrlfmd.org/ref-lab-reports>).

During 2023, FMD outbreaks have continued to affect countries in the established endemic regions of the world. Particular attention has been focussed upon new FMD outbreaks and

events that have occurred at the margins of these endemic regions (reported on the WOAHS WAHIS Interface: <https://wahis.woah.org/#/home>, summarised in Figure 2-3, Table 2-1 and described elsewhere in this report). Further details of many of the characterisation of viruses retrieved from these outbreaks are provided later in this report.

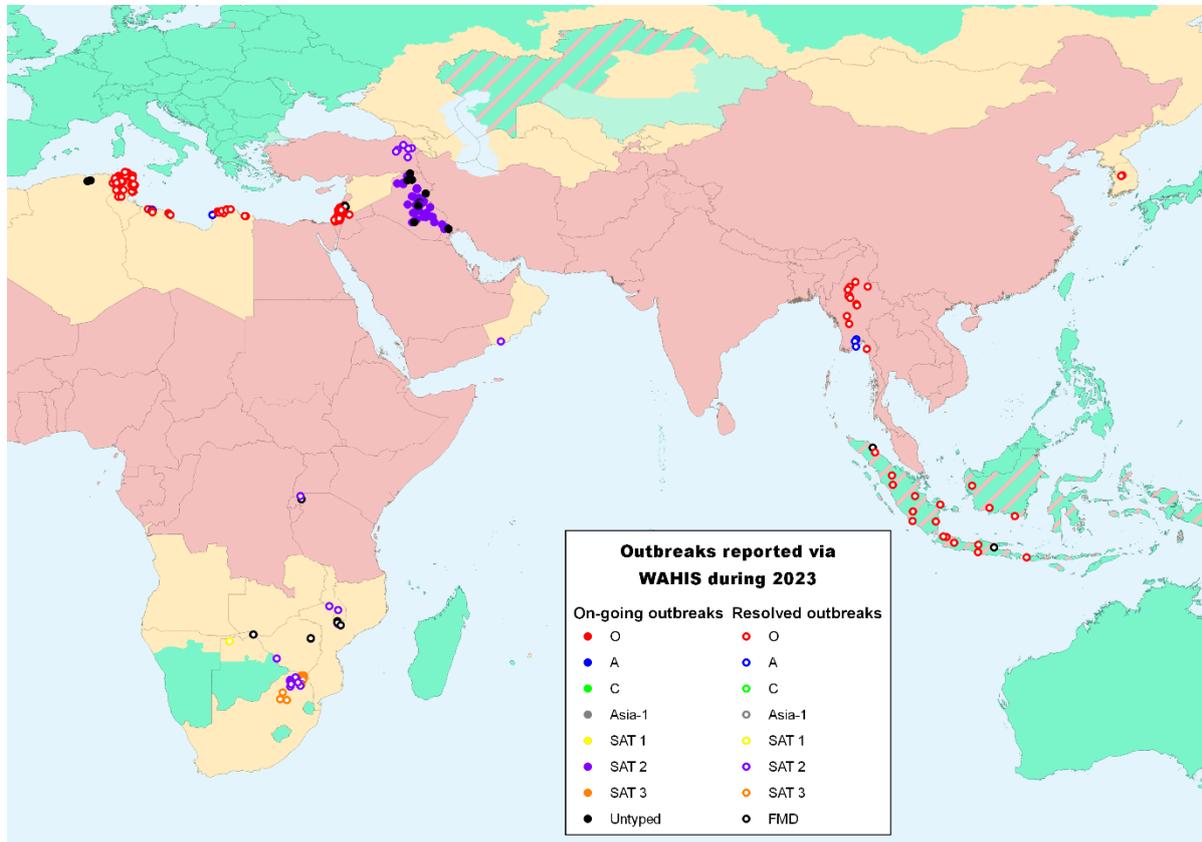


Figure 2-3: Map indicating the location of significant epidemiological events and disease outbreaks reported to WOAHS in immediate notifications or follow-up reports in 2023 (data, available from: <https://wahis.woah.org/#/home>, downloaded on 14 May 2024)

**Table 2-1:** New FMD outbreaks reported to WOAHS during 2023 (data retrieved from WAHIS on [www.oie.int](http://www.oie.int) on 14<sup>h</sup> May 2024).

Country	New outbreaks	Susceptible	Cases	Number of Animals			
				Killed and disposed of	Slaughtered	Deaths	Vaccinated
Algeria	2	187	10	0	0	0	0
Cambodia	3	1015	111	0	0	2	0
China (People's Rep. of)	4	282	22	282	0	0	0
Egypt	12	1296	17	0	0	1	108401

Country	Number of Animals						
	New outbreaks	Susceptible	Cases	Killed and disposed of	Slaughtered	Deaths	Vaccinated
Ethiopia	41	1486132	34318	0	11	121	16960
Indonesia	4126	0	19007	0	0	0	0
Iraq	52	51913	28406	0	0	261	0
Israel	1	920	1	0	0	0	0
Jordan	2	19000	11433	0	0	381	78600
Kenya	14	509	40	0	0	8	0
Korea (Rep. of)	11	1571	33	1571	0	0	0
Libya	22	14436	1159	0	0	211	0
Malawi	3	19714	75	0	0	0	9894
Malaysia	7	602	88	0	0	0	0
Nepal	15	59737	1364	0	0	20	55483
Nigeria	24	4345	1994	0	20	17	0
Oman	1	5100	23	0	0	0	5100
Pakistan	272	19629	1308	0	0	34	33920
Palestine	4	231	49	0	0	28	12
Rwanda	1	43178	197	0	197	0	43178
Saudi Arabia	129	27750	794	0	0	159	0
Somalia	3	1714	15	0	0	2	0
South Africa	13	6977	74	0	0	0	0
Sudan	2	186	82	0	0	0	0
Thailand	10	6631	722	0	0	23	0
Tunisia	16	1694	187	0	0	12	4201
Türkiye (Rep. of)	200	140694	6799	0	0	798	1276440
Uganda	0	18375	84	0	0	0	12714
United Arab Emirates	2	1900	5	5	0	0	0

## 2.2 Overview of the activities of the WOA/FAO FMD Laboratory Network during 2023

The WOA/FAO FMD Reference Laboratory Network provides important support to the global control of FMD and provides opportunities and expertise for developing and sustaining laboratory capacity and capability, exchange of materials and technologies, harmonising approaches to diagnosis and supporting complementary research. Laboratories within the Network regularly receive samples for FMD diagnosis from many parts of the world. The *in*

*vitro* antigenic properties of selected isolates are assessed for vaccine matching and nucleotide sequencing allows precise characterisation of new isolates and tracing of their origin by comparison with viruses held in virus collections. This analysis assists the monitoring of the 'real time' emergence and spread of FMD virus globally.

1402 clinical samples from suspect cases of FMD were tested by laboratories in the Network (and associated laboratories) during 2023. These samples were collected from 34 countries from all seven FMD endemic pools (Figure 2-4). **However, sampling within these pools is not equivalent:** and efforts are currently underway with the Network to improve sample collection in regions where sampling is under-represented.

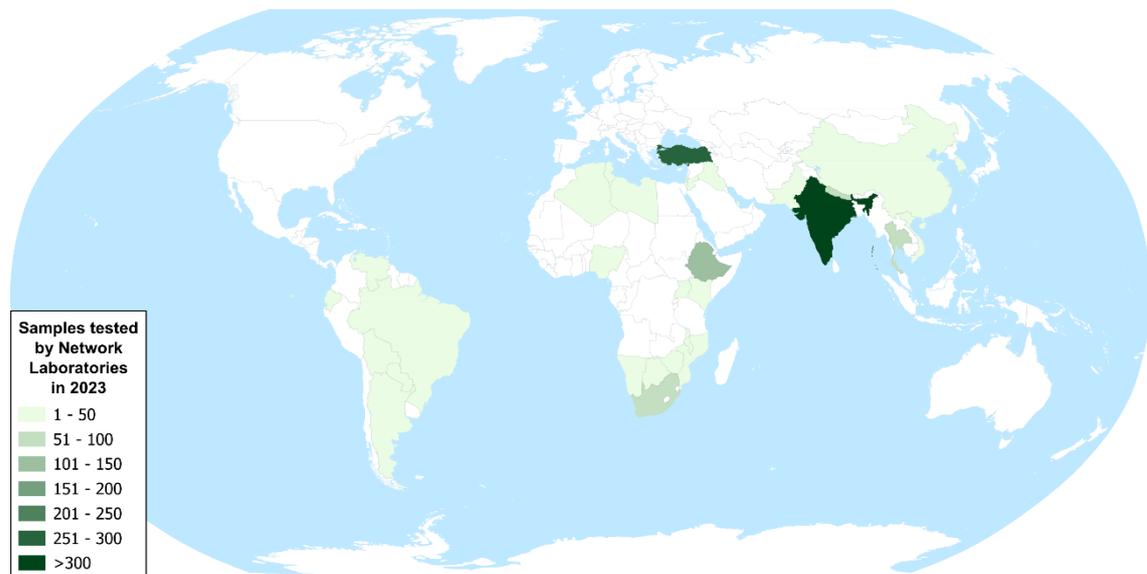
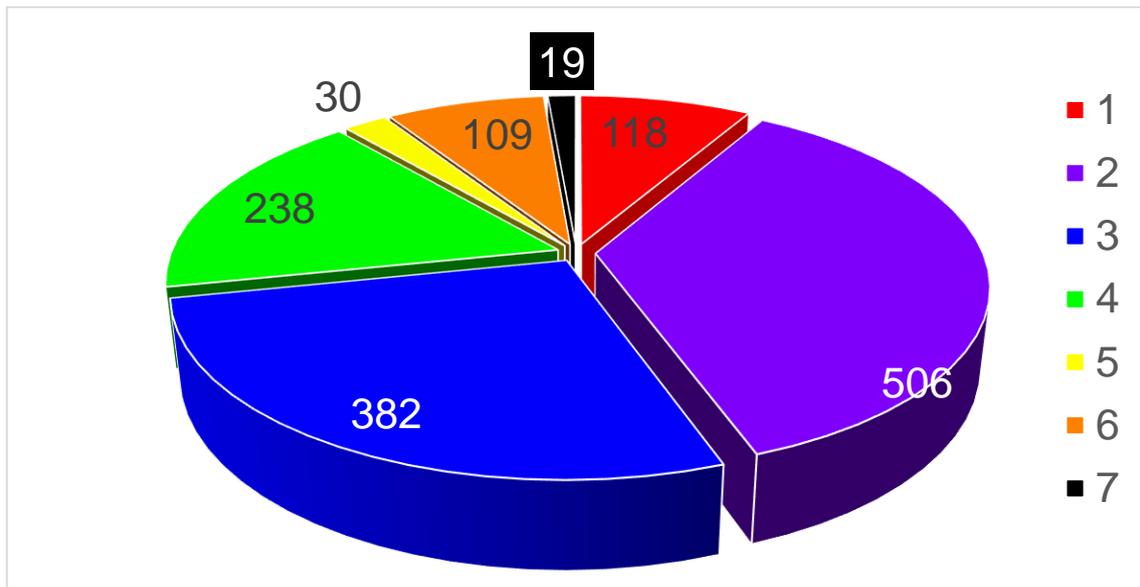
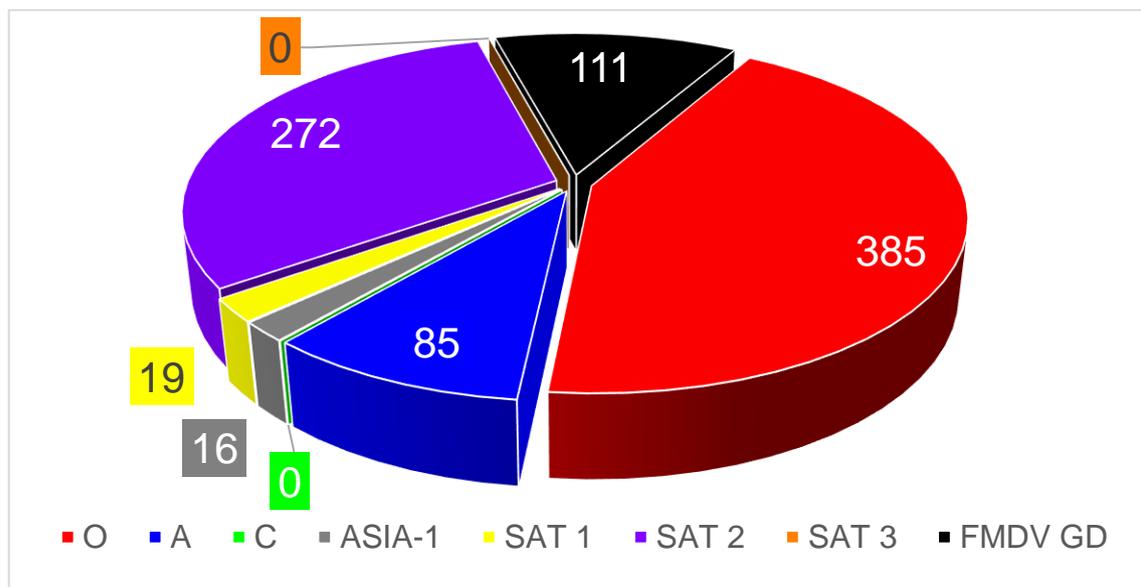


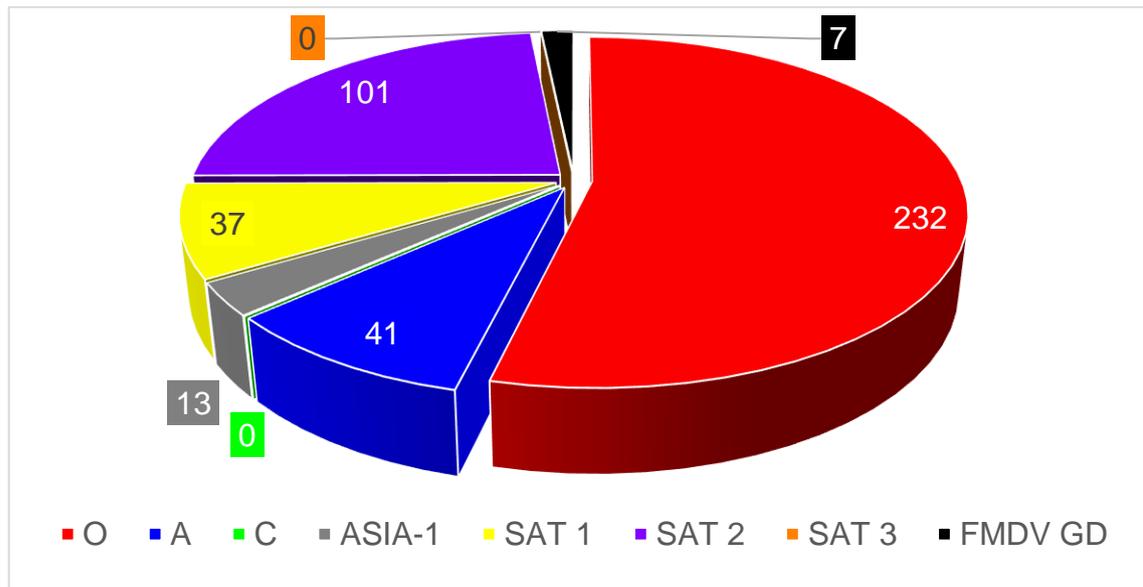
Figure 2-4: Distribution of samples collected from suspect cases of FMD and reported by the WOAH/FAO FMD Laboratory Network during 2023. Routine surveillance that is undertaken in countries that are FMD-free without vaccination is not shown.



**Figure 2-5:** Clinical samples (n=1402) tested for FMD investigation (virology) by the WOA/FAO FMD Laboratory Network from FMD endemic countries during 2023 and their distribution across the seven FMD endemic pools (see Figure 2-1).



**Figure 2-6:** Summary of results for characterised isolates (n=888) from FMD endemic countries were reported by the Network during 2023. FMDV GD denotes samples that were only positive using molecular (RT-PCR methods), while a further 515 samples were tested but found to be negative for FMDV using all diagnostic methods.



**Figure 2-7:** Summary of 431 samples (viruses and field isolates) that were sequenced (VP1/capsid/complete genome) during 2023 (see Appendix 3).

The results for the individual samples are reported later in this report. Characterization results obtained on samples received by WRLFMD and PANAFTOSA can also be found respectively at: <http://www.wrlfmd.org/> and at: <http://new.paho.org/panaftosa>.

### 2.3 Regional distribution of different FMD viral lineages

In regions where FMD is endemic, continuous evolution of the virus generates geographically discrete lineages that are genetically distinct from FMD viruses found elsewhere. The conjectured global status for FMD (see Figure 2-1) masks the underlying complexity of FMDV virus distribution in the different pools (at serotype, toptype and lineage levels). This report showcases a new format to display how different FMD lineages circulate in different regions of the world. Using a new tool (called PRAGMATIST) that has been developed in partnership between WRLFMD and EuFMD, analyses accommodate the latest epidemiological data collected by the Network and presented in this report regarding FMDV lineages detected in samples to assess the relative importance of the viral strains circulating within each *source regions* (see Table 2-2 below).

Table 2-2: Conjectured distribution of important FMDV lineages in different endemic regions. For each of the regions, data represent the relative importance of the different lineages [prevalence score estimated as a proportion (%) of total FMD cases that occur in domesticated host animals]. NB: Arrows highlight changes from the figures published in this table in last year's report; Number in subscript is the previously published figure.

FMDV Lineage	West Eurasia	Southeast/ East Asia	North Africa	South Asia	East Africa	West & Central Africa	Southern Africa	South America
O/ME-SA/PanAsia-2	↓ 30 <sub>35</sub>							
O/ME-SA/PanAsia		10						
O/SEA/Mya-98		21.5						
O/ME-SA/Ind-2001	↓ 5.5 <sub>7</sub>	40	↓ 0 <sub>2</sub>	↓ 76 <sup>1</sup> <sub>11</sub>				
O/EA or WA	↓ 1.5 <sub>3</sub>		↑ 60 <sub>55</sub>		↓ 53.5 <sub>55</sub>	↑ 69 <sub>63</sub>	16	
O/EAURO-SA								90
O/CATHAY		10.5						
A/ASIA/Sea-97		18						
A/ASIA/Iran-05	↓ 28 <sub>32</sub>							
A/ASIA/G-VII	↓ 5 <sub>10</sub>			↑ 20 <sub>10</sub>				
A/AFRICA			↓ 30 <sub>33</sub>		↓ 17 <sub>22</sub>	↓ 15 <sub>17</sub>		
A/EURO-SA								10
Asia-1	↓ 10 <sub>12.5</sub>			4				
SAT 1	↑ 1 <sub>0</sub>				↑ 15 <sub>8</sub>	↓ 1 <sub>3</sub>	16	
SAT 2	↑ 19 <sub>0.5</sub>		10		14	15	52	
SAT 3					0.5		16	
C								

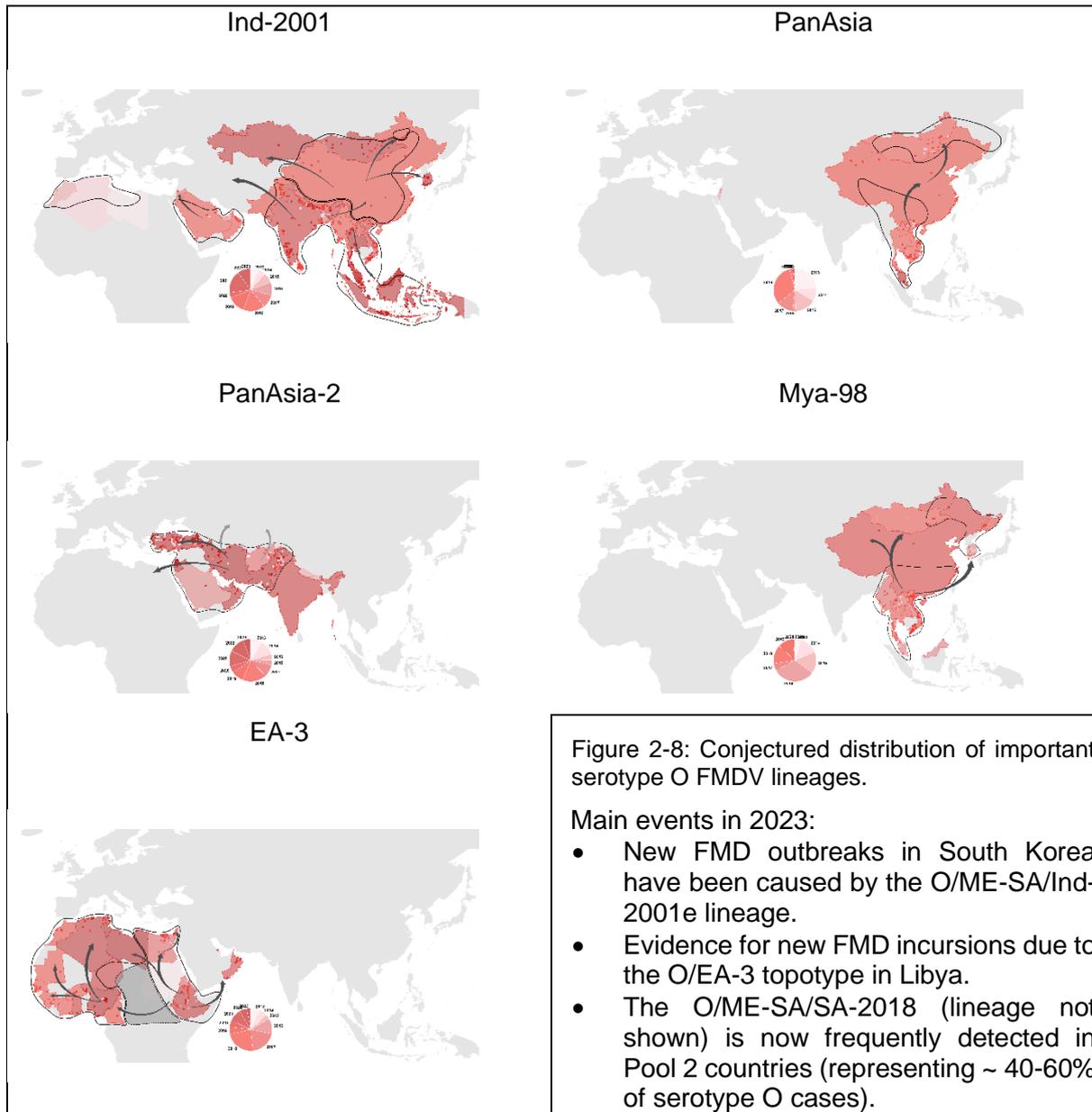
<sup>1</sup> Includes cases due to the emerging O/ME-SA/SA-18 lineage that has been recently detected in Pool 2.

Based on these data, a *prevalence score* is defined by estimating the proportion of each of the local viral strains that would be represented if 100 animals infected with FMDV were randomly selected from each source area.

