The 16th Annual Meeting of the OIE/FAO FMD Reference Laboratory Network

23rd and 24th November 2021

Core Members

	OIE Reference Laboratory for Foot and Mouth Disease, Dirección de Laboratorio Animal, SENASA, Argentina Participants: Sabrina Galdo
	OIE 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 Participants: David Lefebvre, Nick De Regge
	OIE Regional Reference Laboratory for Sub-Saharan Africa (RRLSSA) Botswana Vaccine Institute (BVI), Botswana Participants: Elliot Fana, Mokganedi Mokopasetso
	Centro Panamericano de Fiebre Aftosa (PANAFTOSA) and PAHO /WHO and OIE Reference Laboratory for FMD, Brazil Participants: Edviges Maristela Pituco
	FAO FMD Reference Laboratory, National Centre for Foreign Animal Disease National Centres for Animal Disease, Canadian Food Inspection Agency, Canada Participants: Charles Nfon
	OIE and China National FMD Reference Laboratory, Lanzhou Veterinary Research Institute (LVRI), CAAS, People's Republic of China Participants: Haixue Zheng , Jianhong Guo, Jijun He, Wen Dang, Xiangtao Liu
	OIE FMD Reference Laboratory, French Agency for Food and, Environmental and Occupational Health & Safety (ANSES), France Participants: Labib Bakkali Kassimi, Souheyla Benfrid
	FAO Reference Centre for FMD in South Asia, ICAR – Directorate of Foot-and-Mouth Disease, Indian Council for Agricultural Research, Mukteswar, Nainital (Uttarakhand), India Participants: J. K Biswal, Jajati Keshari Mohapatra, Rabindra Prasad Singh, Samir Kumar Sarangi, Saravanan Subramaniam
	OIE/FAO FMD Reference Laboratory, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Italy Participants: Efram Foglia, Giulia Pezzoni, Santina Grazioli
	OIE Reference laboratory for Foot and Mouth Disease, Animal and Plant Quarantine Agency (QIA), Republic of Korea Participants: Jong-Hyeon Park, Sang-Ho Cha, Soyoon Ryoo
	FAO FMD Reference Laboratory, Wageningen University & Research (WUR), The Netherlands Participants: Aldo Dekker, Phaedra Eble
	FAO Reference Centre for FMD for Central Asia and West Eurasia and OIE Reference Laboratory for FMD, Federal Governmental Institute, Centre for Animal Health (FGI ARRIAH), Vladimir, Russian Federation Participants: Svetlana Fomina, Ms Karaulov
	FAO Reference Laboratory for FMD in Africa and OIE FMD Reference Laboratory, Transboundary Animal Diseases Programme, ARC-Onderstepoort Veterinary Institute (ARC-OVI), South Africa Participants: Livio Heath, Melanie Chitray, Pamela Opperman
	OIE Regional Reference Laboratory for Foot and Mouth Disease in the South East (RRLSEA) Department of Livestock Development, Thailand Participants: Kingkarn Boonsuya Seeyo, Sahawatchara Ungvanijban

	FAO World Reference Laboratory and OIE FMD Reference Laboratory The Pirbright Institute Pirbright, United Kingdom Participants: Abdelaziz Yassin, Ali Burman, Amin Asfor, Antonello Di Nardo, David Paton, Donald King, Julie Maryan, Madeeha Afzal, Nick Knowles, Sarah Belgrave, Valérie Mioulet
	FAO Reference Centre for FMD and other vesicular diseases for the Americas and the Caribbean and OIE FMD Reference Laboratory Foreign Animal Disease Diagnostic Lab, Plum Island Animal Disease Center (PIADC), United States of America Participants: Robin Holland, Sulee Robbe-Austerman

Affiliates

	Australian Centre for Disease Preparedness (ACDP), Australia Participants: Nagendra Singanallur, Petrus Jansen van Vuren, Wilna Vosloo
	NATIONAL Animal Health Diagnostic & Investigation Center (NAHDIC), Ethiopia Participants: Daniel Gizaw
	Foot and Mouth Disease Laboratory, Kenya Participants: Abraham Sangula
	National Veterinary Research Institute, Vom, Plateau State, Nigeria Participants: Hussaini Ularamu, Wungak Yiltawe
	Şap Institute (and WELNET FMD), Ankara, Turkey Participants: Can Cokcaliskan, Unal Parlak
AU-PANVAC	Pan African Veterinary Vaccine Center for African Union (AU-PANVAC), Ethiopia Participants: Ethel Chitsungo, Cisse Rahamatou