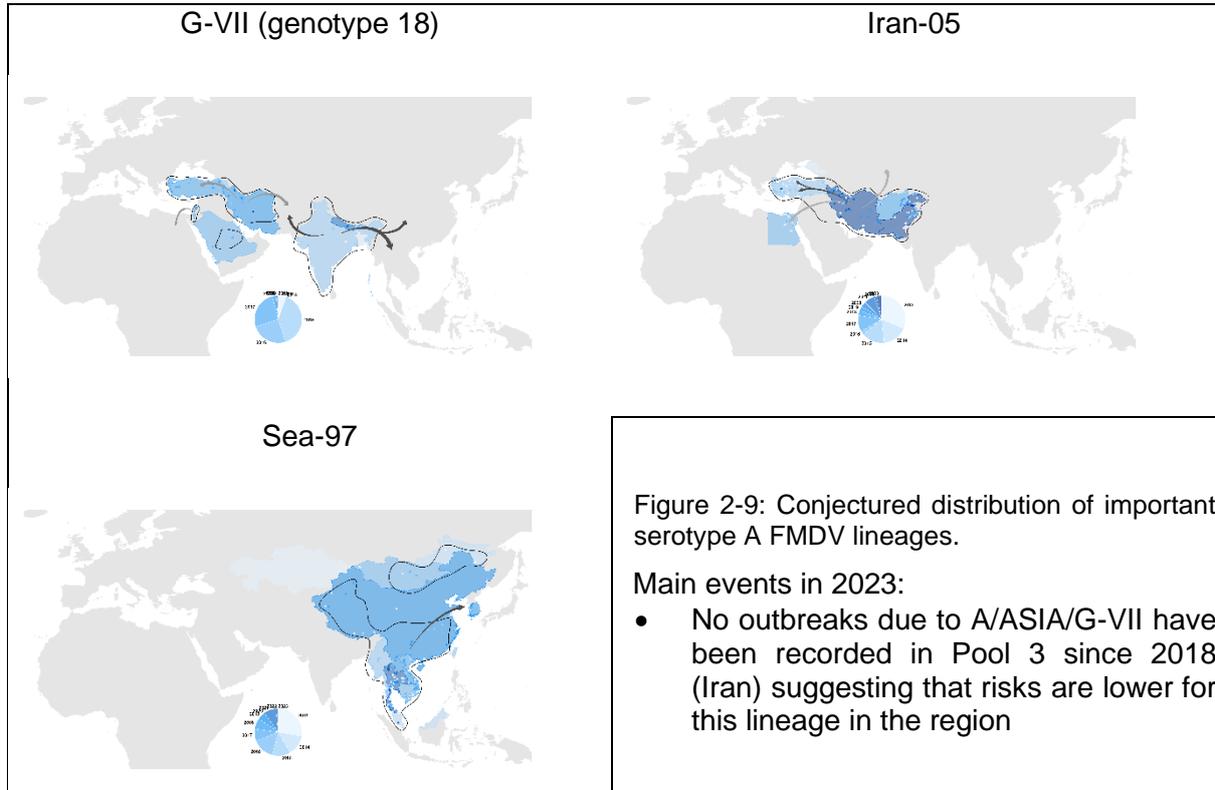
In order to help visualise the changing patterns in FMDV distribution and recognise risks for the emergence of new lineages, the Network has reviewed available intelligence for epidemiologically important FMDV lineages (Table 2-2), focussing on those that have already demonstrated a potential for long-distance trans-pool spread: O/ME-SA/Ind-2001, O/ME-SA/PanAsia, O/ME-SA/PanAsia-2, O/SEA/Mya-98, O/EA-3, A/ASIA/G-VII, A/ASIA/Iran-05, A/ASIA/Sea-97 and SAT 2/VII.

**The current known and conjectured distribution of these different FMD viral lineages are represented in the maps below:** The extent of current distribution for each of the viral lineages is represented within the black lines, while the location of individual outbreaks (dots) and affected countries (shaded colours, according to dates) are shown. NB: Arrows are drawn to highlight the regions that are now threatened by these lineages and text boxes highlight some of the headline events and changes that have occurred during 2022-23.

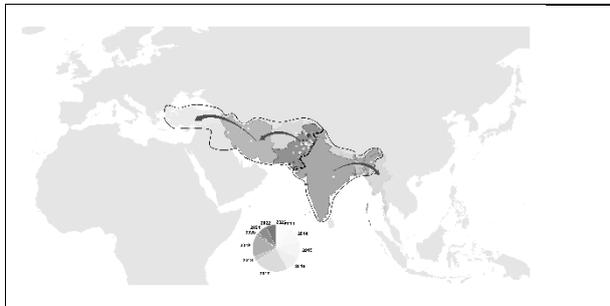
## FMDV O



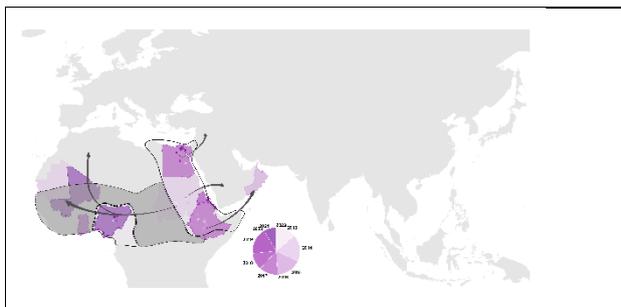
## FMDV A



## FMDV Asia 1

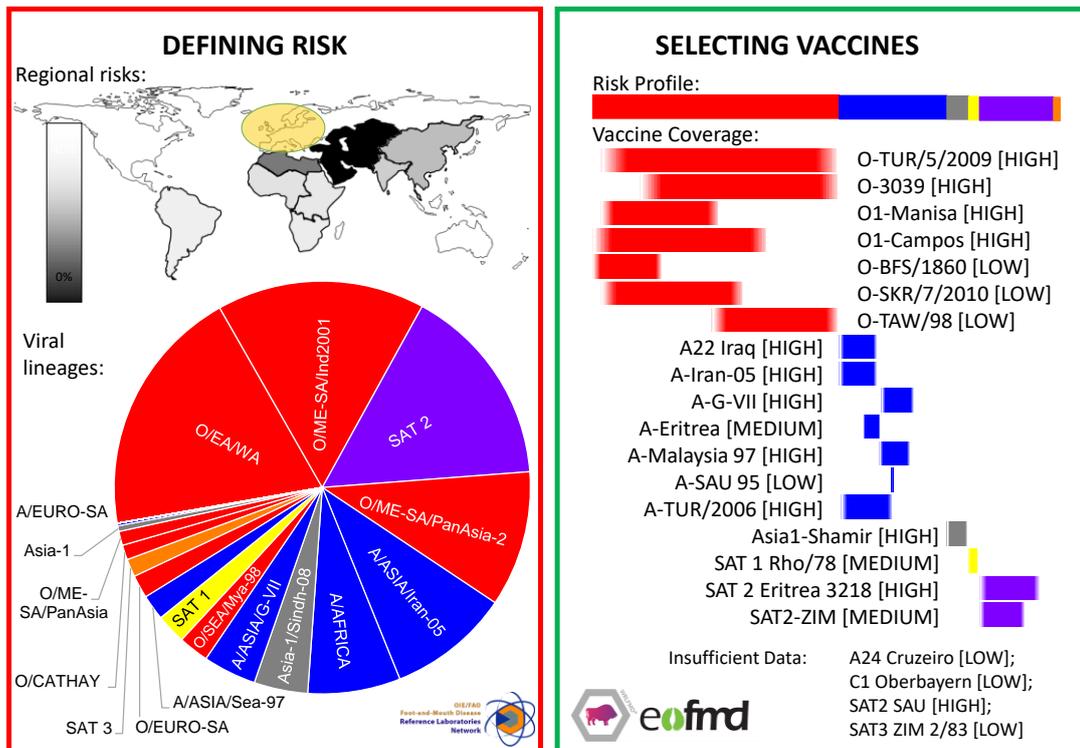


## FMDV SAT 2



## 2.4 Vaccine matching and recommendations

These take two forms: regional recommendations and details of locally produced vaccines for each of the FMD endemic pools are summarised later in this report, whilst the WRLFMD recommendations for FMD free countries are given in Figure 2-12 below. Details of vaccine matching work undertaken by the Network are summarised in Appendix 2.



NB: Analyses uses best available data, however there are gaps in surveillance and vaccine coverage data

Figure 2-12: Recommendations from WRLFMD on FMD virus strains to be included in FMDV vaccine antigen bank for Europe (January 2024)

Outputs from WRLFMD are generated with a tool (called PRAGMATIST<sup>1</sup>) that has been developed in partnership between WRLFMD and EuFMD. These analyses accommodate the latest epidemiological data collected by the Network regarding FMDV lineages that are present in different source regions (see Table 2-2 above), as well as available *in vitro*, *in vivo* and field data to score the ability of vaccines to protect against these FMDV lineages. Further information about FMD vaccine producers is available on the Network website: <https://www.foot-and-mouth.org/fmd-vaccine-producers>

The figure highlights the importance of these source regions for Europe (using data collected at the EU-RL Workshop); please contact WRLFMD/EuFMD for assistance to tailor these outputs to other geographical regions. NB: Vaccine-coverage data presented is based on available data and may under-represent the true performance of individual vaccines.

<sup>1</sup> Ludi *et al.*, (2022) PRAGMATIST: A tool to prioritize foot-and-mouth disease virus antigens held in vaccine banks. *Front Vet Sci.* 9:1029075. doi: [10.3389/fvets.2022.1029075](https://doi.org/10.3389/fvets.2022.1029075)

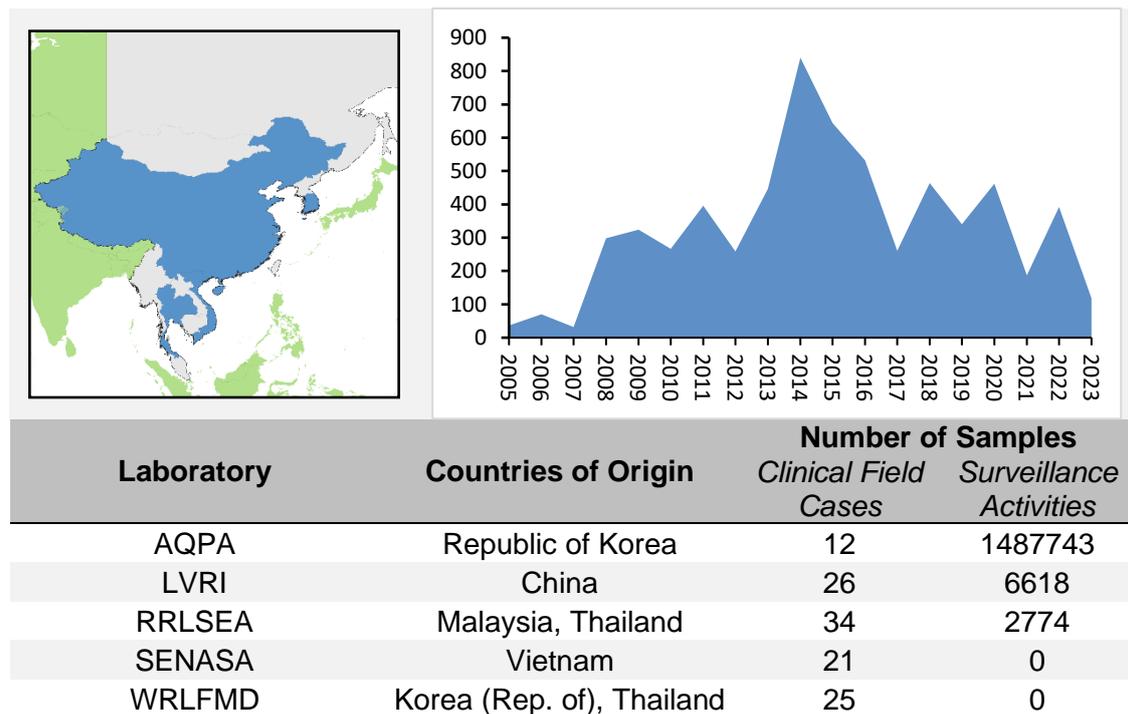
### 3 Overview of Network surveillance activities in each of the regional endemic pools

#### 3.1 Pool 1 Regional synopsis

##### 3.1.1 Conjectured circulating FMD viral lineages in Pool 1 during 2023

- Serotype O:
  - SEA/Mya-98
  - ME-SA/PanAsia
  - ME-SA/Ind2001e
  - CATHAY
- Serotype A:
  - ASIA/Sea-97
- Serotype Asia-1 (no outbreaks detected since 2017, Myanmar)

**Table 3-1:** Overview of clinical samples collected and tested from Pool 1 in 2023 (countries highlighted in blue; graph represents clinical submissions to Network laboratories since 2005)



### Pool 1 headlines:

- During May 2023, 11 FMD outbreaks were detected on cattle and goat farms located in Cheongju and Jeungpyeong, Republic of Korea. Sequencing showed that the causative FMDV belonged to the O/ME-SA/Ind-2001e lineage, sharing the closest nucleotide identity (97.95-99.21%) to viruses from Mongolia and Pool 1 countries. These sequences were distinct to those collected from the previous (2019) outbreaks in the Republic of Korea (see: Appendix 4.1). Prompt implementation of emergency vaccination using serotype O vaccines, together with intensive active surveillance on farms surrounding the infected premises successfully prevented any further spread of FMD.
- In China, four new FMD outbreaks (3 in cattle, 1 in pig) have been detected, all serotype O (see: Appendix 4.2). Surveillance using lymph node samples has detected additional FMDV positive animals (n=40). The dominant strain in China is O/ME-SA/Ind-2001e; serotype A has not been detected since 2019.
- No new outbreaks due to serotype Asia1 were detected in 2021. This serotype has been absent from Pool 1 since 1998, except for outbreaks in Vietnam (2006) and Myanmar (2017).

### 3.1.2 Vaccine recommendations for Pool 1

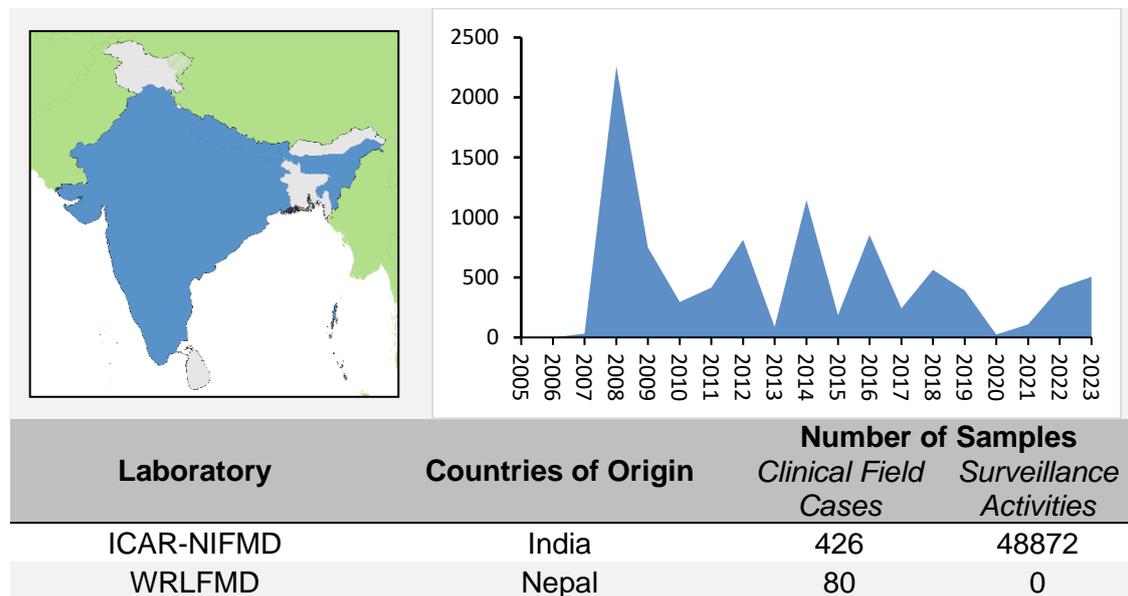
- Internationally produced vaccines:
  - O: Campos, O<sub>1</sub> Manisa, Primosky, TUR/5/2009 & 3039
  - A: Arg2001, A24 Cruzeiro, Iran/05, A22/Iraq/64, Malaysia/97, TUR/20/06 & Zabaikalsky.
  - Asia 1: Shamir
- Locally produced vaccines (in Thailand):
  - O: 189/87 (Udornthani/87), O/CATHAY
  - A: Lopburi/12, Sakolnakorn/97
- Locally produced vaccines (at FGBI ARRIAH):
  - O: Ind-2001, Mya-98, 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).

## 3.2 Pool 2 Regional synopsis

### 3.2.1 Conjectured circulating FMD viral lineages in Pool 2 during 2023

- Serotype O:
  - ME-SA/Ind-2001e
  - ME-SA/SA-2018
- Serotype A:
  - ASIA/IND (genotype VII also known as genotype 18)
- Serotype Asia-1

Table 3-2: Overview of clinical samples collected and tested from Pool 2 in 2023 (countries highlighted in blue; graph represents clinical submissions to Network laboratories since 2005)



**Pool 2 headlines:**

- The O/ME-SA/SA-2018 lineage is now frequently detected in India and the surrounding countries.
- A new clade within the O/ME-SA/SA-2018 lineage has been described in Bangladesh (called MYMBD21; Hossain et al., 2023).
- Serotype Asia1 Group VIII has been detected in India (see Appendix 4.3), the first cases since the last reports in 2018, while in Bangladesh another serotype Asia 1 clade has been detected (G-IX).
- Locally produced FMD vaccines do not appear to be well matched against the serotype A viruses that are circulating in the region (see comment below).

Hossain et al., (2023) Emergence of a novel sublineage, MYMBD21 under SA-2018 lineage of Foot-and-Mouth Disease Virus serotype O in Bangladesh. Sci Rep. 13: 9817.

**3.2.2 Vaccine recommendations for Pool 2**

- Internationally produced vaccines:
  - O/ME-SA/PanAsia-2 (or suitable alternative). *In vitro* vaccine matching data for O/ME-SA/Ind2001 provides evidence for an antigenic match with O/TUR/09 vaccine (MSD) and O-3039 (Boehringer Ingelheim).
- Locally produced vaccines (by Indian suppliers):
  - O/IND/R2/1975
  - IND40/2000 \*
  - Asia1/IND/63/1972

\* Serotype A FMD virus strains circulating in India since 2012–13 have been found to be antigenically divergent from the currently used vaccine strain (IND40/2000). Taking

into account the studies carried out by ICAR-NIFMD regarding the selection of suitable (alternate) FMDV serotype A vaccine strains, A/IND27/2011 emerged as the candidate strain of choice out of a panel of 8 strains (Mohapatra et al., 2023).

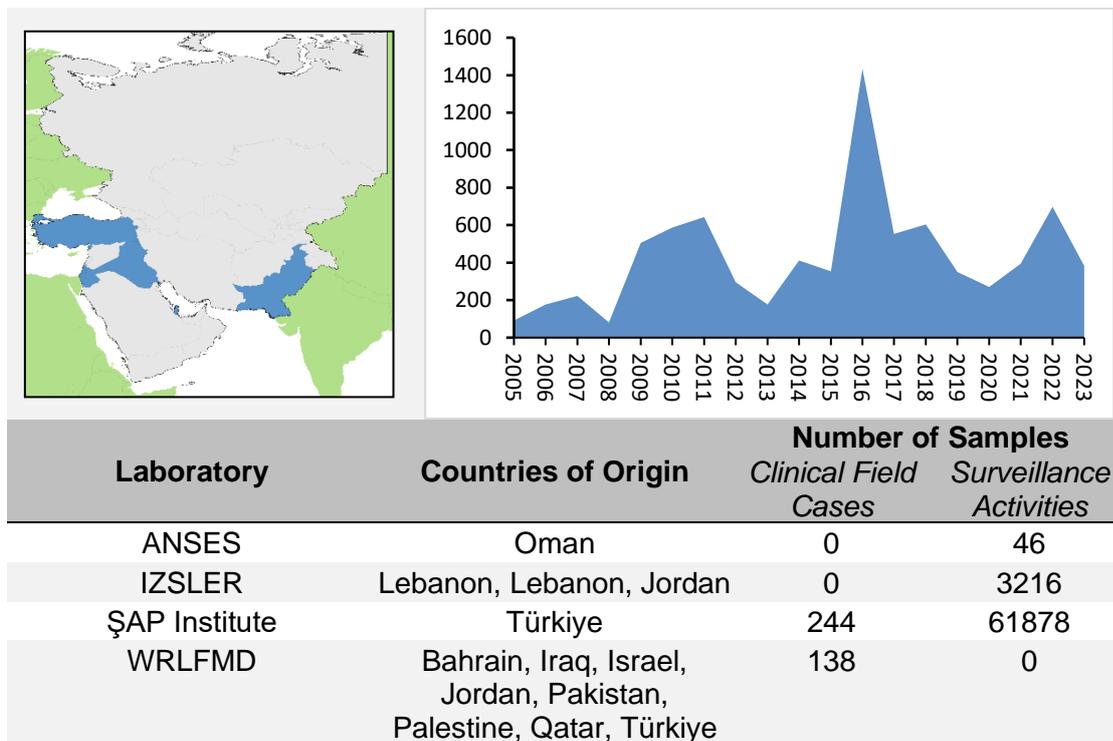
Mohapatra et al., (2023) Emergence of a novel genetic lineage 'A/ASIA/G-18/2019' of foot and mouth disease virus serotype A in India: A challenge to reckon with. *Virus Res.* 333: 199140.

### 3.3 Pool 3 Regional synopsis

#### 3.3.1 Conjectured circulating FMD viral lineages in Pool 3 during 2023

- Serotype O:
  - ME-SA/PanAsia-2 [comprising at least two viral sublineages (ANT-10 and QOM-15) present in different countries].
  - ME-SA/Ind-2001 (via introductions from South Asia: Pool 2)
  - ME-SA/SA-2018 in UAE and Oman (2022)
  - EA-3 (in Israel & Palestinian Autonomous Territories)
- Serotype A:
  - ASIA/Iran-05 [comprising 4 predominant viral sublineages (SIS-10, SIS-12, SIS-13 and FAR-11)]
  - ASIA/G-VII
- Serotype Asia-1:
  - Sindh-08
- Serotype SAT 2:
  - XIV in Turkey

**Table 3-3:** Overview of clinical samples collected and tested from Pool 3 in 2023 (countries highlighted in blue; graph represents clinical submissions to Network laboratories since 2005)



### Pool 3 headlines:

- The emergence of the SAT2/XIV topotype in Iraq, Jordan, Türkiye, Bahrain and Oman). These are the first ever reported cases of serotype SAT 2 in some of these countries (Iraq, Jordan, Türkiye). Analyses of sequences collected from these cases confirm that they are caused by viruses which are closely related to viruses collected from Ethiopia during 2022 (see: Appendix 4.4). Clinical disease in large ruminants was reported to be severe, associated with higher-than-expected mortality levels in older animals. There were obvious concerns about the potential for rapid onward spread to other countries in the region and to the FMD-free buffer zone in Thrace via east-to-west virus conveyers that have been described for other FMDV lineages. FMD vaccines were donated from the EU antigen bank to increase immunity within livestock population in Türkiye and a homologous FMD vaccine was produced in Türkiye to help control the spread of this lineage. A qualitative risk assessment was prepared by FAO to cover the onward risk of this lineage spreading in the region (McLaws et al., 2023).
- The complexity of FMDV circulation in the Gulf States was highlighted by the unexpected detection of the SAT1/I topotype in Qatar (samples collected during April 2023; see Appendix: 4.5). This virus shares closest sequence identity to a virus from Kenya, further reinforcing the epidemiological connections between East Africa and the Gulf States.
- The O/ME-SA/PanAsia-2<sup>ANT-10</sup> sub-lineage is still circulating in Israel and Palestine.
- Serotypes A and Asia 1 have not been detected in Türkiye since 2015 and January 2018, respectively.
- The A/ASIA/G-VII lineage has not been detected in the region since 2018 (Iran) and Israel (2017).
- Retrospective analyses has identified a new clade of serotype Asia 1 viruses circulating in Pakistan that are distinct to other Asia 1 serotype viruses, including the Asia1/IX genotype that has been reported recently in Pool 2.

McLaws et al., (2023) Risk of foot-and-mouth disease SAT2 introduction and spread in countries in the Near East and West Eurasia. October 2023. Rome, FAO. <https://doi.org/10.4060/cc8173en>

### 3.3.2 Vaccine recommendations for Pool 3

Internationally produced vaccines

- MSD and Boehringer-Ingelheim:
  - O/ME-SA/PanAsia-2 (or suitable alternative)
  - O/Manisa
  - A Iran-05 (or A TUR 06)
  - A22/Iraq
  - Asia-1 Shamir
  - A/G-VII
  - SAT2 (Eritrea-98 or ZIM 83)

Vaccines from FGBI ARRIAH):

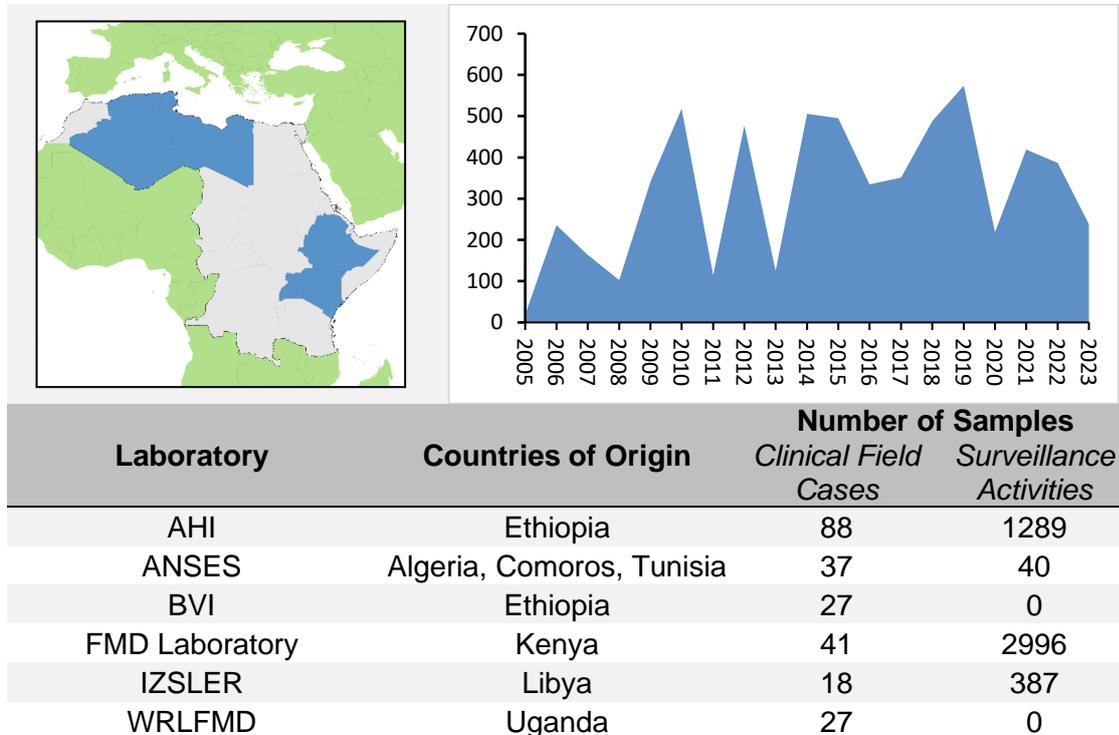
- O: Ind-2001, Mya-98, PanAsia-2
- A; G-VII, Iran-05, Sea-97
- Asia1: Shamir, Sindh-08
- SAT2
- Locally produced vaccines:
  - O/TUR/07 (PanAsia 2)
  - A05 (A/IRN/17)
  - A/ASIA/Iran 05<sup>FAR-11</sup>
  - A/Asia/G-VII
  - Asia 1/Sindh-08
  - SAT2/XIV
- Locally produced vaccines (other suppliers in the region):
  - Vetal
  - MEVAC
- Turvac Oil Tetravalent, containing:
  - SAT 2/ XIV [IRQ23]
  - O/PanAsia-2 [O/TUR/07]
  - A/ASIA/Iran 05<sup>FAR-11</sup> [A/IRN/21]
  - Asia 1/Sindh-08 [Asia1/TUR/15]

### 3.4 Pool 4 Regional synopsis

#### 3.4.1 Conjectured circulating FMD viral lineages in Pool 4 during 2023

- Serotype O:
  - EA-2 ( Kenya, Tanzania, DR Congo, Uganda)
  - EA-3 (Libya, Tunisia)
  - EA-4 (Ethiopia, Kenya, Uganda)
  - ME-SA/Sharqia-72 (detected in samples collected in Egypt in 2009)
- Serotype A
  - AFRICA/I (Kenya, Tanzania, D.R. Congo)
  - AFRICA/IV (Algeria, Sudan, Eritrea, Egypt)
  - AFRICA/VII (Ethiopia, Egypt)
  - ASIA/Iran-05<sup>BAR-08</sup> (Egypt)
- Serotype SAT 1
  - I (Kenya, Tanzania, Comoros)
  - IX (Ethiopia)
- Serotype SAT 2:
  - IV (Kenya, Tanzania)
  - V (Algeria)
  - VII (Sudan, Egypt, Mauritania)
  - XIII (Ethiopia, Sudan)
  - XIV (Ethiopia)
- Serotype SAT 3
  - Only detected in African buffalo in the south of the Queen Elizabeth National Park, Uganda in 1970, 1997 and 2013).

**Table 3-4:** Overview of clinical samples collected and tested from Pool 4 in 2023 (countries highlighted in blue; graph represents clinical submissions to Network laboratories since 2005). *Note: These figures include samples collected in countries in North Africa where FMD outbreaks have occurred since 2013.*



**Pool 4 headlines:**

- Detection of serotype SAT 2 in North Africa. Sequence data shared demonstrates that FMD cases detected in Algeria during December 2023, were due to an unusual SAT2 toptype (SAT2/V: see Appendix 4.6). These cases represent the first time that serotype SAT 2 has been detected in any of the Maghreb countries (Tunisia, Algeria and Morocco). Viruses from this lineage were last found in Ghana (1991), Togo (1990) and Ivory Coast (1990) and further work is now urgently needed to understand the source of this virus (presumably from Central/West Africa), and the risk pathways by which SAT2/V has been introduced into North Africa; where previous and other ongoing outbreaks have involved the O/EA-3 and A/AFRICA/G-IV lineages which originate from West Africa.
- The detection of serotype SAT1/I in the Comoros Islands (representing the second incursion after O/EA-2 was detected in 2019). These sequences (See Appendix 4.7) are distinct to the FMD virus detected in Qatar (with >10 percent nucleotide difference); however, the concurrence of these outbreaks in two locations may reflect a general upsurge in SAT 1 in East Africa.
- Serotype O (topotype EA-3) has been reintroduced into Tunisia due to a virus most closely related to FMDVs in Sudan and Ethiopia (see: Appendix 4.8). Surveillance has been facilitated using FTA cards that have been shipped to a FMD Reference Laboratory for testing.

### 3.4.2 Vaccine recommendations for Pool 4

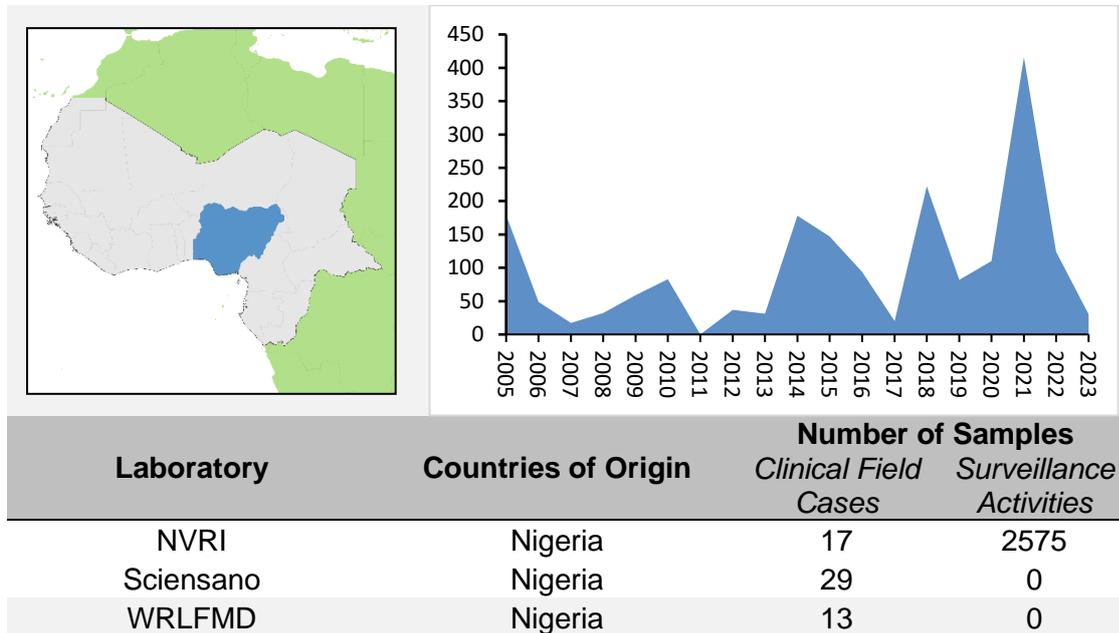
- Internationally produced vaccines:
  - O: Manisa, 3039
  - O: PanAsia-2 (or equivalent)
  - A: Eritrea
  - SAT 1: Sat105, SAT109
  - SAT 2: SAT251, Eritrea
  - SAT 3: SAT306, SAT309
- Locally produced vaccines from KEVIVAPI (Kenya):
  - O: K 77/78 - EA1
  - A: K5/80 - G1
  - SAT1: T155/71 - NWZ
  - SAT2: K52/84 - IV
- Locally produced vaccines from Ethiopia:
  - Serotype O (EA-3)
  - Serotype A (Africa/G-III)
  - Serotype SAT 2 (XIII)
- Locally produced vaccines from BVI (Botswana including the following strains O/Manisa 1/78, O/3039, SAT105, SAT109, SAT2035, SAT251, SAT306 & SAT309

### 3.5 Pool 5 Regional synopsis

#### 3.5.1 Conjectured circulating FMD viral lineages in Pool 5 during 2023

- Serotype O:
  - WA and EA-3 (Nigeria)
- Serotype A:
  - AFRICA/G-IV & G-VI
- Serotype SAT 1
  - Topotype X (Nigeria and Cameroon)
- Serotype SAT 2:
  - Topotype VII (Mauritania)

**Table 3-5:** Overview of clinical samples collected and tested from Pool 5 in 2023 (countries highlighted in blue; graph represents clinical submissions sent to Network laboratories since 2005)



**Pool 5 headlines:**

- The O/EA-3 toptype appears to be widely spread across the region – including in Niger where new cases have been detected

**3.5.2 Vaccine recommendations for Pool 5**

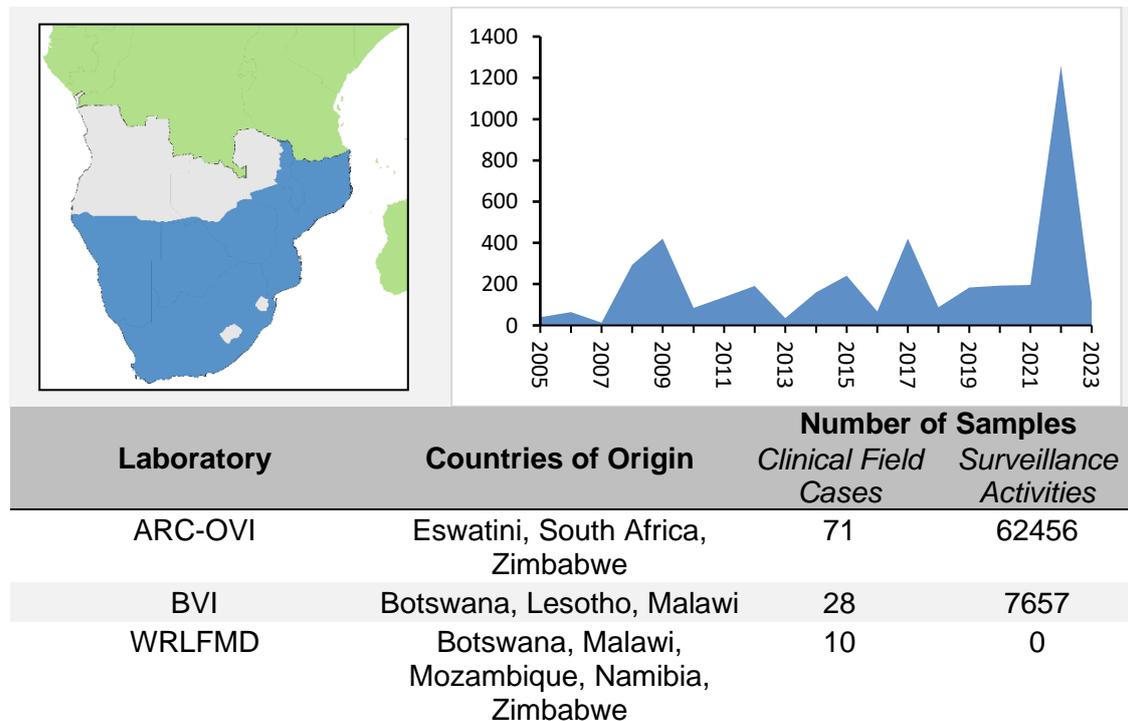
- Internationally produced vaccines:
  - O/Manisa
  - O/Maghreb
  - O/PanAsia-2 (or equivalent)
  - O: 3039
  - A: Eritrea
  - SAT 2: Eritrea & Zimbabwe
- Locally produced vaccines
  - O: NIG 04/14
  - O: WA and EA-3 toptypes
  - A: NIG 07/13
  - A: West Africa (G-IV lineage)
  - SAT 1: Topotype X
  - SAT 2: NIG 03/12
  - SAT 2: Topotype VII
  - O, A, SAT 1 & SAT 2 (Boru-Vacc, Nigeria)

### 3.6 Pool 6 Regional synopsis

#### 3.6.1 Conjectured circulating FMD viral lineages in pool 6 during 2023

- Serotype O
  - O/EA-2 toposotype
- Serotype A
- Serotype SAT 1:
  - Topotypes I, II and III
- Serotype SAT 2:
  - Topotypes I, II and III
- Serotype SAT 3:
  - Topotypes I, II and III

**Table 3-6:** Overview of clinical samples collected and tested from Pool 6 in 2023 (countries highlighted in blue; graph represents clinical submissions since 2005)



#### Pool 6 headlines:

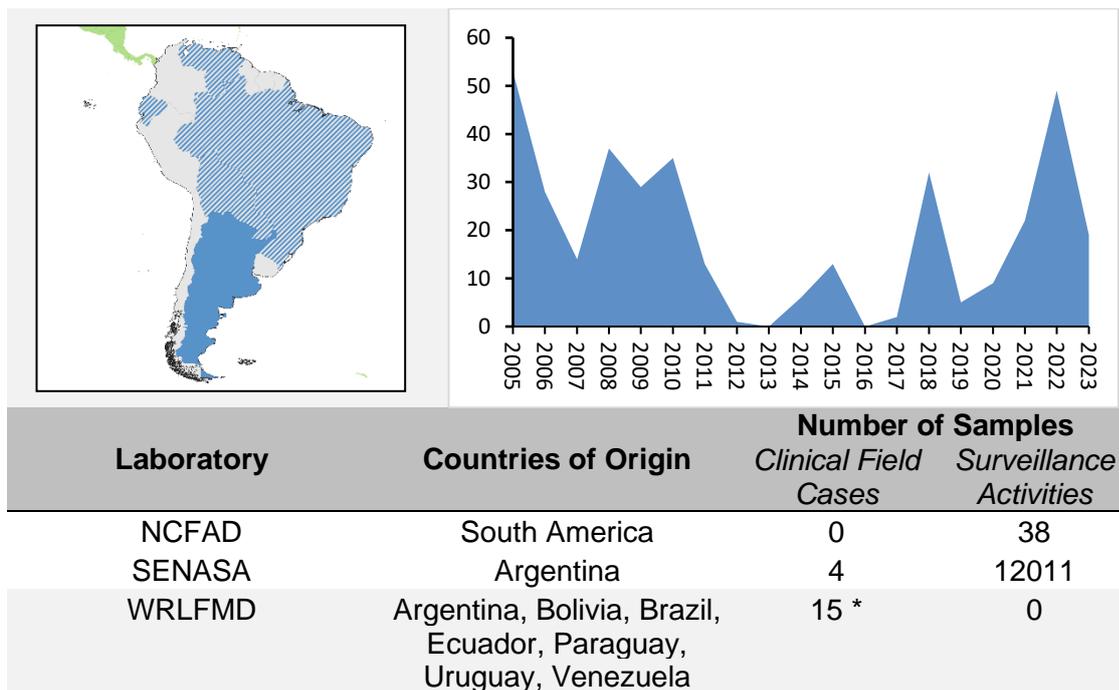
- South Africa has lost its “suspended” WOA status for FMD. No new clinical FMD outbreaks have been reported in South Africa during 2023, although 181 outbreaks that were detected from previous years have not yet been closed. Vaccination (using a trivalent SAT 1-3 vaccine) has been used to control the disease, 650,000 animals were vaccinated. Surveillance of previously affected farms are ongoing.

### 3.6.2 Vaccine recommendations for Pool 6

- Internationally produced vaccines:
  - O: Manisa, 3039
  - A: A Saudi Arabia
  - SAT 1: SAT105, SAT 109
  - SAT 2: SAT251, SAT 2 Eritrea
  - SAT 3: SAT306, SAT 309
- Locally produced vaccines
  - O: Manisa
  - A: A Zambia, A4165, A3238
  - SAT 1: SAT105, SAT109
  - SAT 2: SAT251, SAT2035
  - SAT 3: SAT306, SAT309

### 3.7 Pool 7 Regional synopsis

**Table 3-7:** Overview of clinical samples collected and tested from Pool 7 in 2023 (countries highlighted in blue; graph represents clinical submissions since 2005)



\* All samples tested by the WRLFMD from South America are from historical, and not current, outbreaks

#### Pool 7 headlines:

- Except for Venezuela which has no official FMD status with the WOA, there have been no confirmed cases of FMD anywhere in South America during 2023.

### 3.7.1 Vaccine recommendations for Pool 7

- Internationally produced vaccines:
  - All vaccines used in the region are produced in South America (Argentina, Brazil, Colombia, Paraguay & Venezuela have vaccine manufacturers)
- Locally produced vaccines
  - O: O<sub>1</sub> Campos
  - A: A<sub>24</sub> Cruzeiro, A/Arg/2001
  - C: C<sub>3</sub> Indaial

## 4 Improving the quality of laboratory tests from FMD reference laboratories

### 4.1 Proficiency testing schemes (PTS) organised by the Network Partners

#### ACDP, Australia

- National Terrestrial Program
  - 9 participating laboratories from Australia and New Zealand
  - Support of LEADDR Network

#### AHI, Ethiopia

- NSP 3 ABC ELISA
  - 2 laboratories in Ethiopia

#### ANSES, France

- FMD Proficiency testing scheme 2023
  - 39 laboratories from EU members; EU candidates; 6 Neighbouring countries participated
  - PTS tested FMDV detection & characterisation and antibodies detection

#### APQA, Republic of Korea

- National Proficiency test for Diagnosis of FMD
  - 46 Diagnostic Laboratories from the Republic of Korea.
  - National Proficiency test for Diagnosis of FMD (RT-PCR, SP ELISA, NSP ELISA).

#### IZSLER, Italy

- National (Italy) Proficiency test
  - PTs addressed to maintain and practice preparedness of 10 regional laboratories to support the NRL in case of FMDV emergency.
  - Samples for serology: panel of 20 blind sera, (Tests required: ELISA for SP type A Ab (by competitive ELISA IZSLER kit) and NSP ELISA)
  - Samples for molecular assay: panel of 3 blind samples (Tests: 3D Real-time RT-PCR)"

#### LVRI, China

- Proficiency testing & Interlaboratory comparison
  - 34 participating laboratories from China.
  - 32 of the 34 laboratories returned results in line with the anticipated results.

#### PANAFTOSA/VPH, PAHO/WHO

- Proficiency Testing Program for diagnostic tests for FMD and vesicular stomatitis
  - 26 countries participated:



for use. Laboratories were also asked to use the scenario information as well as the information from the virological panel to select appropriate assays for use with the serological panel including what FMDV serotype(s) should be tested.