OIE/FAO Representatives

eufmd	The European Commission for the Control for Foot-and-Mouth Disease Participants: Bryony Armson, Carsten Potzsh, Fabrizio Rosso, Kees van Maanen
	Food and Agriculture Organization of the United Nations Participants: Melissa McLaws, Samia Metwally
OIE	OIE – World Organisation for Animal Health Participants: Bolortuya Purevsuren, Min-Kyung Park

PCP Support Officers (PSOs) & Heads of Regional Laboratory Networks/Epi/Lab leaders

	Epi Leader, West Eurasia Participant: Satenik Kharatyan
	Epi Leader, Middle East Participant: Rehab Abdelkader
	Lab Leader, SAARC, India Participant: Rajeev Ranjan
	PSO, Azerbaijan, Kyrgystan Participant: Carsten Potzsch
	PSO, South Sudan, Sudan, Palestine Participant: Kees Van Maanen
	PSO, The Gambia, Sierra Leone Participant: Austine Bitek

EPI-interactive

EPI-interactive	New Zealand Participants: Petra Muellner, Uli Muellner

TUESDAY 23rd NOVEMBER 2021, DAY 1

Opening of the 16th annual meeting and adoption of agenda (Don King)

Opening remarks from Dr King provided a brief review of the history and core activities of the Network which was established in 2004. Since the last meeting in 2000, WBR Lelystad (The Netherlands) has been designated as an FAO Reference Laboratory for FMD and has signed the MoU as a core member of the Network. In view of the COVID-19 situation, this is the second meeting of the Network in a "virtual format" which allows wider participation from FAO, OIE and affiliate laboratories, LabNet and EpiNet leads from the FMD regional roadmaps and PCP Support Officers (PSOs).

Recent achievements of the Network were reviewed, including:

- Publication of the 2020 Annual Network Report summarising the global situation regarding the distribution of FMD in different regions of the world.
    - Circulation of a new simple-to-complete Excel spreadsheet to help with analysis of trends and the preparation of the annual report
- Tools to improve surveillance in endemic pools via the development of cost-effective pipelines to ship samples and translation of the e-learning course for FMD diagnostics into French
- Coordinated work to develop improved methods to assess serological responses that can be used for post-vaccination testing and FMD surveillance in endemic countries
- Two manuscripts have been published in peer-reviewed journals during 2021:
    - History of serotype C and recommendations to prevent re-introduction of the serotype – published in *Virus Evolution*
    - FMD Reference materials highlighting current gaps in available reagents – published in *Scientific and Technical Review of the OIE*
- Development of a FMDV sequence database (https://www.fmdbase.org/) and work to establish web-based dashboards to facilitate data exchange and analyses (discussed on Day 2)
- Distribution of a survey to collected views on FMDV strain nomenclature
- Other reference laboratory networks are currently being developed by the OIE/FAO using the FMD Reference Laboratories Network as a positive example.

Update on OIE activities on FMD (Min Kyung Park)

Twenty-four applications for disease-free status were received in May 2021; thirteen of these were for FMD. Six FMD applications across three countries were successful; in Brazil, the whole country is now free from FMD covered by different zonal statuses free with and without vaccination with a larger part of the country free with vaccination. Russia has two newly recognised zones free with vaccination while the rest of the country is FMD free without vaccination. The FMD-free with vaccination zone has been extended in Colombia at the border with Venezuela.

A revision of the Terrestrial Animal Health Code Chapter 8.8 Infection of foot and mouth disease has been circulated to members for comment. The introduction of vaccinated animals into an FMD-free zone or country where vaccination is not practiced is under discussion, along with alternative surveillance measures to demonstrate freedom from FMD after an outbreak in a shorter recovery period from six to three months. It is also proposed that the Protection Zone (Article 4.4.6) be used as a risk management strategy to minimise the impact of disease introduction. Chapter 3.1.8 Foot and mouth disease (infectious with foot and mouth disease virus) in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animal 2019 has two proposed revisions.

Action 021-01 coordinate contributions from the network to the OIE code and OIE Manual – Led by David Lefebvre, Charles Nfon, Labib Bakkali-KassimiKassimi and Sabrina Galdo. The harmonisation of heterologous post-vaccination responses (Paragraph "D. Vaccine matching tests" of Chapter 3.01.08 of the OIE Manual) will be reviewed by the new working group (see Action 021-09)

Update from FAO (Samia Metwally)

Dr Metwally provided thanks to everyone attending the meeting; the network is doing a great job in understanding and controlling FMD. The global action plan for regional epi and lab networks was outlined as a way to manage FMD at the regional level for the next 5 years with reference laboratories and the Network continuing to work as collaborative partners. The Emergency Management Centre for Animal Health (EMC-AH) has been introduced to help countries respond to disease emergencies.

An initiative towards eliminating residual risks associated with FMD, serotype C is underway (under umbrella of GF-TADs) based on compiling evidence that this virus serotype no longer circulates in livestock populations. This initiative (2021-26) will review surveillance evidence that serotype C is extinct and will aim to reduce the risk of FMD-C being released and also adopt a system to respond if this serotype reappears.

Regional roadmap meetings for SADC, W. Africa and Middle East have taken place along with Regional Advisory Group Meetings for E. Africa, W. Eurasia, SADC and W. Africa. Epidemiology and Laboratory Network meetings for W. Africa, W. Eurasia and Middle East have been strengthened by the work of PCP-FMD support officers within countries.

The Global Coordination Committee of FMD (GCC-FMD) met in September 2021 and is working to harmonise an FMD control strategy across the world.

Action 021-02 Plan to improve ongoing connectivity of the Network to regional laboratories (e.g regional roadmaps) and the inclusion of more laboratories in the proficiency testing schemes

Update from WRLFMD (Don King)

The number of samples (n=451) received to the WRLFMD in 2021 has increased compared to 2020 when overseas shipments were adversely affected by COVID-19. A coordinated approach by Valérie Mioulet, Kees van Maanen and Paolo Motta to target priority FMD endemic countries was acknowledged as responsible for a larger number of samples received by the WRLFMD this year. Shipments include those from the DR Congo (where samples have been received for the first time in 10 years) and Nigeria where large batches of samples have been collected. Real-time exchange of viral sequences for FMD outbreaks in Israel, Iran, Malawi, Mauritius, Namibia and Turkey has benefited from the connections established within this Network.

Headline events in 2021 include:

- The identification of serotype O (O/EA-2 topotype) in Namibia, where cases in July represent the continued spread of this lineage that originates from East Africa. Together with cases due to the O/EA-2 topotype that have also been detected recently in Zambia, these outbreaks pose new threats to Southern Africa where serotype O is not normally resident.
- Increasing dominance of the O/ME-SA/Ind-2001e lineage in Pool 1 where it appears to be supplanting previously circulating serotype O lineages (including O/SEA/Mya-08 and O/ME-SA/PanAsia). This lineage continues to be detected in Pakistan (where it was first detected in 2019) and there has also been a second detection of the O/ME-SA/Ind-2001e lineage in Mauritius in 2021 that appears to be distinct to viruses that caused outbreaks in 2016.

- Detection of SAT 2 (topotype I) in KwaZulu-Natal in May 2021 that represents an FMD outbreak that has occurred away from the usual areas of high concern in southern Africa.
- FMD viruses detected in animals imported from East Africa to Bahrain were characterised as O/EA-3 and A/AFRICA/G-1 highlighting the ease by which FMD viruses can be imported into new areas via the trade in live animals.

A new research paper (Di Nardo et al., 2021 – *Molecular Biology and Evolution*) from the WRLFMD and collaborators has studied VP1 sequences collected over a 20-year period for lineages endemic in the Middle-East. These results help us to understand how FMD is maintained (as periodic waves) in endemic countries and identifies important viral reservoirs for serotypes O, A and Asia-1 in Afghanistan, Pakistan and Iran.

Vaccine matching data at WRLFMD have been generated for 27 field strains against the vaccines from MSD and BI and now also includes O1 Campos from Biogenesis Bago. Vaccine selection for endemic settings is assisted by continued work to measure heterologous antibody responses – where the use of regional reference antigens is increasingly important.