## 4.2 Supply of reagents

### ANSES, France

Type of reagent	Quantity	Recipient of the reagent (Laboratories/Countries)
kit one-step RT-PCR	6	Armenia, Azerbaijan, Georgia, Jordan, Lebanon, Turkey
FMDV Primers	10	Armenia, Azerbaijan, Georgia, Jordan, Lebanon, Turkey
rtRT-PCR 3D/ $\beta$ -actin	300 tests	Jordan
rtRT-PCR Type O/ $\beta$ -actin	100 tests	Jordan
rtRT-PCR Type A/ $\beta$ -actin	100 tests	Jordan
rtRT-PCR Type SAT2-XIV/ $\beta$ -actin	100 tests	Jordan

### APQA, Republic of Korea

Type of reagent	Quantity	Recipient of the reagent (Laboratories/Countries)
VDRD FMDV 3Diff/PAN Rapid kit	10 tests	Malaysia
VDRD FMDV 3Diff/PAN Rapid kit	10 tests	Sri Lanka

### BVI, Botswana

Type of reagent	Quantity	Recipient of the reagent (Laboratories/Countries)
FMD virus antigens [O, SAT 1, SAT 2 & SAT 3]	200ml per each serotype	Namibia
FMD antisera (rabbit) [O, SAT 1, SAT 2 & SAT 3]	50ml per each serotype	Namibia
FMD anti sera (guinea pig) [O, SAT 1, SAT 2, SAT 3]	50ml per each serotype	Namibia
FMD positive bovine serum [O, SAT 1, SAT 2, SAT3]	50ml per each serotype	Namibia
FMD negative bovine serum	20ml	Namibia

## ICAR-NIFMD, India

Type of reagent	Quantity	Recipient of the reagent (Laboratories / Countries)
DIVA NSP ELISA	For testing 81194 serum samples	State FMD regional and collaborating centres, India
SPCE SP ELISA	For testing 148500 serum samples	State FMD regional and collaborating centres, India
Sandwich Ag ELISA	For testing 2800 serum samples	State FMD regional and collaborating centres, India

## IZSLER, Italy

Type of reagent	Quantity	Recipient of the reagent (Laboratories / Countries)
Antigen Detection ELISA and serotyping (FMDV O, A,C, Asia 1, SAT1-2) kit	171	Albania, Algeria, Argentina, Armenia, Australia, Austria, Azerbaijan, Bahrain, Botswana, Bulgaria, Cyprus, Croatia, Egypt, France, Georgia, Germany, Jordan, Greece, Iran, Iraq, Ireland, Kenya, Kyrgyzstan, Kosovo, KSA, Latvia, Lebanon, Libya, Lithuania, Macedonia, Malta, Morocco, Mongolia, Montenegro, Pakistan, Poland, Czech Republic, Saudi Arabia, Serbia, Sierra Leone, Singapore, Sweden, Taiwan, Turkey, UAE
SPCE for antibodies specific to FMDV serotype O	169	Albania, Algeria, Argentina, Armenia, Austria, Bangladesh, Botswana, Bulgaria, Croatia, Egypt, Georgia, Germany, Greece, Iran, Iraq, Ireland, Kyrgyzstan, Kosovo, Latvia, Macedonia, Malta, Morocco, Mongolia, Montenegro, Nigeria, Pakistan, Poland, Czech Republic, Serbia, Saudi Arabia, Sweden, Taiwan, Turkey, UAE
SPCE for antibodies specific to FMDV serotype A	178	Albania, Argentina, Armenia, Austria, Bangladesh, Botswana, Bulgaria, Croatia, Egypt, Georgia, Germany, Greece, Iran, Iraq, Ireland, Kyrgyzstan, Kosovo, Latvia, Macedonia, Malta, Morocco, Mongolia, Montenegro, Nigeria, Pakistan, Poland, Czech Republic, Serbia, Saudi Arabia, Sweden, Taiwan, Turkey, UAE
SPCE for antibodies specific to FMDV serotype Asia 1	106	Albania, Algeria, Argentina, Armenia, Austria, Bangladesh, Bulgaria, Croatia, Georgia, Germany, Greece, Iran, Iraq, Ireland, Kyrgyzstan, Kosovo, Latvia, Macedonia, Malta, Mongolia, Montenegro, New Zealand, Pakistan, Poland, Czech Republic, Serbia, Sweden, Taiwan, Turkey, UAE
SPCE for antibodies specific to FMDV serotype SAT 1	44	Algeria, Austria, Azerbaijan, Botswana, Croatia, Ireland, Malta, Poland, Czech Republic, Sweden, Taiwan, UAE

Type of reagent	Quantity	Recipient of the reagent (Laboratories / Countries)
SPCE for antibodies specific to FMDV serotype SAT 2	123	Algeria, Argentina, Armenia, Austria, Azerbaijan, Botswana, Bulgaria, Croatia, Egypt, Georgia, Germany, Jordan, Greece, Iran, Iraq, Malta, Nigeria, New Zealand, Palestine, Poland, Czech Republic, Saudi Arabia, Sweden, Taiwan, Turkey, UAE
FMDV 3ABC trappig ELISA (NSP-ELISA kit)	69	Armenia, Austria, Bahrain, Egypt, France, Jordan, Iraq, Iran, Ireland, Kyrgyzstan, Lebanon, Nigeria, Czech Republic, Saudi Arabia, Senegal, Sierra Leone, Turkey
LFD1 (typing O, A, Asia 1 and Pan-FMD)	206	EuFMD, Iraq, Syria, Comoros, Uganda, Pakistan
LFD2 (typing SAT 1 and SAT 2, Pan-FMD)	106	Jordan, Iran, Iraq, Uganda
Tissue extraction kits	143	EuFMD, Iraq, Syria, Jordan, Iran, Uganda
Ready-to-use master mix (3D pan-FMDV real-time RT-PCR)	1150 (reactions)	Serbia, N. Macedonia, Montenegro, Kosovo, Albania, Bosnia and Herzegovina (Sarajevo lab), Bosnia and Herzegovina (Banja Luka lab), Greece, Bulgaria, Moldova, Kenya
positive control (inactivated viruses)	21 mL	Serbia, N. Macedonia, Montenegro, Kosovo, Albania, Bosnia and Herzegovina (Sarajevo lab), Bosnia and Herzegovina (Banja Luka lab), Greece, Bulgaria, Moldova, Kenya

#### FADDL, USA

Type of reagent	Quantity	Recipient of the reagent (Laboratories / Countries)
Proficiency Test Panels for FMD/CSF	350 (400 PACs)	State Diagnostic Laboratories, USA
Proficiency Test Panels for FMD/SVA	250 (300 PACs)	State Diagnostic Laboratories, USA
FMDV Positive Amplification Control	160 vials (25 µl/vial)	State Diagnostic Laboratories, USA
Positive Amplification Controls for ASF/CSF/FMD	20	Dominican Republic

## PANAFTOSA/VPH, PAHO/WHO

Type of reagent	Quantity	Recipient of the reagent (Laboratories/Countries)
FMDV kits for the detection of antibodies against non-structural protein (NSP): NCPanaftosa System (3ABC ELISA and EITB)	302 (188 3ABC ELISA + 114 EITB)	Argentina, Brazil, Colombia, Ecuador, Paraguay, Peru, Uruguay and South Korea
FMDV kits for the detection of antibodies against structural protein (SP): LP-ELISA FMD O and A	215	Brazil, Colombia, Ecuador, Paraguay, Peru and Uruguay
FMDV antigen typing ELISA kits ( <i>IS-ELISA for differential typing of FMDV/VSV</i> )	21	Argentina, Brazil, Colombia, Ecuador, Morocco, Paraguay, Peru and Uruguay
Cell lines ( <i>BHK-21 and IBRS-II</i> )	20	Argentina, Brazil, Paraguay and Uruguay
FMDV seeds	19	United Kingdom
Antisera NSP and SP ( <i>reference material</i> )	9	Colombia
Molecular biology ( <i>reference material</i> )	25	Colombia and Morocco

### RRLSEA, Thailand

Type of reagent	Quantity	Recipient of the reagent (Laboratories / Countries)
Rabbit trapping Ab for O, A, and Asia 1	20	National Laboratories in Thailand
Guinea Pig Anti Ab for O, A, and Asia 1	20	National Laboratories in Thailand
Concentrated Inactivated antigen for O, A, and Asia 1	20	National Laboratories in Thailand
Strong positive Control serum	20	National Laboratories in Thailand
Weak positive Control serum	20	National Laboratories in Thailand
Negative Control serum	20	National Laboratories in Thailand

### Sciensano, Belgium

Type of reagent	Quantity	Recipient of the reagent (Laboratories / Countries)
BEI inactivated FMDV (6 serotypes)	77 ml	NRL in the EU
Inactivated serum against FMDV (6 serotypes from vaccinated animals)	26 ml	NRL in the EU
Inactivated NSP positive serum	39 ml	NRL in the EU

### SENASA, Argentina

Type of reagent	Quantity	Recipient of the reagent (Laboratories/Countries)
Guinea pig complement	256	Paraguay and Argentina
Guinea pig hyperimmune sera	371	Paraguay and Argentina
inactivated viral antigen	6030	Argentina
Viral suspension production	32950	Argentina

### WRLFMD

Type of reagent	Quantity	Recipient of the reagent (Laboratories/Countries)
FMDV antigens, FMDV antisera and serum controls	1424 ml	Czech Republic, France, Germany, Morocco, Republic of Korea, Slovakia, Switzerland, United Kingdom, USA, Vietnam
FMD virus isolates	166 ml	Egypt, Germany, Israel, Italy, Jordan, United Kingdom

### 4.3 Training courses organised by Network partners

#### ANSES, France

- Emergency diagnosis & post vaccination monitoring
  - Online course, 10-26 May 2023.
  - Attended by 12 participants from Middle Eastern countries
- FMDV detection, sequencing and sequence analyses
  - Course held at ANSES:
    - 12-16 June 2023; 4 participants from North African countries
    - 26-30 June 2023; 2 participants from Middle Eastern countries
    - 3-7 July 2023; 4 participants from SEEN countries
- Emergency response preparedness.
  - Course held in Lyon, France, 14-06 June 2023.
  - 20 participants from France.

#### APQA, Republic of Korea

- The 10<sup>th</sup> Workshop on Diagnosis of Animal Diseases supported by WOAHP Reference Laboratories of APQA
  - Course held at APQA in the Republic of Korea from 17<sup>th</sup> to 26<sup>th</sup> October 2023.
  - Course attendee by 12 delegates from Malaysia (2), Vietnam (3), Kazakhstan (2), Philippines (3) and Sri Lanka (2).
  - The workshop on diagnosis of animal diseases supported by WOAHP Reference Laboratories with the aim of providing scientific and technical training for other WOAHP member countries

#### FADDL, USA

- Foreign Animal Disease Diagnostician Training Course
  - This course trains participants in field identification skills to determine a differential diagnosis of disease in poultry and livestock foreign to the United States, and the steps to perform field investigations and correct sample submission techniques.
  - 25 participants from the USA for each course at Plum Island, Orient, New York, USA or virtually.
  - Courses run on:
    - 23<sup>rd</sup> to 27<sup>th</sup> January 2023
    - 27<sup>th</sup> February to 3<sup>rd</sup> March 2022 (*Virtual course*)
    - 13<sup>th</sup> to 17<sup>th</sup> March 2023
    - 26<sup>th</sup> to 30<sup>th</sup> June 2023
    - 5<sup>th</sup> to 9<sup>th</sup> December (*Virtual course*)

#### ICAR-NIFMD, India

- FMD serosurveillance, seromonitoring, serotyping and hands on training on DIVA ELISA, SPC ELISA, and by sandwich ELISA

- Eight training multi-day programs run between January and October 2023 at ICAR-NIFMD, Bhubaneswar.
- Nine participants from state FMD regional and collaborating centres in India.
- Routine training/capacity building programs on FMD diagnosis, serosurveillance and seromonitoring. Hands on training was given for DIVA ELISA, SPC ELISA and by sandwich ELISA.
- Application of multiplex PCR for sensitive and rapid detection of FMDV serotypes
  - Run on 21-24 February 2023 at ICAR-NIFMD, Bhubaneswar. For 9 participants from state FMD regional and collaborating centres in India.
  - Hands-on training programme on multiplex PCR for detection of FMDV serotypes circulating in India in order to strengthen the state FMD regional centres.
- Epidemiological Approaches to Prevent and Control Transboundary Animal Diseases with Special Focus on Zoonotic Diseases and FMD
  - Online training on 3-12 October given to 14 participants from BIMSTEC member states.
  - Series of online session on different topics were conducted in collaboration with ICAR-NIVEDI, Bengaluru.
- Vaccine QC harmonization workshop
  - Run on 22-25 August 2023 at ICAR-NIFMD, Bhubaneswar for 25 participants from three vaccine manufacturers and three testing laboratories.
  - Conducted for harmonization of *in vitro* neutralization test for use in vaccine QC potency test. This has been adopted for vaccine QC experiments under LHDCP.
- Country wide Capacity Building Programme on probang/ oesophageal-pharyngeal fluid (OPF) collection, dispatch and follow-up of FMD NSP reactors
  - Training given to staff of the State Animal Husbandry Department and regional FMD Centres.
    - Two online workshops (on 1-2 June and 3-4 July) for more than 230 participants were conducted on follow up investigation of FMD virus non-structural protein (NSP)-seroreactors by oropharyngeal fluid (OPF) sampling and testing.
    - Six hands-on training sessions on Probang/Oesophageal-pharyngeal fluid (OPF) collection, dispatch at various locations across India, each for ~20 participants, were run between June and September.

### IZSLER, Italy

- Laboratory training on FMD diagnosis
  - 15<sup>th</sup> to 26<sup>th</sup> May 2023
  - Training provided to 2 vets from Jordan and 2 vets from Iraq.
  - Topics covered during the training:
    - Overview of epidemiology and Laboratory Diagnosis of FMD;
    - Concepts for the differentiation between FMDV vaccinated and infected animals;
    - FMDV NSP-ELISAs for anti-NSP Antibody detection: test execution and results interpretation;

- Detection of Antibodies to FMDV Structural Proteins (SP) by Solid Phase Competitive ELISA (SPCE): test execution and results interpretation;
- Penside test for FMD diagnosis and Ag detection ELISA: sample preparation, testing and results interpretation;
- Concepts of Biosecurity for FMD Laboratory;
- Demonstration of VNT, VNT reading and interpretation;
- Demonstration of FMDV RNA extraction by affinity columns and FMDV pan Real-time RT-PCR based on 3D gene; Execution of FMDV RNA extraction and Real-time RT-PCR;
- Discussion of the results obtained from the Real-time RT-PCR;
- Introduction to FMDV sequencing and phylogenetic analysis

### **LVRI, China**

- FMD vaccines, vaccination and post-vaccination monitoring training courses

### **PANAFTOSA/VPH, PAHO/WHO**

- Simulation exercise: health emergency and response to notification of suspected vesicular disease
  - Run from 27<sup>th</sup> to 31<sup>st</sup> March 2023 in Sergipe, Brazil
  - 60 participants from Brazil
  - The exercise promoted theoretical review and practical training on clinical and epidemiological research, collection and shipment of samples to the laboratory, FMD diagnosis, biosecurity and dissemination control procedures, information system and communication flow, as well as discussion and evaluation of strategies and methods to contain the spread of FMD.
- Simulation exercise: health emergency and response to notification of suspected vesicular disease
  - Run from 14<sup>th</sup> to 18<sup>th</sup> August 2023 in Goiás, Brazil
  - 5 participants from Brazil
  - The exercise promoted theoretical review and practical training on clinical and epidemiological research, collection and shipment of samples to the laboratory, FMD diagnosis, biosecurity and dissemination control procedures, information system and communication flow, as well as discussion and evaluation of strategies and methods to contain the spread of FMD.
- Simulation exercise: health emergency and response to notification of suspected vesicular disease
  - Run from September 30<sup>th</sup> to 6<sup>th</sup> October 2023 in Napo, Ecuador
  - 60 participants from Ecuador
  - The exercise promoted theoretical review and practical training on clinical and epidemiological research, collection and shipment of samples to the laboratory, FMD diagnosis, biosecurity and dissemination control procedures, information system and communication flow, as well as discussion and evaluation of strategies and methods to contain the spread of FMD.
- Training in diagnostic tests
  - Run from 19<sup>th</sup> to 30<sup>th</sup> June 2023 in São Paulo, Brazil

- 2 participants from Guyana
- Training in serological and molecular diagnostic methods for vesicular diseases (RT-qPCR and RT-PCR, 3ABC ELISA, EITB, viral neutralization and vaccine matching).
- Training in diagnostic tests
  - Run from 14<sup>th</sup> to 25<sup>th</sup> August 2023 in Pedro Leopoldo, Brazil
  - participant from Uruguay
  - Training in serological and molecular diagnostic methods for vesicular diseases (RT-qPCR and RT-PCR, 3ABC ELISA, EITB, viral neutralization and vaccine matching).

### **RRLSEA, Thailand**

- C-ELISA workshop Support by DTRA
  - Three-day course held at RRLSEA.
  - Attended by 22 participants
    - 18 from Thai National Laboratories
    - 2 from the Bureau of Veterinary Biologics (BVB)
    - 2 from Indonesian laboratories
  - The training objective was to train participants in the serology technique for antibody detection for FMD to effectively analyse and evaluate it in their laboratory. Participants were guided on establishing a C-ELISA method for antibody detection under the ISO 17025:2017 quality system. The workshop data and the results from the laboratories using the test in their laboratories will be used as part of the test validation. The aim is to build technical relationships and a knowledge network among the national laboratories and inform them of technical requirements and management measures for the new test in their laboratory setting.

### **Sciensano, Belgium**

- Training on molecular and serological diagnostic techniques for LSDV and FMDV.
  - Course held from 30<sup>th</sup> January 2023 to 3<sup>rd</sup> March 2023 at Sciensano, Belgium.
  - Attended by two participants from Algeria.

### **SENASA, Argentina**

- FMD: Clinical and laboratory diagnosis. Collection, storage and sending of samples.
  - Run in July 2023 in Argentina.
  - Attended by 66 professionals and technicians from the National Animal Health Service, SENASA.
  - Course content: detection of suspected cases, detection at the field, differential diseases that can be detected, material and sample packaging, *etc.*
- CRISPR-Cas and lamp techniques
  - Run in August 2023 in Argentina.
  - Attended by 22 professionals and technicians from the National Animal Health Service, SENASA.

- Course content: CRISPR-Cas and LAMP as biotechnological tools for the diagnosis and development of reagents in the area of microbiology.
- Molecular patterns of viral infection: entry to the cell
  - Run in August 2023 in Argentina.
  - Attended by 26 professionals and technicians from the National Animal Health Service, SENASA.
  - Course content: Components of the basic structure of the virus, stages of replication, different mechanisms of viral entry and exit and entry of virus.
- Viral isolation
  - Run in April 2023 in Argentina.
  - Attended by 14 professionals and technicians from the National Animal Health Service, SENASA.
  - Course content: Viral isolation.

### WRLFMD

- Technical visits to
  - Thailand
    - Training 6 delegates
  - Indonesia
    - Training 10 delegates
- Seminar on FMD risks for Iran and neighbouring countries
  - Given to 20 delegates from Iran
- The WRLFMD has hosted delegates from [Argentina, Georgia, Germany, Portugal, Thailand, USA] for hands-on training in FMD diagnostic methods.

## 4.4 Collaborative projects

### ANSES, France

Collaborators	Purpose of collaboration	Outcomes
FLI, CIRAD, CICbioGUNE, IDvet, BI, NOTT, CSIC, CISA-INIA, WBVR, UP, UoS, ISRA, LCV	Improved control of priority animal diseases: Novel vaccines and companion diagnostic tests for African horse sickness, peste des petits ruminants and foot and-mouth disease (SPIDVAC)	Develop innovative vaccines and companion diagnostic tests for three priority animal diseases listed as notifiable terrestrial diseases by the World Organisation for Animal Health (WOAH): African horse sickness (AHS), peste des petits ruminants (PPR) and foot and mouth disease (FMD)

Collaborators	Purpose of collaboration	Outcomes
SLU, FLI, SAP, Sciensano	From proteogenomic host response signatures of persistent foot-and-mouth disease virus (FMDV) infection to diagnostic markers and therapeutic control (FMDV_PersistOmics)	Identification of host response signatures infection, diagnostic markers and therapeutic control of persistent foot-and-mouth disease virus (FMDV)

#### APQA, Republic of Korea

Collaborators	Purpose of collaboration	Outcomes
National Center for Veterinary Diagnosis, Department of Animal health, Hanoi, Vietnam	Studies on genetic characterization of FMD viruses and avian influenza virus in Vietnam	Virus isolates, Reports
(National Animal Health and Production Research Institute, Phnom Penh, Cambodia	Studies on genetic characterization of FMD viruses and avian influenza virus in Cambodia	Virus isolates, Reports
National Animal Health Laboratory, Vientiane, LAO PDR	Studies on genetic characterization of FMD viruses and avian influenza virus in LAO PDR	Virus isolates, Reports
Central Disease Investigation Laboratory, Dhaka, Bangladesh	Studies on genetic characterization of FMD viruses in Bangladesh	Virus isolates, Reports

#### ICAR-NIFMD, India

Collaborators	Purpose of collaboration	Outcomes
WRLFMD	FMD Vaccine Quality Testing and Enhancing India's Animal Vaccine Testing Capabilities	

#### IZSLER, Italy

Collaborators	Purpose of collaboration	Outcomes
The Pirbright Institute	Development of assays for FMD vaccine quality using VP4 as a marker for intact antigen'	Diagnostic Assay to evaluate the vaccine quality

Collaborators	Purpose of collaboration	Outcomes
The Pirbright Institute	Development of prototype ELISA tests for antibodies and antigen detection based on MAb, to fill gaps in the FMDV-SAT 3 diagnostic	Diagnostic Assay based on Mabs

## FADDL, USA

Collaborators	Purpose of collaboration	Outcomes
Central Luzon State University, Philippines	Surveillance and management strategies for ASF, CSF, JEV. Global Partnership for Animal Zoonotic Disease Surveillance.	The primary goal of the international collaboration is to establish a global network of partners for the surveillance and timely detection of high-consequence foreign animal and zoonotic disease threats to US agriculture and public health. Provided consultation and transferred technologies.
Makerere University, Uganda	Distribution, genetic diversity, and transmission dynamics of ASF	The primary goal of the international collaboration is to establish a global network of partners for the surveillance and timely detection of high-consequence foreign animal and zoonotic disease threats to US agriculture and public health. Provided consultation and transferred technologies.
African Center of Excellence for Genomics of Infectious Diseases, Nigeria	Next generation sequencing and genomic surveillance of ASF and JEV	The primary goal of the international collaboration is to establish a global network of partners for the surveillance and timely detection of high-consequence foreign animal and zoonotic disease threats to US agriculture and public health. Provided consultation and transferred technologies.
Department of Livestock Services, The Gambia	Surveillance of FMDV infectious in livestock	The primary goal of the international collaboration is to establish a global network of partners for the surveillance and timely detection of high-consequence foreign animal and zoonotic disease threats to US agriculture and public health. Provided consultation and transferred technologies.

Collaborators	Purpose of collaboration	Outcomes
West Africa Livestock Innovation Center, The Gambia	Epidemiology of Peste des Petits Ruminants	The primary goal of the international collaboration is to establish a global network of partners for the surveillance and timely detection of high-consequence foreign animal and zoonotic disease threats to US agriculture and public health. Provided consultation and transferred technologies.
University of Buea, Cameroon	Epidemiology of Peste des Petits Ruminants	The primary goal of the international collaboration is to establish a global network of partners for the surveillance and timely detection of high-consequence foreign animal and zoonotic disease threats to US agriculture and public health. Provided consultation and transferred technologies.
Canada	International partnership through the North American Foot and Mouth Disease Vaccine Bank (NAFMDVB)	Preparedness and response capabilities against FMD outbreaks in North America.
Republic of Georgia	Evaluation of RT-qPCR for differential detection of FMDV Serotypes	Establish the protocol / assay in the Republic of Georgia for use in surveillance or epidemiological studies. Building capacity to run the assay by conducting training.
Accra Veterinary Laboratory, Ghana	Surveillance and Molecular serotyping of FMD Samples collected during FMD outbreak.	The primary goal of the international collaboration is to establish a global network of partners for the surveillance and timely detection of high-consequence foreign animal and zoonotic disease threats to US agriculture and public health. Provided consultation and transferred technologies.

#### LVRI, China

Collaborators	Purpose of collaboration	Outcomes
Private sector	Private sector consultative committee (PSCC) virtual meeting	Meeting on 16 March 2023
CAAS-PAEC	CAAS-PAEC Collaboration meeting	Meeting on 19 May 2023

Lao People's Democratic Republic Cambodia	People's Republic of China-Lao People's Democratic Republic-Cambodia Livestock Sector Institutional Meeting	Meeting on 26 May 2023
SEACFMD	26th SEACFMD National Coordinators Meeting (2023.8.22-24)	Meeting on 22-24 August 2023
FMD sub-Association, CAAV	18th National Conference of FMD sub-Association, CAAV	Meeting

### NVRI, Nigeria

Collaborators	Purpose of collaboration	Outcomes
FADDL, USA	Global Partnership on Animal and Zoonotic Disease Surveillance	Expanding laboratory capacity

### PANAFTOSA/VPH, PAHO/WHO

Collaborators	Purpose of collaboration	Outcomes
Instituto Biológico – SP, Brazil	Post-vaccination monitoring. Percentage expectation of protection against FMD virus in systematically vaccinated cattle herds in Brazil	Considering serotype O, it was observed that animals between 0 and 6 months old, 30 DPV, had an EPP of 65.5%. Animals over 6 months old, with more than one dose of vaccine (30 DPV), had an EPP of over 77%, reaching 91.9% in animals over 24 months old. For serotype A, it was observed that the animals, regardless of age group, showed high EPP (30 DPV), with 78.71% in animals aged 0 to 6 months, reaching 98.75% in animals over 24 months. At the same time, the samples were analyzed using the ELISA 3ABC/EITB methodology and all the animals were negative for NSP.

Collaborators	Purpose of collaboration	Outcomes
Instituto Biológico – SP, Brazil  Ministry of Agriculture and Livestock of Brazil, Brazil	Production of reference serum panels for vaccine matching studies using bivalent and monovalent vaccines with serotypes O1 Campos and A24 Cruzeiro.	Twenty animals were vaccinated and followed for 60 days, with samples collected at 7 DPV, 14 DPV, 28-30 DPV, 7DPR, 14 DPR and 28-30 DPR.  FMD vaccination (primary vaccination) resulted in a mean EPP of 98.16% for serotype A and a mean EPP of 93.47% for serotype O at 28 days post-vaccination by ELISA CFL (liquid phase competition).  Considering VN, the results were slightly lower than those found in ELISA, but reached values above 70% from 7 DPR, both for serotype O and serotype A, rising to 80% from 14 DPR.
Ministry of Agriculture and Livestock of Brazil, Brazil	Evaluation of FMD virus inactivators in field samples.	This work aims to evaluate the efficacy of buffers in the inactivation of FMDV. The buffers have so far shown the ability to inactivate the virus, where the cells showed no cytopathic effect after exposure to the isolate with the buffer, but further analysis will be necessary to confirm this result.
Ministry of Agriculture and Livestock of Brazil, Brazil	Validation of rapid (commercial) tests for the detection of foot-and-mouth disease.	In progress.

#### RRLSEA, Thailand

Collaborators	Purpose of collaboration	Outcomes
DLD	Research collaboration	Research proposal
WOAH	Research collaboration/Support collaboration	PT support/Training support
WRL; Pirbright	Research collaboration/training/PT	PT support/Training support
ACDP	Research collaboration/training	Research collaboration/Training support
SATREPS	Research collaboration	Research collaboration/Training

Collaborators	Purpose of collaboration	Outcomes
(JICA; Japan)		support
NIAH; Japan	Research collaboration	Research collaboration

### Sciensano, Belgium

Collaborators	Purpose of collaboration	Outcomes
ANSES (France)	Joint EU-RL FMD	<a href="https://eurl-fmd.anses.fr/en/minisite/lrue-fievre-aphteuse/welcome-website-european-union-reference-laboratory-foot-and-mouth">https://eurl-fmd.anses.fr/en/minisite/lrue-fievre-aphteuse/welcome-website-european-union-reference-laboratory-foot-and-mouth</a>
BVI (Botswana)	Bilateral collaboration	Participation of Sciensano to interlaboratory comparison organised by BVI
NVI (Nigeria)	Bilateral collaboration	No projects ongoing in 2023
LNV (Burundi)	Bilateral collaboration	No projects ongoing in 2023

## SENASA, Argentina

Collaborators	Purpose of collaboration
USDA - INTA	Determination of the improvement of the antigenic spectrum by heterologous strains
RA.HO. 6	Genetic and antigenic characterization of circulating strains
Biogenesis Bago	Genetic and antigenic studies in new vaccine development
INTA	Induced immunity against FMDVS by formulations containing chimeric Virus Like Particles in the murine model
INTA	Molecular epidemiology models for the control of viral epidemics of livestock interest

## WBVR-Lelystad, Netherlands

Collaborators	Purpose of collaboration	Outcomes
Vaccine producers	Improve vaccine	Confidential
Nagendra Singanallur	Evaluation of NS ELISA in small ruminants	Similar sensitivity and specificity as in cattle
Chinese Accademia of Science	PhD project	characterisation of VHHs and use of VHH for serology in vaccinated animals

## WRLFMD

Collaborators	Purpose of collaboration	Outcomes
APQA , Republic of Korea	Molecular epidemiology of FMD in Asia	Collection of sequence data and phylogenetic analyses
IZSLER, Italy	IZSLER/Pirbright collaboration	Development of immunoassays for vesicular diseases
IZSLER, Italy	Improved tools for FMD vaccine quality control	Immunoassays for FMD vaccine integrity
ANSES, France	SAT2/XIV	Origin and spread of SAT2/XIV

Collaborators	Purpose of collaboration	Outcomes
RRLSEA, Thailand	Research collaboration / training / PT	PT support/Training support
ICAR-NIFMD, India	FMD Vaccine Quality Testing and Enhancing India's Animal Vaccine Testing Capabilities	

#### 4.5 *In vivo* potency studies undertaken during 2021

##### APQA, Republic of Korea

Purpose of study	Study design	Vaccine strain used in Korea	Challenge strain	Result
Protection test in the pigs of commercial vaccine or candidate	Single dose challenge (Challenged at 28 dpv)	O1 Manisa + O 3039 + A22 Iraq	O/SKR/6-2/2023/APQA	100 % (5/5)
		O/RUS/Primorsky/2014 + A/RUS/Zabaikalsky/2013	(O/ME-SA/Ind-2001e)	100 % (5/5)
		O1 Compos + A24 Cruzeiro + A/ARG/2001		100 % (5/5)
		O/SKR/BE/2017 + A/SKR/YC/2017 (Korean Vaccine candidate)		100 % (5/5)

##### FADDL, USA

Purpose of study	Study design (PD <sub>50</sub> )	Name of Vaccine strain (Lots)	Name of Challenge strain	PD <sub>50</sub> Value
NAVVCB (completed 21 March 2023)	PD <sub>50</sub>	Serotype A	Sensitive/CUI	Pass
NAFMDVB (completed Dec 2023)	PD <sub>50</sub>	Serotype SAT	Sensitive/CUI	Pass

##### FMD NRL, Kenya

Purpose of study	Study design (PD <sub>50</sub> )	Name of Vaccine strain (Lots)	Name of Challenge strain	PD <sub>50</sub> Value
Vaccine Lot* potency test	PD <sub>50</sub>	O K77/78 (2/23)	O K77/78	43.0

Purpose of study	Study design (PD <sub>50</sub> )	Name of Vaccine strain (Lots)	Name of Challenge strain	PD <sub>50</sub> Value
Vaccine Lot* potency test	PD <sub>50</sub>	A K5/80 (7/22 & 3/23)	A K5/80	29.5 & 43.0
Vaccine Lot* potency test	PD <sub>50</sub>	SAT1 T155/71 (1/23)	SAT1 T155/71	43.0
Vaccine Lot* potency test	PD <sub>50</sub>	SAT2 K52/84 (8/22 & 4/23)	SAT2 K52/84	29.5

\*using Kenya Veterinary Vaccines Production Institute (KEVEVAPI) vaccine

### ICAR-NIFMD, India

Purpose of study	Study design (i.e., PD <sub>50</sub> , PGP etc..)	Name of Vaccine strain	Name of Challenge strain	Result
Vaccine QC testing	A group of 12 FMD sero-negative calves were selected for each batch of testing consisting of non-vaccinated control (2), safety testing (2) and potency test (8). The calves were vaccinated 2 times at 28 days interval and pre vaccination (0 day) as well as 28 days post vaccination serum was evaluated for serotype specific FMD antibody titer using VNT for potency. A booster was administered to calves in the potency group at 28 days post primo vaccination and animals were sampled at 28 days post booster (56 days post primo-vaccination) and tested in 3AB3NSP ELISA for NSP antibody purity.	O/INDR2/1975, A/IND40/2000 and As/IND63/1972	N/A	During the year 2023, seven batches of FMD vaccines have been tested and reports were communicated to DAHD, GoI.

### LVRI, China

- In vivo potency study
  - 16 cattle vaccinated with O/MAY98/BY/2010+Re-A/WH/09
  - 2 control animals unvaccinated.
  - They were all challenged with field isolate (2023015 [O/Ind-2001e])
  - 15 of the 16 animals were protected

## WBVR-Lelystad, Netherlands

Purpose of study	Study design	Name of Vaccine strain	Name of Challenge strain	Result
Dose finding study	4 x 5 vac + 2 control	Confidential	Confidential	Confidential
PD <sub>50</sub> study	3 x 5 vac + 2 control	Confidential	Confidential	Confidential
Dose finding study	4 x 5 vac + 2 control	Confidential	Confidential	Confidential
Dose finding study	3 x 8 vac + 2 control	Confidential	Confidential	Confidential

## Appendix 1 Details of clinical samples from field cases from countries in FMDV endemic regions tested during 2023

Laboratory	Samples from	Total	O	A	C	Asia 1	Sat 1	Sat 2	Sat 3	Untyped	NVD	Comments
AHI	Ethiopia	88	16	10	-	-	4	30	-	-	28	
ANSES	Comoros	1	-	-	-	-	1	-	-	-	-	
	Algeria	22	-	-	-	-	-	22	-	-	-	
	Tunisia	14	-	-	-	-	-	-	-	14	-	
APQA	Korea (Rep. of)	12	12	-	-	-	-	-	-	-	-	
ARC-OVI	South Africa	71	-	-	-	-	-	-	-	-	71	
BVI	Botswana	21	-	-	-	-	2	-	-	-	19	
	Ethiopia	27	4	8	-	-	-	-	-	-	15	
	Malawi	7	-	-	-	-	-	3	-	-	4	
FGI-ARRIAH	-	-	-	-	-	-	-	-	-	-	-	
FMD laboratory	Kenya	41	16	2	-	-	5	1	-	-	17	
ICAR-NIFMD	India	426	102	42	-	4	-	-	-	-	278	
IZSLER	Libya	18	6	-	-	-	-	-	-	5	7	
LVRI	China	26	-	-	-	-	-	-	-	18	8	
NCFAD	None from Endemic countries	-	-	-	-	-	-	-	-	-	-	
NVRI	Nigeria	17	7	3	-	-	-	1	-	-	6	
RRLSEA	Thailand	34	13	-	-	-	-	-	-	10	11	
Şap Institute	Türkiye	244	88	-	-	-	-	156	-	-	-	
SENASA	Argentina	4	-	-	-	-	-	-	-	-	4	Poxvirus detected
	Vietnam	21	21	-	-	-	-	-	-	-	-	
WRLFMD	Argentina	4	1	3	-	-	-	-	-	-	-	
	Bahrain	10	-	-	-	-	-	5	-	3	2	
	Bolivia	2	1	1	-	-	-	-	-	-	-	Historical sample
	Botswana	4	-	-	-	-	-	-	-	1	3	
	Brazil	1	-	1	-	-	-	-	-	-	-	Historical sample
	Ecuador	1	1	-	-	-	-	-	-	-	-	Historical sample
	Iraq	12	-	-	-	-	-	10	-	2	-	
	Israel	6	6	-	-	-	-	-	-	-	-	
	Jordan	27	-	-	-	-	-	23	-	4	-	
	Malawi	2	2	-	-	-	-	-	-	-	-	
	Mozambique	1	-	-	-	-	-	1	-	-	-	
	Nepal	80	36	-	-	-	-	-	-	31	13	
	Nigeria	13	10	-	-	-	-	-	-	3	-	
	Namibia	2	-	-	-	-	-	2	-	-	-	
Pakistan	50	11	5	-	12	-	-	-	12	11	1 sample pos. for O & A	

Laboratory	Samples from	Total	O	A	C	Asia 1	Sat 1	Sat 2	Sat 3	Untyped	NVD	Comments
	Paraguay	2	2	-	-	-	-	-	-	-	-	Historical sample
	Palestine, State of	6	4	-	-	-	-	-	-	2	-	
	Qatar	7	-	-	-	-	7	-	-	-	-	
	Korea, Republic Of (South)	1	1	-	-	-	-	-	-	-	-	
	Thailand	24	17	7	-	-	-	-	-	-	-	
	Türkiye	20	3	-	-	-	-	17	-	-	-	
	Uganda	27	4	-	-	-	-	-	-	5	18	
	Uruguay	3	1	2	-	-	-	-	-	-	-	Historical sample
	Venezuela	2	-	1	-	-	-	-	-	1	-	Historical sample
	Zimbabwe	1	-	-	-	-	-	1	-	-	-	

## Appendix 2 Vaccine matching studies undertaken by Network partners during 2023

Vaccine efficacy is influenced by both vaccine potency and vaccine match and it is possible that a poor match may to some extent be compensated by high potency vaccines and by administering more than one dose at suitable intervals. The use of oil adjuvant is also expected to improve efficacy. Thus, a vaccine with a weak antigenic match to a field isolate, as determined by serology, may nevertheless afford some protection if it is of sufficiently high potency. Therefore, in the absence of a good match, or where the match is unknown, vaccines of high potency should preferably be used. The  $r_1$  values shown below, represent the one way serological match between vaccine strain and field isolate, calculated from the comparative reactivity of an antiserum, raised against the vaccine in question, to the vaccine virus and the field isolate.

### Key:

M	Matched with the vaccine
B	Borderline
N	Not matched with the vaccine

### For VNT:

$r_1$  greater than 0.3 - suggest that there is a close antigenic relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection.

$r_1$  less than 0.3 - suggest that the field isolate is antigenically different to the vaccine strain. Where there is no alternative, the use of this vaccine should carefully consider vaccine potency, the possibility to use additional booster doses and monitoring of vaccinated animals for heterologous responses.

### For LB-ELISA:

$r_1 \geq 0.4$  – suggest that there is a close relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection

$r_1 \leq 0.4$  - suggest that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect.

### APQA, Republic of Korea

Country/Year	Field virus		Vaccine strain				
	Serotype / Topotype / Genotype	O/3039	O/RUS/Primorsky/2014	O1/Campos	O/PanAsia2 (Korean Vaccine candidate)	O/SKR/BE/2017 (Korean Vaccine candidate, Ind2001)	
O/SKR/6- 2/2023/APQA	O/ME-SA/Ind-2001e	M	M	M	M	M	

### BVI, Botswana

Virus Isolate	Vaccine strain (r <sub>1</sub> Value)	
	SAT 2035	SAT 251
MAL 05/2023	0.82	0.65

### ICAR-NIFMD, India

Sample Name	Serotype/Topotype/ Genotype	Vaccine Strains			
		O/INDR2/1 975	A/IND40/20 00	A/IND27/20 11	As/IND63/1 972
O/ICFMD47/2022	O/ME-SA/SA-2018	M			
O/ICFMD/79/2022	O	M			
O/ICFMD/160/2022	O/ME-SA/SA-2018	M			
O/ICFMD/182/2022	O/ME-SA/SA-2018	M			
O/ICFMD/325/2022	O	M			
O/ICFMD/49/2023	O/ME-SA/Ind2001e	M			
O/IC 83/2023	O/ME-SA/Ind2001e	M			
O/IC 86/2023	O/ME-SA/Ind2001e	M			
O/IC 91/2023	O/ME-SA/SA-2018	M			
O/IC 92/2023	O/ME-SA/SA-2018	M			
O/IC 109/2023	O/ME-SA/SA-2018	M			
O/IC 129/2023	O/ME-SA/SA-2018	M			
O/IC 130/2023	O/ME-SA/SA-2018	M			
O/IC 131/2023	O/ME-SA/SA-2018	M			
O/IC 195/2023	O/ME-SA/SA-2018	M			
A/ICFMD195/2022	A/ASIA/G-18/2019		N	M	
A/ICFMD196/2022	A/ASIA/G-18/2019		N	M	

A/ICFMD197/2022	A/ASIA/G-18/2019	N	M	
A/ICFMD117/2023	A/ASIA/G-18/2019	N	M	
As/ICFMD 45/2023	Asia-1/ASIA/IX			M

### LVRI, China

Field isolate	Lineage	Vaccine strain		
		O/MAY98/BY/2010	Re-O	re-O/17002
2022-031	O/CATHAY	N	N	M
2023007-013	O/CATHAY	M	M	M
2023039-28	O/CATHAY	N	M	M
2023015	O/Ind-2001	M		
2023025	O/Ind-2001	M		

### RRLSEA, Thailand

#	Location	Date submitted	Vaccine matching by LP ELISA with Thai seed virus vaccine	
			O/189	O/189 + O/CATHAY
1	Prachuap Khiri Khan	10/12/2022	0.25	0.5
2	Chiang Rai	10/12/2022	0.38	1
3	Mae Hong Son	10/12/2022	0.5	1
4	Lop Buri	10/12/2022	0.75	1
5	Nakhon Si Thammarat	5/1/2023	0.25	0.5
6	Nakhon Si Thammarat	5/1/2023	0.19	0.38
7	Saraburi	10/1/2023	0.75	1

### Şap Institute, Türkiye

Sample Name	VNT r1 value SAT2 XIV
SAT2/TUR/613/23	0.92
SAT2/TUR/629/23	1
SAT2/TUR/782/23	0.712
SAT2/TUR/785/23	1

### SENASA, Argentina

Sample Name	VNT r1 value
-------------	--------------

	BB O1 CAMPOS monovalent - swine	CDV O1 CAMPOS monovalente - cattle	CDV O/A/A trivalent - cattle
O/VIT/15/19	0.43	0.42	
O/VIT/13/20	0.77	0.61	1
O/VIT/19/19	1	1	1
O1 MANISA		0.45	1
O/GAM/1/18		1	
O/PAK/3/20		0.78	0.3

### WRLFMD

Isolate	Serotype O		O Campos Boehringer Ingelheim		O Manisa Boehringer Ingelheim		O Panasia 2 Boehringer Ingelheim		O/TUR/5/09 MSD Animal Health		O1 Campos Biogènesis Bagó		O-3039 Boehringer Ingelheim	
	Topotype	Lineage	r <sub>1</sub>	titre	r <sub>1</sub>	titre	r <sub>1</sub>	titre	r <sub>1</sub>	titre	r <sub>1</sub>	titre	r <sub>1</sub>	titre
UGA 17/2022	EA-2	-	0.40	1.98	0.57	2.15	0.49	2.03	0.49	2.03	0.35	2.43	0.75	1.88
UGA 27/2022	EA-2	-	0.32	1.89	0.52	2.11	0.43	1.97	0.53	2.06	0.44	2.53	0.95	1.97
EGY 8/2021	EA-3	-	0.69	2.2	0.54	2.32	0.73	2.51	0.89	2.44	0.76	2.81	0.66	1.99
EGY 10/2021	EA-3	-	0.39	1.96	0.48	2.27	0.39	2.23	0.39	2.08	0.46	2.6	0.36	1.73
NEP 58/2021	ME-SA	Ind-2001	0.16	1.87	0.49	2.22	0.21	1.87	0.78	2.21	0.20	2.28	0.51	1.7
SKR 1/2023	ME-SA	Ind-2001	0.77	2.23	0.74	2.23	0.98	2.37	0.8	2.24	0.88	2.74	0.93	2.02
TAI 5/2022	ME-SA	Ind-2001	0.32	2.22	0.55	2.27	0.60	2.31	1	2.39	0.85	2.83	0.91	2
TAI 1/2023	ME-SA	Ind-2001	0.15	1.9	0.4	2.14	0.33	2.06	0.63	2.16	0.35	2.44	0.44	1.69
ISR 12/2022	ME-SA	PanAsia-2	0.24	1.72	0.38	1.89	0.34	1.9	0.43	1.81	0.59	2.52	0.32	1.66
ISR 2/2023	ME-SA	PanAsia-2	0.26	1.75	0.32	1.85	0.28	1.8	0.47	1.84	0.45	2.4	0.38	1.74
PAK 9/2022	ME-SA	PanAsia-2	0.16	1.66	0.28	1.91	0.27	1.88	0.26	1.88	0.13	2.04	0.41	1.79
PAK 2/2023	ME-SA	PanAsia-2	0.36	2	0.35	1.98	0.36	2.01	0.90	2.22	0.35	2.48	0.41	1.8
PAT 3/2022	ME-SA	PanAsia-2	0.29	1.79	0.52	1.9	0.27	1.93	0.59	2.02	0.51	2.43	0.65	1.82
PAT 2/2023	ME-SA	PanAsia-2	0.30	1.76	0.37	1.76	0.15	1.68	0.58	2.01	0.34	2.26	0.52	1.62
TUR 1/2023	ME-SA	PanAsia-2	0.28	1.93	0.51	2.04	0.37	2.14	0.56	1.97	0.63	2.61	0.37	1.65
TUR 2/2023	ME-SA	PanAsia-2	0.25	1.88	0.34	1.86	0.34	2.06	0.67	2.05	0.56	2.56	0.33	1.6
TUR 3/2023	ME-SA	PanAsia-2	0.32	1.99	0.48	2.01	0.31	2.02	0.55	1.96	0.62	2.6	0.45	1.73
NEP 13/2022	ME-SA	SA-2018	0.40	2.28	0.63	2.33	0.57	2.35	1	2.32	0.51	2.65	1	2.02
NEP 26/2022	ME-SA	SA-2018	0.23	2.03	0.32	2.04	0.32	2.1	0.68	2.15	0.20	2.29	0.66	1.82
TAI 16/2022	SEA	Tai-87	0	0	0.06	1.3	0.11	1.57	0.18	1.61	0.08	1.78	0.18	1.29

Isolate	Serotype A		A Eritrea 98 Boehringer Ingelheim		A GVII 2015 Boehringer Ingelheim		A Iran 05 Boehringer Ingelheim		A Malaysia 97 Boehringer Ingelheim		A Saudi 95 Boehringer Ingelheim		A/TUR/20/0 6 MSD Animal Health		A22 Iraq Boehringer Ingelheim	
	Topotype	Lineage	r <sub>1</sub>	titre	r <sub>1</sub>	titre	r <sub>1</sub>	titre	r <sub>1</sub>	titre	r <sub>1</sub>	titre	r <sub>1</sub>	titre	r <sub>1</sub>	titre
EGY 7/2021	AFRICA	G-IV	0.21	1.98	0.04	0.62	0.07	1.47	NT		0.11	1.51	0.05	1.26	0.08	1.62
EGY 1/2022	AFRICA	G-IV	0.16	1.86	0.01	0.14	0.22	1.98	NT		0.03	0.92	0.03	1.03	0.13	1.86
PAK 4/2023	ASIA	Iran-05	NT		0.20	1.24	0.16	1.77	0.15	1.72	NT		0.26	1.85	0.25	2.05
PAK 5/2023	ASIA	Iran-05	NT		0.14	1.08	0.23	1.91	0.11	1.59	NT		0.25	1.83	0.20	1.96
TAI 8/2021	ASIA	Sea-97	NT		0.54	1.75	0.14	1.67	0.45	2.06	NT		0.17	1.64	0.25	1.95
TAI 1/2022	ASIA	Sea-97	NT		0.38	1.6	0.08	1.46	0.32	1.92	NT		0.19	1.7	0.18	1.8
TAI 14/2022	ASIA	Sea-97	NT		0.71	1.87	0.17	1.76	0.66	2.23	NT		0.29	1.89	0.28	2
EGY 2/2022	EURO-SA	-	0.10	1.65	0.13	1.13	0.06	1.42	NT		0.1	1.48	0.07	1.42	0.07	1.57

Isolate	Serotype Asia 1		Asia 1 Shamir <i>Boehringer Ingelheim</i>	
	Topotype	Lineage	r <sub>1</sub>	titre
PAK 26/2022	ASIA	Sindh-08	0.40	2.14
PAK 39/2022	ASIA	Sindh-08	0.33	2.06

Isolate	Serotype SAT 1		SAT1 Rho 78 <i>Boehringer Ingelheim</i>	
	Topotype	Lineage	r <sub>1</sub>	titre
QTR 5/2023	I (NWZ)	-	0.39	1.85
QTR 6/2023	I (NWZ)	-	0.41	1.86
QTR 7/2023	I (NWZ)	-	0.35	1.8

Isolate	Serotype SAT 2		SAT2 Eritrea 98 <i>Boehringer Ingelheim</i>		SAT2 OMN 2015 <i>Biogénesis Bagó</i>		SAT2 Zim 83 <i>Boehringer Ingelheim</i>	
	Topotype	Lineage	r <sub>1</sub>	titre	r <sub>1</sub>	titre	r <sub>1</sub>	titre
BAR 2/2022	XIV	-	0.40	1.5	NT		0.40	1.9
BAR 7/2022	XIV	-	0.50	1.6	NT		0.50	2.0
ETH/2/2022	XIV	-	0.80	1.6	NT		0.50	2.2
ETH/3/2022	XIV	-	0.50	1.7	NT		0.20	1.7
ETH/3/2022	XIV	-	0.50	1.7	NT		0.20	1.7
IRQ/2/2022	XIV	-	0.70	1.8	NT		0.40	2.0
IRQ/5/2023	XIV	-	0.50	1.6	NT		0.70	2.3
IRQ/9/2023	XIV	-	0.40	1.5	NT		0.30	2.0
JOR 11/2023	XIV	-	0.60	1.7	NT		0.20	1.7
JOR 20/2023	XIV	-	0.80	1.9	NT		0.20	1.8
JOR 26/2023	XIV	-	0.90	1.9	0.40	2.4	0.30	1.9
TUR 4/2023	XIV	-	1	1.7	NT		0.30	2.0
TUR 17/2023	XIV	-	0.70	1.5	NT		0.30	1.9

NT – not tested

## Appendix 3 Nucleotide sequence analysis

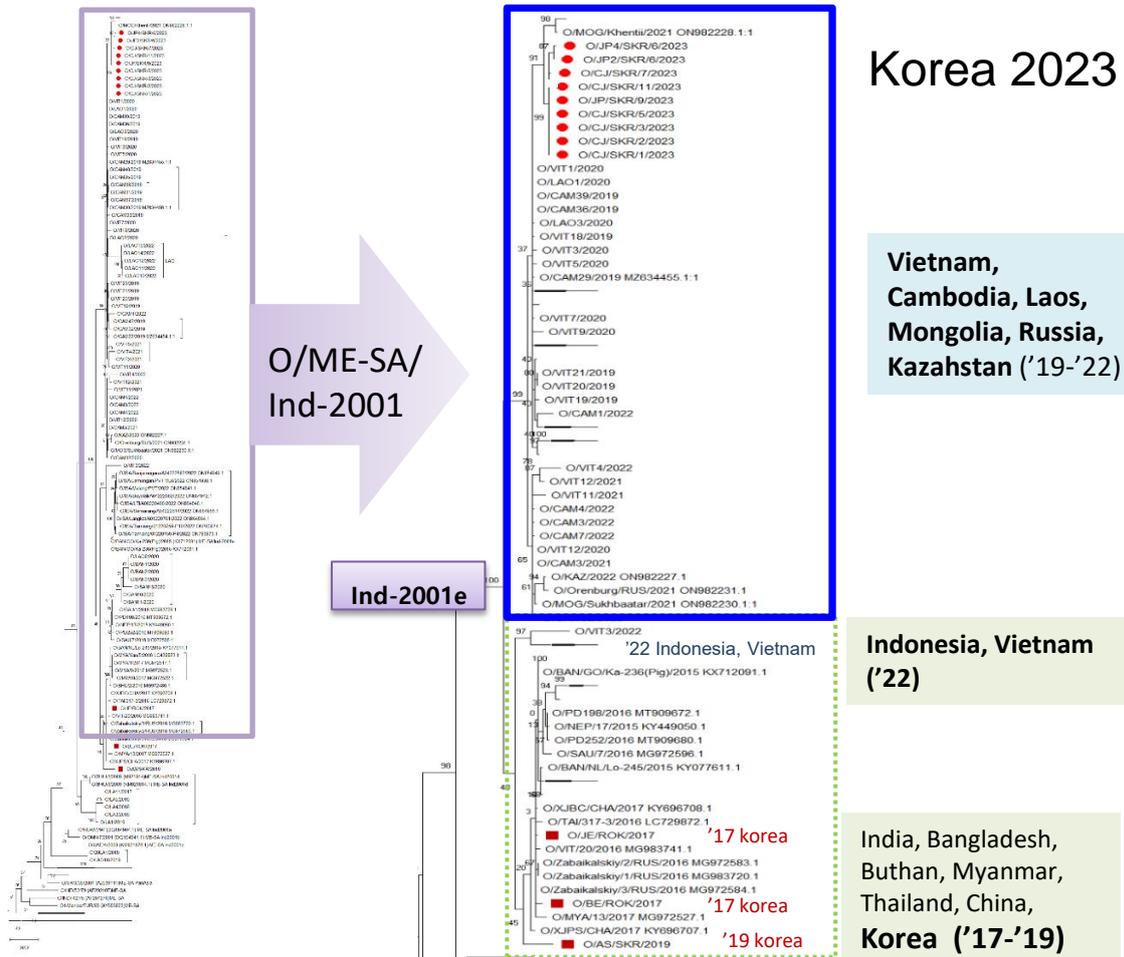
FMDV nucleotide sequence data for phylogenetic analysis

Testing Laboratory	Sample Country	Region Sequenced	Total	O	A	C	ASIA-1	SAT 1	SAT 2	SAT 3	FMDV GD	Notes
APQA	Korea (Rep. of)	VP1	9	9	-	-	-	-	-	-	-	
ARC-OVI	Botswana		4	-	-	-	-	2	2	-	-	
	Kenya		1	-	-	-	-		1	-	-	
	Malawi		1	-	-	-	-		1	-	-	
	Mozambique		6	-	-	-	-	4	2	-	-	
	Namibia		2	-	-	-	-	2	2	-	-	
	Nigeria	Complete	1	-	-	-	-	1		-	-	Historical samples. Draft data from meeting
	South Africa	Genome	21	-	-	-	-	12	9	-	-	
	Sudan		1	-	-	-	-	1		-	-	
	Eswatini		1	-	-	-	-	1		-	-	
	Tanzania		1	-	-	-	-	1		-	-	
	Zambia		4	-	-	-	-	2	2	-	-	
Zimbabwe		3	-	-	-	-	2	1	-	-		
BVI	Botswana	VP1	2	-	-	-	-	2	-	-	-	
	Ethiopia	VP1	12	11	1	-	-	-	-	-	-	
	Malawi	VP1	3	-	-	-	-	-	3	-	-	
FGI-ARRIAH	-	-	-	-	-	-	-	-	-	-	No data from meeting or returned	
ICAR-NIFMD	India	VP1	55	38	16	-	1	1	1	1	1	
IZSLER	Libya	VP1	6	6	-	-	-	-	-	-	-	
LVRI	China	VP1	60	60	-	-	-	-	-	-	-	
NCFAD	-	-	-	-	-	-	-	-	-	-	-	Draft data from meeting
ŞAP Institute	Türkiye	VP1	30	12	-	-	-	-	18	-	-	
SENASA	PANAFTOSA	VP1	2	1	1	-	-	-	-	-	-	
	Vietnam	VP1	21	20	-	-	-	-	-	-	1	
	SENASA Ceparium	VP1	7	7	-	-	-	-	-	-	-	
WRLFMD	Argentina	VP1	4	1	3	-	-	-	-	-	-	Historical sample
	Bahrain	VP1	5	-	-	-	-	-	5	-	-	
	Bolivia	VP1	2	1	1	-	-	-	-	-	-	Historical sample
	Brazil	VP1	1	-	1	-	-	-	-	-	-	Historical sample
	Colombia	VP1	5	4	1	-	-	-	-	-	-	Historical sample
	Ecuador	VP1	1	1	-	-	-	-	-	-	-	Historical sample
	Iraq	VP1	10	-	-	-	-	-	10	-	-	
	Israel	VP1	6	6	-	-	-	-	-	-	-	
	Jordan	VP1	23	-	-	-	-	-	23	-	-	
Malawi	VP1	2	2	-	-	-	-	-	-	-		

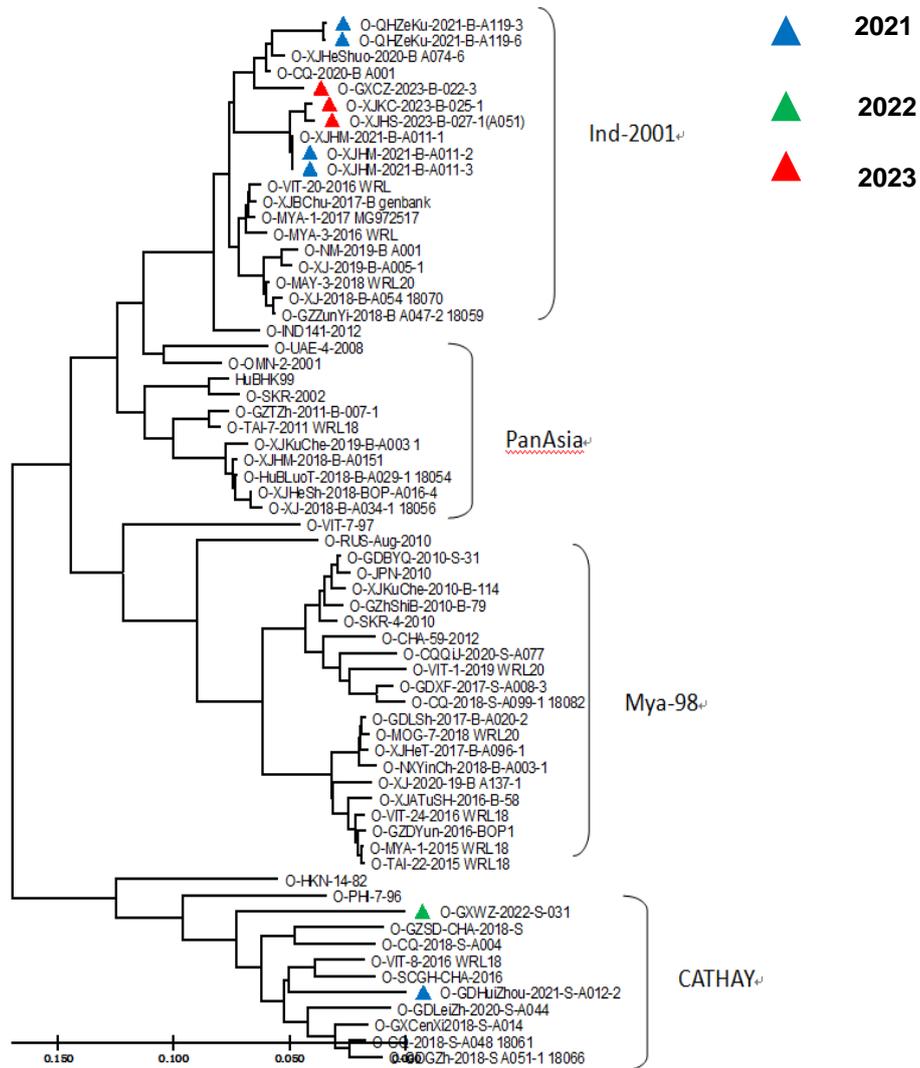
Testing Laboratory	Sample Country	Region Sequenced	Total	O	A	C	ASIA-1	SAT 1	SAT 2	SAT 3	FMDV GD	Notes
	Mozambique	VP1	1	-	-	-	-	-	1	-	-	
	Nepal	VP1	37	37	-	-	-	-	-	-	-	
	Nigeria	VP1	10	10	-	-	-	-	-	-	-	
	Namibia	VP1	2	-	-	-	-	-	2	-	-	
	Pakistan	VP1	29	11	6	-	12	-	-	-	-	
	Paraguay	VP1	2	2	-	-	-	-	-	-	-	Historical sample
	Palestine, State of	VP1	4	4	-	-	-	-	-	-	-	
	Qatar	VP1	7	-	-	-	-	7	-	-	-	
	Korea, Republic Of (South)	VP1	1	1	-	-	-	-	-	-	-	
	Thailand	VP1	24	17	7	-	-	-	-	-	-	
	Türkiye	VP1	21	3	-	-	-	-	18	-	-	
	Uganda	VP1	4	4	-	-	-	-	-	-	-	
	Uruguay	VP1	3	1	2	-	-	-	-	-	-	Historical sample
	Venezuela	VP1	2	-	2	-	-	-	-	-	-	Historical sample
	Zimbabwe	VP1	1	-	-	-	-	-	1	-	-	

## Appendix 4 Selected phylogenetic trees for 2023

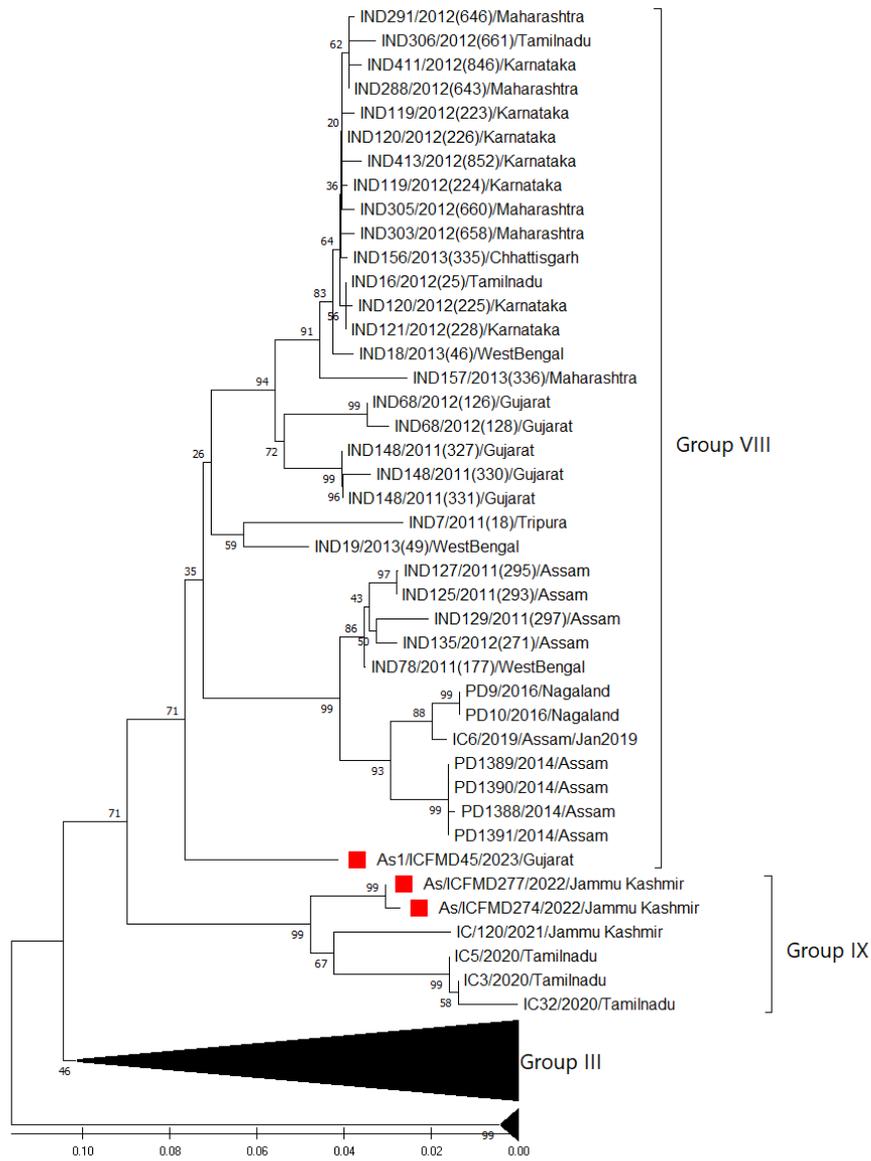
### A4.1 – O/ME-SA/Ind2001e outbreaks in the Republic of Korea (APQA)



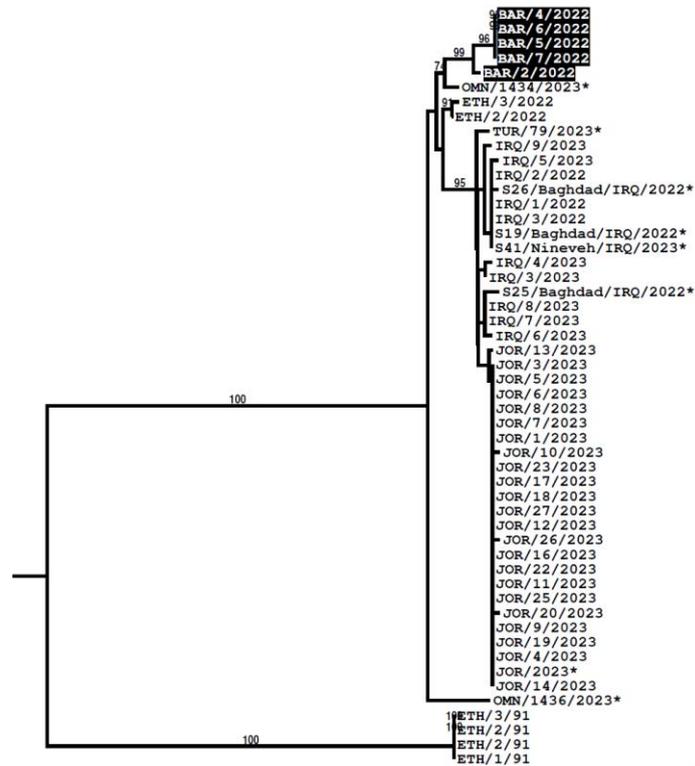
### A4.2 – O/ME-SA/Ind2001e outbreaks in China (LVRI)



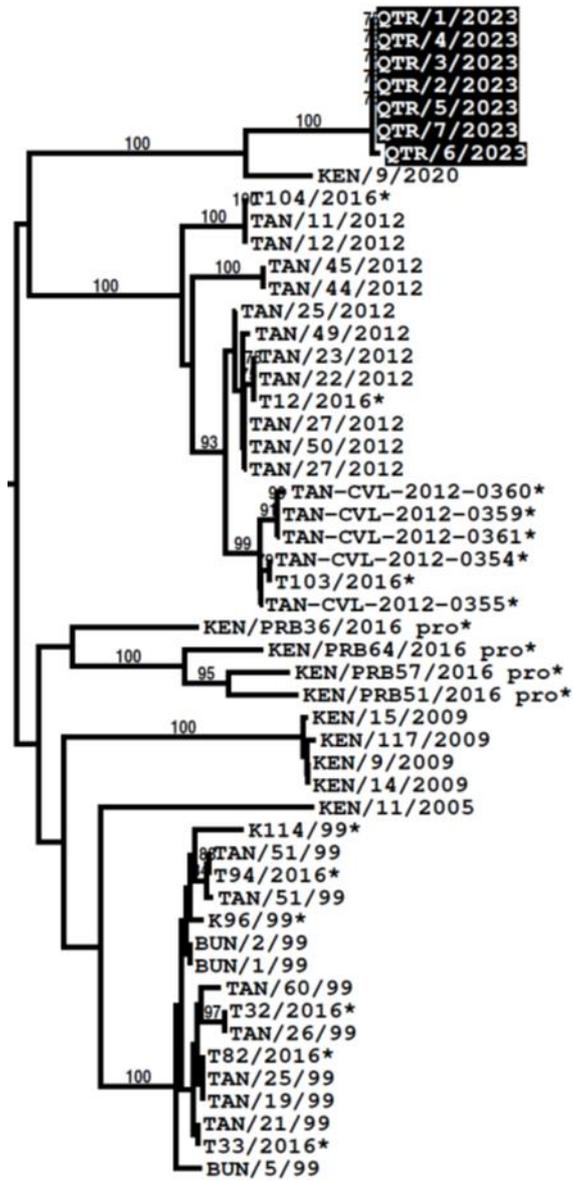
### A4.3 – Serotype Asia 1 lineages in India (ICAR-NIFMD)



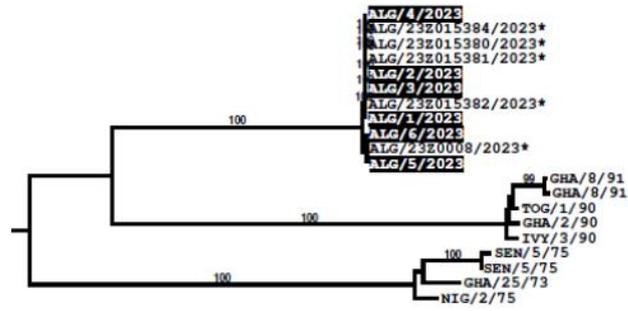
#### A4.4 – SAT2/XIV topotype sequences from the Middle East (WRLFMD with data shared from other partners)



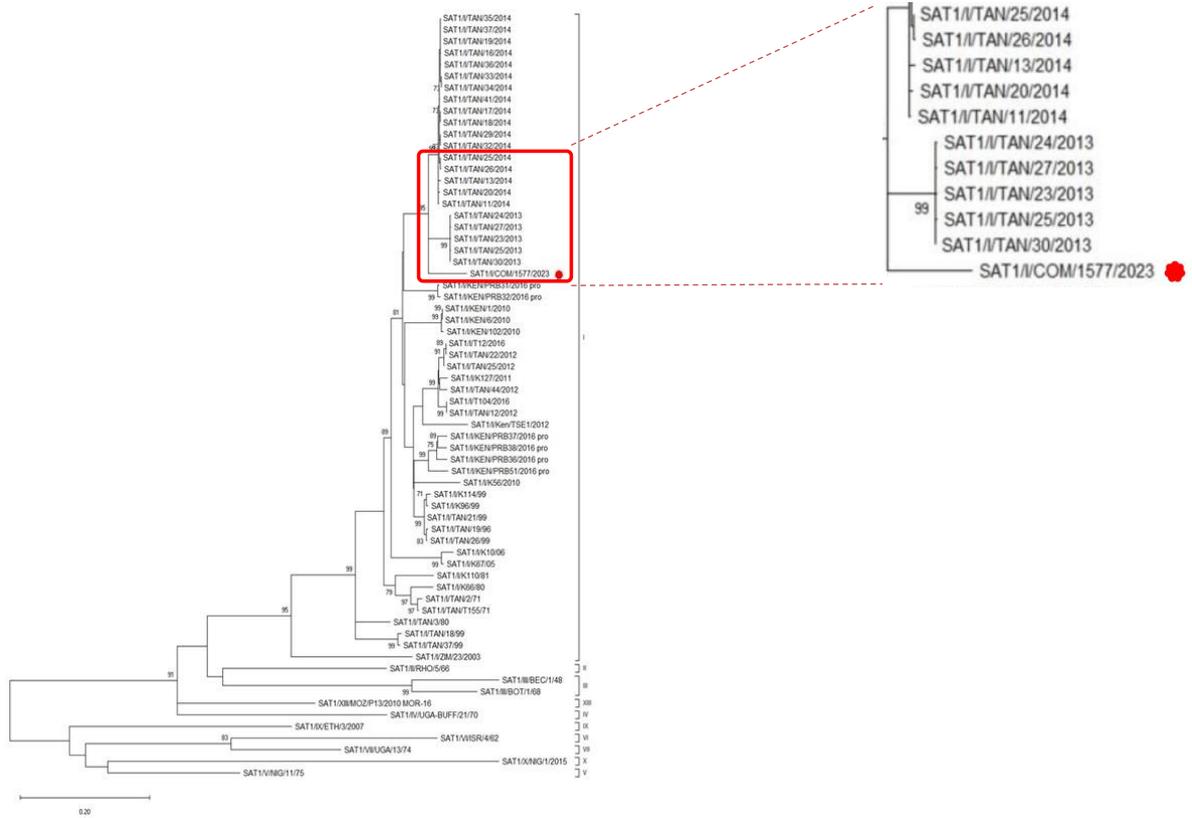
### A4.5 – SAT1/I topotype sequences from Qatar (WRLFMD)



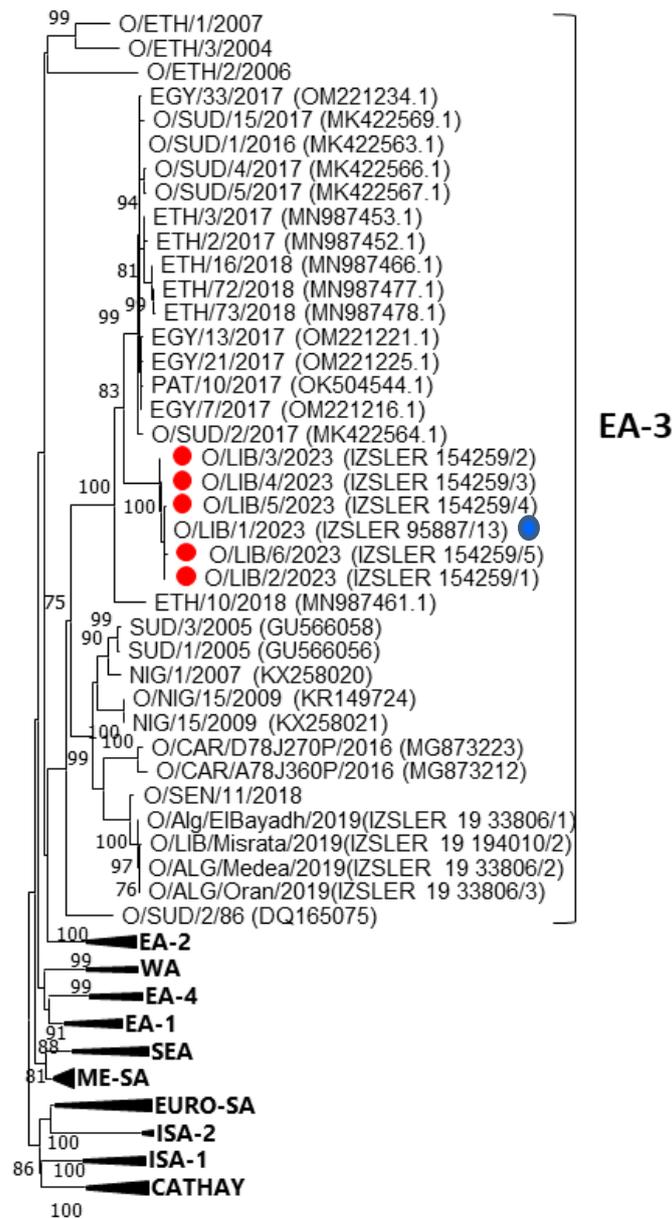
#### A4.6 – SAT2/V topotype sequences from Algeria (ANSES/WRLFMD)



### A4.7 – SAT1/I toptotype sequences from the Comoros (ANSES)



### A4.7 – O/EA-3 sequences from Libya (IZSLER)



## Appendix 5 The 18<sup>th</sup> Annual Meeting of the WOA/FAO FMD Reference Laboratory Network

10<sup>th</sup> to the 12<sup>th</sup> October 2023

Hosted by: NCFAD, Winnipeg Canada



## Core Members

	WOAH Reference Laboratory for FMD, Dirección de Laboratorio Animal, SENASA, Argentina Speaker: Sabrina Galdo Novo
	WOAH collaborating Centre for validation, quality assessment and quality control of diagnostic assays and vaccine testing for vesicular diseases in Europe, and FAO Reference Centre for Vesicular Diseases Sciensano, Belgium Speaker: David Lefebvre; Participant: Nick De Regge
	WOAH Reference Laboratory for FMD Botswana Vaccine Institute (BVI), Botswana Speaker: Elliot Fana
	Centro Panamericano de Fiebre Aftosa (PANAFTOSA) and PAHO /WHO and WOA Reference Laboratory for FMD, Brazil Speaker: Edvigés Maristela Pituco
	FAO and WOA FMD Reference Laboratory, National Centre for Foreign Animal Disease National Centres for Animal Disease, Canadian Food Inspection Agency, Canada Speaker: Shawn Babiuk; Participants: Charles Nfon, Michele Roy
	WOAH and China National FMD Reference Laboratory, Lanzhou Veterinary Research Institute (LVRI), CAAS, People's Republic of China Speaker: Wen Dang
	WOAH FMD Reference Laboratory, French Agency for Food and, Environmental and Occupational Health & Safety (ANSES), France Speaker: Guillaume Girault; Participant: Labib Bakkali Kassimi
	FAO Reference Centre for FMD in South Asia, ICAR – Directorate of Foot-and-Mouth Disease, Indian Council for Agricultural Research, Mukteswar, Nainital (Uttarakhand), India Speaker: Rabindra Prasad Singh
	WOAH/FAO FMD Reference Laboratory, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Italy Speaker: Santina Grazioli
	WOAH Reference laboratory for FMD, Animal and Plant Quarantine Agency (APQA), Republic of Korea Speaker: Jong-Hyeon Park; Participants: Soyeon Ryoo, Sung-Han Park
	FAO FMD Reference Laboratory, Wageningen Bioveterinary Research, Lelystad, Netherlands Speaker: Aldo Dekker; Participant: Phaedra Eble
	FAO Reference Centre for FMD for Central Asia and West Eurasia and WOA Reference Laboratory for FMD, Federal Governmental Institute, Centre for Animal Health (FGI ARRIAH), Vladimir, Russian Federation Speaker: Viktor Nikiforov
	FAO Reference Laboratory for FMD in Africa and WOA FMD Reference Laboratory, Transboundary Animal Diseases Programme, ARC-Onderstepoort Veterinary Institute (ARC-OVI), South Africa Speaker: Livio Heath
	Department of Livestock Development, Pakchong, Thailand Speaker: Kingkarn Boonsuya Seeyo
	FAO World Reference Laboratory (WRLFMD) and WOA FMD Reference Laboratory The Pirbright Institute Pirbright, United Kingdom Speakers: Donald King, Anna Ludi, Antonello Di Nardo

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 WOAHD FMD Reference Laboratory, Foreign Animal Disease Diagnostic Lab, Plum Island Animal Disease Center (PIADC), United States of America  
Speaker: Vivienne O'Donnel, Participant: Fawzi Mohammed

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### Affiliates

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	Animal Health Institute (AHI), Ethiopia Speaker: Daniel Gizaw
	Foot and Mouth Disease Laboratory, Kenya Speaker: Abraham Sangula
	National Veterinary Research Institute, Vom, Plateau State, Nigeria Speaker: Hussaini Ularanu
	Şap Institute (and WELNET FMD), Ankara, Turkey Speaker: Naci Bulut

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### WOAH/FAO Representatives

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	The European Commission for the Control of Foot-and-Mouth Disease Speaker: Donal Sammin Participant: Fabrizio Rosso
	Food and Agriculture Organization of the United Nations Speakers: Melissa McLaws, Samia Metwally Participant: Metlin Artem
	World Organisation for Animal Health Speakers: Min-Kyung Park

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### Invited Speakers

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	USDA, Fort Collins USA Speaker: Sarah Mielke
	The Pirbright Institute Speakers: Ryan Waters, Toby Tuthill
	FAO and WOAHD FMD Reference Laboratory, National Centre for Foreign Animal Disease National Centres for Animal Disease, Canadian Food Inspection Agency, Canada Speaker: Oliver Lung

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### Vaccine Producers

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	Boehringer-Ingelheim, VPH Veterinary Public Health Participants: Pascal Hudelet
	Biogenesis-Bago Participants: Romina Scian
	MERCK MSD, MERCK Participants: Chriche du Plessis

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Apologies from: Australian Centre for Disease Preparedness (ACDP), Australia

## TUESDAY 10<sup>th</sup> NOVEMBER 2023, DAY 1

### Opening of the 18<sup>th</sup> annual meeting and adoption of the agenda

Dr Mary-Jane Ireland (CVO Canada) opened the meeting and welcomed all participants to Canada. She noted that FMD is an important transboundary disease that poses risks for all endemic and free countries. She acknowledged the importance of the meeting to exchange and facilitate collaboration.

Dr Silva Primal (CFIA/ACIA) thanked the meeting organisers (Charles Nfon, Michele Roy, and Sarah Belgrave), sponsors (EuFMD, WOA, Biogenesis Bago, Boehringer Ingelheim and MSD) and participants. He applauded the efforts of the Network to provide sound advice on how FMD spreads around the globe and what vaccines and diagnostic techniques to use.

### Adoption of agenda and overview of lab network - Don King

The Network was established in 2004 with two core activities: (1) to understand global FMD virus distribution patterns and use these data to inform vaccine recommendation and (2) to harmonise and improve the quality of laboratory testing carried out by international and national reference laboratories. Dr King acknowledged the continued essential support for the Network from EuFMD and WOA and sponsorship from vaccine companies (Biogenesis Bago, Boehringer Ingelheim and MSD) to help offset the costs of the Annual meeting. The meeting agenda was unanimously adopted.

### Brief update from WOA – Min-Kyng Park

The first part of the presentation reviewed recent changes to the WOA official animal health status. In the 2022/2023 evaluation cycle, a total of 11 new applications were received. Four of these were for FMD: FMD free without vaccination status (1 zone), FMD free with vaccination status (1 country + 2 zones). No applications were received for the WOA endorsement of an official FMD control programme. At the WOA General Session in May 2023, the newly recognised FMD-free status includes: (1) Bolivia, one new zone recognised FMD-free without vaccination, (2) Colombia, one new zone recognised FMD-free with vaccination (with this entire territory now free from FMD), and (3) Russia, one new zone recognised as FMD-free with vaccination. The FMD status of Indonesia and the zones in Kazakhstan remain suspended. After the suspension of the FMD-free status of Zone 6b of Botswana in August 2022, a Containment Zone within the zone was approved in March 2023.

The presentation included an update on work to revise the Terrestrial Animal Health Code Chapter 8.8 *Infection with foot-and-mouth disease virus*. A revised chapter will be submitted for adoption in May 2024, where changes to the text include: (1) provision on introduction of vaccinated animals into countries/zones free from FMD where vaccinated is not practised (only from countries/zones free with vaccination), (2) elaborated provisions regarding the establishment of a protection zone in face of threat, (3) options for shorter waiting periods for recovery/reinstatement of FMD-free status, and (4) recommendations for importation of fresh meat of small ruminants from FMD infected countries/zones.

Network partners are requested to support work in parallel to update the FMD Chapter in the Terrestrial Manual – where the deadline is mid-July 2024 for adoption in May 2025 (discussed later in the meeting).

### **Brief Update from FAO - Samia Metwally**

The FAO is looking to expand the capacity of the eight regional leading FMD laboratories (five in Africa, two in West Asia and one in the Middle East). There are changes to Reference Center Designation: (1) 5-year term instead of 4 years, (2) updated guidelines for application (these have become more stringent), (3) annual reports will now be mandatory and will be posted on website with agreement and (4) joint FAO review panel at mid-term and end of designation for extension. Upcoming events in 2024 include establishing a FAO Reference Center Epidemiology Network and a FAO Global Conference for Animal Health/One Health Reference Centres. The presentation also briefly explained that the FAO procurement process for vaccines (including FMD vaccines) is being reviewed to enable the rapid response to events in the field and better maintain the cold chain for the vaccine products.

### **Brief Update from the GF-TADs FMD Working Group - Melissa Mclaws**

The GF-TADs FMD Working Group was established in 2011 to promote and monitor the Global FMD control strategy. The group acts as the secretariat for the GCC-FMD – Global Coordination Committee for FMD (a new committee that was set up in 2021) that aims to bring regions together to share success and brainstorm challenges relating to FMD control.

To monitor the progress of countries along the PCP pathway, virtual and in-person roadmap meetings have continued to take place during 2023 (meetings in Azerbaijan ([https://rr-europe.woah.org/wp-content/uploads/2023/07/report\\_9th\\_wea\\_rmm\\_fmd\\_april\\_2023\\_final\\_en.pdf](https://rr-europe.woah.org/wp-content/uploads/2023/07/report_9th_wea_rmm_fmd_april_2023_final_en.pdf)), Bhutan (<https://rr-asia.woah.org/en/news/first-tads-coordination-meeting-for-south-asia/>) and a virtual meeting for West Africa). A new development is the inclusion of other diseases such as PPR and LSD in some of these meetings.

FAO, WOA and EuFMD continue to work to develop new tools which include: (1) PCP-FMD dashboard, (2) PCP-FMD Self-Assessment Tool, and (3) Laboratory Mapping Tool (pilot stage). Three tailored risk assessment documents are being drafted: incursion of SAT 2 to pool 3 (<https://www.fao.org/documents/card/en?details=CC8173EN>), incursion of serotype O into Indonesia and incursion of serotype O into southern Africa.

FAO and WOA reminded the participants that there are strict rules regarding the use of their logos on products such as diagnostic tests and vaccines.

### **Brief update from EuFMD – Donal Sammin**

The Network welcomed Dr Donal Sammin, the new Executive Secretary of EuFMD to the meeting. His presentation outlined the new EuFMD Work programme for 2023-2027 which has three objectives: (1) protection of livestock (risk monitoring and risk mitigation), (2) response to crisis (largely focussing on training and developing tools) and (3) greater control of FAST Diseases (global FMD control, FAST control and vaccine security). These activities include continued engagement with the FMD Reference Laboratories, and further work to develop PRAGMAST (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9798001/>) is anticipated so that the tool can be used for endemic regions. EuFMD has introduced a system for prequalification of FAST vaccines to ensure that they meet WOA standards, and this is now available online (<https://www.fao.org/eufmd/global-situation/prequalification-veterinarymedicines/en/>). One vaccine has been registered using this system.

EuFMD will be 70 years old next year! The first 35 years focussed on the coordination of FMD control in Europe which led to the successful eradication of the disease; since then, EuFMD has expanded to include focus on surveillance in the European neighbourhood and high-risk areas, and has also recently increased scope beyond FMD to cover FAST diseases.

## WOAH Code and Terrestrial Diagnostic Manual Chapter - anticipating updates during 2023/24

David Lefebvre provided some observations on areas of the Terrestrial Manual that require attention from the Network partners. Summary of these points:

- Summary and Introduction sections: cosmetic changes are anticipated but information will stay the same.
- Sample/Collection and transportation, to include (1) LFDs and FTA cards and (2) milk as sample matrix. The inclusion of 50% glycerol in transport media should also be defined in the text.
- Identification of the agent: add Seneca Valley virus (SVV) and highlight an improved schema for differential diagnosis of vesicular diseases.
- The section on *Virus Isolation* could benefit from improvement to: (1) suggest washing epithelial samples 2x in medium with antibiotics, (2) add supplementary cell lines (LFBKaVb6 and ZZ-R 127), (3) include transfection and electroporation of cell lines. Routine testing to confirm that cells with different passage histories maintain sensitivity to FMDV could also be included.
- Serotyping FMDV: In addition to the use of antigen ELISA, this section could explain that alternative molecular methods including VP1 sequence and topotype/lineage specific RT-PCRs provide valuable information. While the complement fixation test is not widely used, it is suggested to keep this method in the chapter.
- Nucleic acid recognition methods: It is suggested to keep the section on agarose gel-based RT-PCR assay but add in manual and (semi-automated) RNA extraction methods. There is benefit in keeping both real-time RT-PCR methods (3D and 5'UTR) since redundancy in molecular tests is useful, although the 3D assay outperforms the 5'UTR. The revised text could also outline the possibility to include an internal control to validate negative results, although these controls are not used in all laboratories. Further information could be included to outline how to interpret real-time RT-PCR data as well as other factors that define best practice for routine molecular testing.
- Molecular Epidemiology: This section should include whole genome sequencing and next generation sequencing, but these methods should not necessarily be defined as official methods.
- Serological tests: The text could be improved to mention the use of bovine standard sera for the tests as well as more details about how to read CPE in the VNT. For SPCE, information could be included to explain how “running means” can be used to monitor cross-session assay performance in the laboratory. Validation criteria for commercial NSP/SP ELISAs could be included and recommendations for the use of these tests in different geographical regions where different FMDV serotypes may be circulating.
- Vaccine selection and vaccine matching – it is not anticipated that this section will be modified during the current review of the text.
- Vaccine potency tests: Don King provided an update from a small working group (Donald King, Phaedra Eblé, Aldo Dekker and Michael Eschbaumer) to harmonise the texts of the fixed dose (PGP/PPG) and titrated dose (PD50) studies used to assess FMD vaccine potency. Harmonisation of texts that define non-protected animals in these FMD potency is urgently required and WOAH and the European Pharmacopeia have requested that Network laboratories clarify this issue since these texts are not completely aligned: viz, the PGP method outlined in the WOAH Manual (Section 5.3.2)

describes unprotected animals as those that “show lesions on the feet within 7 days after inoculation”, while the other three texts define non-protected animals as those that “show lesions at sites other than the tongue”.

**ACTION 23-1:** The Network to prepare a clear plan that prioritises parts of the text that will be updated for the July 2024 deadline (David Lefebvre et al.). NB: The text above should be considered as a proposed draft of the points that will be addressed during the revision of the WOA text (for July 2024)

### **Nagoya Protocol – Don King**

The Nagoya Protocol has been a standing agenda item at the Network Meeting since 2017. A multi-stakeholder workshop hosted by EuFMD was held in March 2023, with a report produced for long-term solution (<https://www.fao.org/eufmd/meetings-and-events/detail/en/c/1619589/>). Additionally, a manuscript co-authored by Network members to raise awareness of the Nagoya protocol has been submitted to *Frontiers in Veterinary Science*.

These are generic issues that impact many other livestock/human diseases. The Network partners supported the idea that the work for FMD could be publicised and used to bridge to other diseases (such as avian influenza and ASF).

### **Global Review from WRLFMD – Don King**

So far, 295 samples have been received this year at the WRLFMD for testing. Additional FMDV sequences have been received for the preparation of genotyping reports. This information is published in the WRLFMD/EuFMD Quarterly reports (<https://www.wrlfmd.org/ref-lab-reports#panel-8431>).

During 2023, the headline events have included the emergence of SAT2/XIV (unlike the more common toptype SAT/VII) in West Asia (Iraq, Jordan, Türkiye, Bahrain and Oman). Phylogenetic analysis suggests that there may have been multiple introductions of SAT2/XIV into these countries from an origin in East Africa. Vaccine matching with Boehringer Ingelheim vaccine suggest both SAT 2 Eritrea 98 and SAT 2 ZIM/2/93 should offer protection. However, some field data suggest that these vaccines may offer less protection than the *in vitro* testing suggests; therefore, an *in-vivo* study will be conducted at Pirbright in December to test these vaccines. Another unexpected event in West Asia has been the detection of SAT1/ in Qatar, where viruses are genetically distinct from SAT1/I viruses identified in 2023 in Comoros.

Globally, O/ME-SA/Ind-2001e continues to be the most widely-spread FMDV lineage – and has recently caused new FMD outbreaks in South Korea. Another separate serotype O lineage (O/ME-SA/SA-2018) in Pool 2 comprised 40% of all of the serotype O cases in India (in 2022). FMD viruses from this lineage have also recently been detected in UAE and Oman and there is potential that it will move quickly following similar pathways as O/ME-SA/Ind-2001.

### **Russia Pool 1 - Viktor Nikiforov**

Russia is officially WOA FMD free (without vaccination) for the majority of the country with other zones free with vaccination. In May 2023, during the 90<sup>th</sup> General Session, WOA recognised zone 5 as FMD free with vaccination. In August 2023 the Russian Federation

submitted a dossier for recognition of FMD free zones with vaccination for two zones bordering Kazakhstan. During 2023, eleven samples were received from suspect cases and tested negative. In addition, LPBE was used to test 20,562 samples from non-vaccinated animals plus additional sera from vaccinated animals. A joint paper with Kazakh and Mongolian scientists has been published describing outbreaks due to O/ME-SA/Ind-2001e which concludes that new outbreaks (during 2022) are not linked to previous outbreaks with viruses circulating in Pool 1. There are new risks associated with SAT2/XIV incursion into West Asian countries and ARRIAH has written to Commonwealth of Independent States (CIS) to notify them of the risk of SAT2 incursion into their territories. ARRIAH continues to provide diagnostic reagents (including post vaccinal SAT2 serum) and carries out a proficiency testing scheme.

### **Abdulnaci Bulut – Pool 3: Türkiye**

Update on behalf of the West Eurasia Lab Network – WELNET FMD.

During 2023, samples have been received to the laboratory from Türkiye (O/ME-SA/PanAsia-2<sup>QOM-15</sup>, O/ME-SA/PanAsia-2<sup>ANT-10</sup> and SAT2/XIV), Iraq (SAT2/XIV) and Qatar (to be processed). Data suggests that SAT2/XIV may have been circulating for more than a year in the West Asia region before being identified. Spread in Türkiye due to the initial cases of SAT2/XIV were limited due to an animal movement standstill and vaccination, but after the movement restrictions were lifted (so that animals could be released for grazing), the number outbreaks spread to east and central Anatolia. SAT2/XIV outbreaks have also been detected in West Anatolia where spread has been attributed to animal movements. These outbreaks have had impact to increase food prices in the country. Other risks in the region include A/Asia/Iran-05<sup>Far-11</sup>, A/ASIA/GVII and Asia 1/Sind-08

### **Pool 2: India – Rabindra Prasad Singh**

During 2023, there have been more reports of FMD cases due to serotype A than seen in the past years [Serotype O (n=47), Serotype A (n=38) and Asia-1 (n=5)]. However, there has been an overall decrease in the number of outbreaks (almost 7-fold) since 2021 and the national NSP antibody prevalence in cattle is around 9.5%. VP1 sequencing for 2023 indicates that the following virus lineages are present in the country: O/ME-SA/Ind2001e, A/ASIA/G-18/non-deletion/2019 lineage and Asia1 Group VIII. In 2022, many of the isolates belonged to the O/ME-SA/SA-2018 lineage, but this year most outbreaks appear to be caused by O/ME-SA/Ind2001e. There is also a reappearance of Asia1 Group VIII after the last reported case in 2018. Vaccine matching indicates that the Indian vaccine will protect against serotypes O and Asia 1 although for serotype A none of the isolates showed an  $r_1$ -value above 0.3.

### **Pool 4: Ethiopia, East Africa – Daniel Gizaw**

A total of 88 samples have been received to AHI, Ethiopia during the year for testing. Antigen ELISA has been used to characterise these viruses as: O (17), A (10), SAT 1 (6), SAT 2 (30), but no sequencing has been undertaken. SAT 1 has been detected by antigen ELISA which would represent the first time that this serotype has been detected in Ethiopia since 2007.

**ACTION 23-2: WRLFMD to organise a shipment for suspect SAT 1 and SAT 2 samples from Ethiopia**

### **Pool 5: Nigeria – West Africa - Hussaini Ularamu**

Thirty-four samples have been collected during 2023; it has been possible to type some of these as serotype O (n=12) and A (n=6). Two samples were positive by real-time RT-PCR only, but no further material is available for these samples. Samples submitted to the WRLFMD demonstrate the presence of O/EA-3 in the country and have been helpful to reconstruct the recent spread of this lineage from West Africa to North Africa.

#### **Pool 4-6: Sub-Saharan Africa – Livio Heath**

Most of the outbreaks in South Africa are now under control with no virus detected on suspect clinical samples during 2023 (i.e., no new outbreaks have been confirmed during the year). In the next 6 months, it is hoped that these outbreaks will be closed by WOA. Serology (60,456 SPCE, 9,691 NSP) shows that most samples are FMDV-antibody negative except for some post-outbreak surveillance where animals remained FMDV-antibody positive for 6 to 12 months after the outbreak. During 2023, surveillance samples were also received to OVI from Eswatini and Zimbabwe.

#### **Pool 4: Kenya – East Africa – Abraham Sangula**

In 2023, the laboratory has received samples from field cases of FMD where serotypes O has been most frequently detected, followed by SAT1, A and SAT2. Additional samples were received for surveillance in support of animal export to Middle Eastern countries (especially for small ruminants). No vaccine matching has been carried out as funding wasn't available. In vivo potency studies for KEVEVAPI vaccines have been conducted (homologous testing), which showed >PD50 for all four strains (O, A, SAT1 and SAT2). A new WOA Twinning project with the WRLFMD will support the shipment of samples to Pirbright for sequencing and vaccine matching.

**Action 23-3 – Kenya to send SAT 2 viruses from 2021 and 2022 to the WRLFMD.**

#### **Pool 1: Southeast Asia – Kingkarn Boonsuya Seeyo**

Submission of FMD outbreak samples to Pakchong has decreased during the past year where only thirty-six samples were tested - 20 were serotype O (sequenced as O/ME-SA/Ind-2001e). Samples were also received from Malaysia for surveillance (testing ongoing). During 2023 there has been a shift in the distribution of FMD from northern Thailand to middle and now the southern region of Thailand. Currently the Thai-Myanmar border is closed; however, it will soon be opened which could cause an increase in the number of outbreaks. Recent research activities include a study to compare the sensitivity and specificity of NSP ELISA kits (Biovet, ID Screen, VDPPro, IDEXX, PrioCHECK and KUcheck) showing that these test kits had a sensitivity ranging from 97.5-99% and a specificity ranging from 97.5 to 100% (NB: Manuscript is currently in Thai but there are plans to translate this document into English). There is also a project to carry out molecular and antigenic characterisation of FMDV isolates in Thailand over the past 15 years.

#### **Pool 1: East Asia and South Korea – Jong-Hyeon Park**

New FMD outbreaks have been detected in Chungcheong Buk-do during May 2023 on 10 cattle and 1 goat farms. These outbreaks were caused by a O/ME-SA/Ind-2001e lineage virus most closely related to those from Vietnam, Cambodia, Laos, Mongolia, Russia and Kazakhstan. The source of the outbreak is unknown (possibly via import of infected objects since there are many foreign workers on Korean farms) and after nine days there were no additional cases found. Since 2010 South Korea has adopted a national vaccination program

for serotypes O and A, where three different vaccines from different suppliers are used. Vaccine matching suggests that the viruses causing the most recent outbreaks are matched against O/3039, O/RUS/Primorsky 2014 and O1 Campos. Local production of an FMD vaccines is also planned with vaccine candidates for O/PanAsia-2 and O/SKR/BE/2017 (this is an Ind2001 virus) being identified.

### **Pool 1: East Asia and China – Wen Dang**

During 2023, twenty-two samples collected in China have been tested and the country has reported three new outbreaks due to serotype O. Forty additional lymph node samples collected for surveillance purpose have been shown to be FMDV positive. There appear to be fewer lineages circulating in China: currently O/ME-SA/Ind-2001e (2023) and O/CATHAY (2022) as well as O/SEA/Mya-98 which is thought to still be circulating in pigs, although no cases have been reported since 2021. Serotype A has not been detected since 2019. Two commercially vaccines are currently being used: O/MAY98/BY/2010 (pig and cattle) and Re-O (pig). Both vaccines do not elicit good responses against O/CATHAY, but a new candidate strain (Re-O/17002) is well-matched. A national PTS has been organised and 34 Chinese provincial labs took part where 32/34 laboratories gave results that met the expected criteria.

### **Pool 7: South America - PANAFTOSA – Edviges Maristela Pituco**

No samples received during 2023 for FMD diagnosis, but the passive surveillance system has identified both SVA and VSV circulation in South America. In 2023 almost 50% of samples from pigs received were positive for SVA. An inactivated SVA vaccine has been produced in Brazil and has started to be used this year to control SVA cases.

Reference antigens, reference kits (SP and NSP) as well as cell lines, RNA, and SP/NSP positive sera were distributed. The laboratory has carried out two technical missions – Guyana (to strengthen surveillance in animal health) and Venezuela (for evaluation of laboratory capacity) and training in laboratory techniques and field diagnosis for veterinarians in the official service.

In Brazil, there will soon be more cattle in free zones without vaccination than in free zones with vaccination – and it is estimated that FMD vaccination will only continue during 2024 in some northeastern states.

### **Pool 7: South America SENASA – Sabrina Galdo Novo**

Twenty-three samples were received which were all negative for FMDV and positive for a poxvirus. During the past twelve months, samples from Vietnam were also tested: twenty were serotype O and one was unknown. Sequencing for these samples is underway.

### **Update from Sciensano – David Lefebvre**

The laboratory has recently received samples from Nigeria (n=29); all FMDV positive. Twenty-three were typed as serotype O/EA-3 and testing for the remaining 6 is ongoing. The epidemiological patterns in Nigeria seem to be dominated by serotype O where in the past (before 2021) there was a more diverse spread of serotypes and different viral lineages.

### **Update from IZSLER – Santina Grazioli**

During 2023, IZSLER has generated sequences extracted from FTA cards from Libya which confirm the presence of serotype O/EA-3 in the country. These sequences are different to the earlier cases of O/EA-3 in North Africa and represent a new introduction of the virus to the region (most closely related to viruses circulating in Sudan and Ethiopia).

During the year, a total of 860 kits were sold to 51 countries, and an increase in the requests for SAT2 kits has been seen. The laboratory has performed training for scientists from Iraq and Jordan. Serum samples have been collected for FMD surveillance purposes from Lebanon and Libya –and tested by SP and NSP. A post-vaccination study (using a South American vaccine) was carried out in Jordan (during 2022-2023) where higher post-vaccination titres were observed in goats compared to sheep. Booster doses increased both the heterologous and homologous titres and the study will continue into 2024 with further timepoints. A separate PVM study is also being carried out in Northwest Syria.

### **Update from ANSES - Guillaume Girault**

FMD viruses detected in samples submitted to ANSES have been sequenced including those from: FMD outbreaks on the islands of the Comoros (SAT 1/I, second case after O/EA-2 in 2019), Oman (O/ME-SA-2018 and SAT2/XIV) and Tunisia (O/EA-3, possible new introduction of the virus to the region). Sequence data shows that the SAT1/I virus spread from East Africa to Comoros. ANSES has recently concluded a large PTS with 39 international participants. An online training for Middle East (Emergency Diagnosis and Post-Vaccination Monitoring) was also carried out (12 participants from 6 countries) as well as a molecular diagnostics course for North Africa (2 countries), the Middle East (2 countries) and SEEN (4 countries). A training on FMD provided to regional coordinators as part of emergency response preparedness was also carried out.

### **Update from FADDL – Vivienne O’Donnell**

The new USDA NBAF (in Manhattan, Kansas) will include both ARS and APHIS as well as other research areas. It is proposed that the facility will be finished during 2024, but it will take some time to get the laboratory fully commissioned for use. No FMDV positive samples have been detected during 2023 and passive surveillance with RT-PCR, 3ABC ELISA, VIAA AGID have all been negative for FMDV and FMDV-specific antibodies. The laboratory also maintains capacity for testing using immunodiffusion which can be useful to rule out non-negative results on the 3ABC ELISA. Additional testing has recently taken place to compare the performance of the PrioCHECK and VMRD NSP ELISAs. For the North American FMD vaccine bank, three new vaccine antigens have been procured and two homologous PD<sub>50</sub> studies have been carried out (the serotype A passed while the SAT2 study is ongoing). Reagents have been supplied as part of a PTS for FMD and SVA (this included positive controls), six training courses on foreign animal disease diagnostics were also carried out.

### **Update from WBVR – Aldo Dekker**

Fifty-six samples were tested by RT-PCR for absence of FMD. Sera was tested by VNT, but this was mostly for PT samples and export/import of animals. Ten strains were also sequenced to check the nanopore sequencing protocol. PGP and PD<sub>50</sub> studies were carried out using homologous strains. Reference sera calibrated to protection has been made and is available for O Manisa (more sera are planned). The mapping of 146S VHS using XLMS has also been published (<https://pubmed.ncbi.nlm.nih.gov/36699337/>) These VHHs could be used as a semi quantitative diagnostic test to assess the quality of FMD vaccines by comparing 146S and 12S

antigens. Efforts are being made to produce 12S ELISA kits (stable plates coated with VHH M3).

#### **Pool 4-6: Sub Saharan Africa – Botswana – Elliot Fana**

Samples have been received from Botswana (1 neg), Malawi [SAT2/II (n=3), no virus detected (n=4)], Ethiopia [(O (n=4), A (n=8) and no virus detected(n=15)]. Vaccine matching has been carried out for the Malawi samples – both vaccine strains SAT 2035 and SAT105 give a match. Surveillance testing for NSPs was carried out for the 2022 outbreaks (Botswana and Malawi) which are now largely under control. A small number of samples for NSP testing have also been received from Lesotho (n=200); these were all negative supporting the FMD free (without vaccination) status of the country. There has been a recent incursion of buffalos into areas with domesticated animals and sero-monitoring of exposed cattle is ongoing as well as probang sampling of the buffalo. BVI will include all available vaccine matching data in their annual return to the Network.

#### **Update from NCFAD – Shawn Babiuk**

Two hundred and eighteen suspect samples have been tested; however, these were all negative for FMDV (some samples were positive for SVV). The lab plans to carry out vaccine matching and new potency studies next year.

#### **Improved ways to collate and display data from laboratories – Antonello Di Nardo**

There are 4 modules on the openFMD website:

- FMDbase (sequence database from GenBank, WRLFMD, WOA/FAO FMD Lab Network),
- FMDtype (ability to upload own sequences and query against sequences available on database),
- FMDwatch (based on FMDV surveillance, available early 2024)
- PRAGMATIST (FMD vaccine strain evaluation).

It is hoped that these tools will improve timely analysis and communication of FMDV activities at country/regional levels. There is a dedicated webserver to ensure that the data can be visualised and won't be corrupted. These systems are currently being beta-tested and it is planned that they will be released for wider use in 2024.

#### *Nomenclature Steering Group Updates*

The nomenclature steering group request laboratories to follow a harmonised nomenclature: Serotype/CountryCode/Sequencing Sample number/Year/LabCode.

e.g. O/ETH/17/2023/WRLFMD

The sample number (in sequence order) is unique and country specific. If a particular isolate/sample has previously been sequenced, then it is recommended that this name is provided in the metadata of the sequence so that data can be linked.

The FMD Network Data Management spreadsheet used for the Annual Report will now include three pieces of information: clinical/suspected cases, surveillance and sequencing. The new excel datasheet which will be sent out this year and has an information section on how to fill

it in. This is not meant to add more work as the spreadsheet mirrors what is already included in the annual reports to WOA. H.

**Action 23-4 – The network should look at the annual report and comment on what is good and what is redundant (e-mail Antonello, Anna and Don).**

### Review of global epidemiology and recent changes to the distribution of the different FMDV lineages

Using the data to understand global and regional risks; changes to the top 10 lineages that have the potential for trans-movement. Notes from discussion with Network partners and general comments on Maps in the Annual reports (<https://foot-and-mouth.org/woahfao-fmd-reference-laboratory-network/woahfao-fmd-laboratories-network-annual-reports>).

1. Add a comment on the maps that emphasise that the arrows do not define precise origin and destination of viruses.
2. Check for consistency in the way that countries are coloured (and explain in the legend what the grey colouring refers to)
3. **O/EA/3** – Two main pathways into North Africa from East and West Africa need to be clearly defined. Although a degree of mixing is anticipated for Pools 4 and 5, sequence data indicates that for this lineage, this has not recently occurred – therefore the emphasis on connections between these pools should not be recuded [WRLFMD to check whether there was an O/EA-3 outbreak in Mali in 2018].
4. **O/IND-2001d and e** – recently detected in South Korea, Indonesia, Russia (and Pools 1 and 2). Move infected areas in China closer to Mongolia and include another arrow highlighting connections between Pool 2 and the Middle East (e.g., Oman). Arrow into Indonesia could be adjusted (further south) to accommodate recently detected cases (2022).
5. **O/MYA98** – no change. Should there be a risk arrow going to Indonesia?
6. **O/PanAsia** - It was suggested that the arrows should be very similar to O/SEA/Mya-98
7. **Make a new map for O/ME-SA/2018**
8. **A/G-VII** - declining dominance in Pool 3 with fewer reports – last cases detected in 2019. Still present in India – so could still be a risk.
9. **SAT2** figure need to be updated to reflect cases due to SAT2/XIV in 2023

Use of these data to estimate lineage prevalence scores (for PRAGMATIST)

**Table 4:** Conjectured relative prevalence of circulating FMD viral lineages in each Pool.

Lineage	Southeast / Central / East Asia [Pool 1]	South Asia [Pool 2]	West Eurasia & Near East [Pool 3]	North Africa	Eastern Africa [Pool 4]	West / Central Africa [Pool 5]	Southern Africa [Pool 6]	South America [Pool 7]
O ME-SA PanAsia-2			30					
O ME-SA PanAsia	10							
O SEA Mya-98	21.5							
O ME-SA Ind2001	40	76 <sup>1</sup>	5.5 <sup>1</sup>	0				
O EA or O WA			1.5	60	53.5	69	16	
O EURO-SA								90
O CATHAY	10.5							
A ASIA Sea-97	18							
A ASIA Iran-05	0		28					
A ASIA G-VII		20	5					

Lineage	Southeast / Central / East Asia [Pool 1]	South Asia [Pool 2]	West Eurasia & Near East [Pool 3]	North Africa	Eastern Africa [Pool 4]	West / Central Africa [Pool 5]	Southern Africa [Pool 6]	South America [Pool 7]
A AFRICA				30	17	15		
A EURO-SA								10
Asia1	0	4	10					
SAT 1			1	0	15	1	16	
SAT 2			19	10	14	15	52	
SAT 3					0.5		16	
C								

<sup>1</sup> Includes cases due to the emerging O/ME-SA/SA-18 lineage that has been recently detected in Pool 2.

Note: For each of the regions, data represent the relative importance of each viral lineage (prevalence score estimated as a percentage [percent] of total FMD cases that occur in domesticated hosts). Proposed changes to increase risks are shown in **red**, while a reduction in risk is shown in **green**. NB: In response to the FMD cases due to SAT2/XIV, risks in Pool 3 had already been reviewed and revised in April 2023

*Summary of proposed changes (shown in Table 1 above): Pool 1 – no change, Pool 2 – data from India indicates increasing proportion of outbreaks due to serotype A (increased to 20 and decrease O to 76); Pool 3 – add SAT 1 to cover the recent outbreaks in Qatar(1), SAT 2 (19), A/G-VII (5) and A/Iran-05 (28), O/Ind-2001 (3.5) and increase O/PanAsia (30); North Africa – O/EA-3 increase to 60 due to recent events in Libya, Tunisia and Algeria, remove all points for O/Ind-2001, Pool 4 –increase SAT 1 due to cases in the Comoros, Kenya and Ethiopia. Pool 5 – increase to serotype O (to reflect recent reports), decrease SAT 1 (1); Pools 6 and 7: no change is proposed.*

### Update on serotype C Taskforce: how can Network partners contribute? - Molly Dunn

There have been no reports of serotype C for the last 19 years since 2004. The FMD-C Taskforce which includes Pirbright, WOA and FAO, has two goals: (1) gathering evidence and measuring risk and (2) reducing risks and maintaining preparedness for serotype C outbreaks. In the past few months, surveys have been distributed to understand where serotype C stockpiles may still be present - to vaccine producers (n=14), veterinary diagnostic laboratories (n=102) and national veterinary authorities (n=66). For the National Veterinary Authority surveyed, 10% stated that FMD-C sources are still present in their countries and ninety-two veterinary diagnostic Laboratories confirmed that they have not disposed of FMD-C viruses. Most of the serotype C materials comprise reference viruses or virus specimens associated with research activities. Data showed that there are 10 manufactures producing FMD-C vaccine. The Taskforce is currently following up this survey to check that the data is accurate.

### Update on serotype C Taskforce: how can Network partners contribute? - Sarah Mielke

Is FMDV serotype C extinct: what can the data tell us?

This project undertaken by USDA has analysed two datasets – one from the WOA/FAO FMD Ref Lab Network and one from the WRLFMD (data from 1942-2021 comprising 21,909 samples of which 176 were serotype C). Using Bayesian methods, data from the Network were grouped by pool at three scales, (i) stratified by year and country, (ii) stratified by country, and (iii) unstratified. Two measures were used to support or refute claims of serotype C extinction, the detection probability (the probability that FMD virus serotype C will be detected

if present, given a prevalence threshold), and the detection capability (the lowest prevalence value that can be detected by the system at a determined detection probability). The results from this analyses suggest that the 95% detection probability standard is met, (i) at prevalence thresholds (PT) of 1, 2, and 5% in all endemic pools when the data is unstratified, (ii) at the 1% PT in a few countries but in most countries (except RVP West/Central Africa) at the 5% PT when data is stratified by year, and (iii) only in a few countries and years when data is stratified by year or country. The detection capability reflects trends found in the detection probability, and highlights countries with the largest sample submissions. These results provide important information regarding the evidence that serotype C is extinct.

**Action 23-5 - A manuscript has been drafted and feedback from the Network partners is welcome. Anyone can review and make changes to this draft; they will be added as authors if they can contribute to the paper.**

### **Update from the Network Working Group for serology and vaccine evaluation – Anna Ludi**

This presentation provided a brief update in the following four areas: (1) validated ELISA kits, (2) test variability, specifically VNT, (3) antigen Panel and (4) defining “protective” cut-offs.

The meeting participants supported the proposal to organise inter-laboratory NSP/SP workshop similar to what was previously conducted in Brescia in 2006 (see: <https://pubmed.ncbi.nlm.nih.gov/16753241/>).

**Action 23-6 – Anna Ludi to draft a concept note for the SP / NSP inter-laboratory workshop.**

**Action 23-7 – WRL to collate and add the reference sera available for the network on the website.**

**Action 23-8 – The antigen panel for Western Africa will be finalised by the Network in Q1 2024.**

### **New methods to directly assess 146S content – Toby Tuthill**

ELISAs have been established with VP4 and VP2-specific monoclonal antibodies to assess the 146S and 12S content of FMD vaccines. Although VP4 of FMDV is thought to be internal, under certain conditions it can present epitopes that are recognised externally, and measuring VP4 signal has been shown to correlate with 146S content. In comparison VP2 binding can be used to determine total antigen content of a FMD vaccine. The VP4 and VP2 monoclonal antibodies used target highly conserved epitopes so that the tests can be used for vaccines for all FMDV serotypes. Validation data indicates that the test correlate to gradient density sedimentation analyses and can also be used to predict serological responses measured by VNT.

### **Experiences from *in vivo* vaccine potency study – vaccine challenge virus production FMDV myocarditis – Ryan Waters**

This presentation reviewed recent experiences from FMD vaccine potency test that have been conducted at the Pirbright Institute. Cardiac Troponin T in serum (measured with a simple-to-use lateral flow device) has been shown to be a sensitive indicator of heart damage and has been used as a predictor of myocarditis in FMD potency studies. This approach has been

used in recent studies at Pirbright and there is now interest in seeing whether laboratories may be interested in using this method.

**Action 23-9 – Follow-up with other reference laboratories to pull together data for cases of myocarditis in FMD potency challenge tests.**

### **Doing things differently – Don King**

The costs of shipping FMD samples internationally have increased dramatically (~4 fold) since the COVID-19 pandemic in 2020. In view of these increasing costs, international FMD laboratories need to urgently develop new pipelines to collect epidemiological data from field cases. One simple approach to reduce these costs could utilise ambient temperature shipping using lateral flow devices (or other systems such as FTA cards) which allow FMD viral RNA to be detected and characterized. Data from ANSES and other laboratories show that recovered RNA can be sequenced and viral isolates can be generated (if needed for vaccine matching and other viral phenotypical assays), although future work is needed to optimise these methods for routine use. Another suggestion was that PCR amplicons could be prepared in local laboratories and shipped for down-stream analyses (sequencing), so long as concerns relating to cross-contamination could be addressed. In the long-term, sequencing capacity might be established across many different laboratories in FMD endemic countries (perhaps using nanopore methods?). However, these ambitions are currently constrained by the costs of these technologies. In the short term, greater development and use of lineage-specific RT-PCRs could help laboratories in endemic countries to identify FMD viruses causing outbreaks and triage samples for sequencing analyses (<https://foot-and-mouth.org/science/lineage-specific-pcr>).

**Action 23-10 A short survey will be distributed to laboratories to highlight the increase in costs (EuFMD could help with this). Partners should make suggestions of alternative pathways to ship samples. Data from this exercise could be consolidated into a summary or review/vision paper (for publication).**

### **Genomics and Bioinformatics at CFIA NCFAD – Oliver Lung**

This presentation described genomics and bioinformatic work undertaken by the laboratory in Winnipeg. The group at CFIA NCFAD provides support for diagnostics, surveillance and conducts research activities. Each year, there continues to be an increasing focus of the use of Nanopore compared to Illumina and Ion Torrent sequencing methods. For nanopore, ONT provides two types of flow cells: ONT MinION and PromethION. (PromethION produces 10x more reads as compared to MinION). The laboratory have optimised methods that can be adopted to sequence a wide range of viruses (representing seven Baltimore classes) and can automate the process to increase through-put. Specific examples highlighted in the talk included the identification of novel Seneca Valley virus-like viruses in whales and pangolins, influenza virus, Mpox and ASFV.

### **Any other business:**

Drs Min-Kyung Park, Samia Metwally and Donal Samin reinforced the important work that is undertaken by this Network. Delegates were sorry to hear that Dr Monique Eloit (WOAH) and Dr Keith Sumption (FAO) will retire very shortly. The meeting delegates expressed their appreciation for the continued support and guidance provided to the Network over many years and passed on their best wishes to Monique and Keith for their well-earned retirement.

The meeting concluded with a vote of thanks to colleagues at CFIA-NCFAD, Winnipeg, Canada (Charles Nfon, Shawn Babiuk, Michele Roy and colleagues) for hosting the meeting.

Close of meeting.