Pool 1: Southeast Asia (Kingkarn Boonsuya Seeyo)

Samples have been received from Thailand (n=75 total; 17 serotype O and 34 serotype A isolates) and Lao PDR (n = 20 no virus detected). Sequencing characterises the FMD viruses collected from Thailand as belonging to the O/ME-SA/Ind2001e and A/ASIA/Sea-97 lineages. A recent NSP serosurvey in Thailand (n=2,866 sera) has highlighted a seroprevalence of 30.08%. Vaccine matching has been undertaken using LBPE and VNT; where serotype O (O/ME-SA/Ind-2001e lineage) were well-matched against the O/189/87 FMD vaccine from Thailand. Testing of two Thai vaccine strains for serotype A shows that A/Sakolnakorn/97 is well matched for most A/ASIA/Sea-97 strains (6/7 for VNT and LBBE), while the A/Lopburi/2012 responses are less well-matched (only 1/7 generated an r1 value >0.3 using VNT). A renovation project of the BSL-3 facilities at Pakchong is underway and will be ongoing until 2023.

Pool 1: East Asia and China (Wen Dang)

A total of three official FMD outbreaks have been reported in three different provinces in China during 2021. Samples from confirmed cases have been supplemented by other samples from clinical or suspect FMD cases in the country (17 samples in total). National surveillance for FMD has also continued using real-time RT-PCR (n= 1801 lymph node and OPF samples), LPBE and 3ABC ELISA for samples collected in slaughterhouses located in 12 Chinese provinces and from border areas in the southwest of the country. FMD positive samples (n=33, VP1) have been sequenced – representing from serotype O (O/CATHAY and O/ME-SA/Ind-2001e lineages) with close relationships to FMD viruses circulating in China and southeast Asia in recent years. The O/ME-SA/PanAsia strain has not been identified since 2019 and there have been no outbreaks caused by O/SEA/Mya-98, but the lineage is believed to still be circulating in the field, especially in pigs. Vaccine matching (using VNT) shows a good antigenic match for the Re-O vaccine strain against O/ME-SA/Ind-2001e, O/SEA/Mya-98 and O/CATHAY field strains, in contrast to the O/BY/2010 vaccine strains that generated poor results for Mya-98 and CATHAY lineages. In addition, in vivo potency studies have been conducted in pigs to demonstrate protection for the Re-O (and alternative Re-O/17002) vaccine against a recent O/SEA/Mya-98 field isolate.

Pool 1: East Asia and South Korea (Jong-Hyeon Park)

There have been no FMD outbreaks reported in the Republic of Korea during 2021 and the last FMD virus lineage detected was O/ME-SA/Ind-2001e in January 2019. Surveillance activities continue involving the testing of samples from quarantine inspection (n=219 rRT-PCR), Animal disease control (n=160 rRT-PCR) and NSP antibody sero-surveillance (n=449,002). The vaccination policy in the Republic of South Korea uses commercial vaccines from three international suppliers (BI, ARRIAH and Biogenesis) covering serotypes O and A. Sera (n=448,458) have been tested for post-vaccination monitoring purposes using serotype O ELISA demonstrating that antibody levels in cattle, breeding pigs and fattening pigs are all >80% at a population level. Vaccine matching (using VNT) has been performed for four established FMD vaccine strains (O1/Manisa, O/3039, O/Primorsky/2014 and O1/Campos) as two FMD vaccine candidates from South Korea (Om-O/PanAsia-2 and O/BE/SKR/2017) against representative field isolates from four different lineages (O/ME-SA/PanAsia, O/ME-SA/Ind-2001d, O/SEA/Mya-98 and O/CATHAY). The best antigenic match for all eleven isolates was demonstrated for the O1/Manisa vaccine. For serotype A, the A/Zabaikalsky and A22 vaccines provide best coverage for A/ASIA/Sea-97 in contrast to the A24/Cruzeiro that was not matched to any of these field isolates. In vivo potency studies provide confidence that vaccines are fit for use, where new small-scale studies in pigs have indicated that three serotype O vaccines (O1/Manisa+O/3039, O/Primorsky/2014+O/SEA/Mya-98 and O1 Campos) when given as two doses provide protection for challenge with an O/CATHAY field isolate. Similar small-scale studies in pigs have also investigated the cross-protection provided by serotype A vaccines.

Pool 1: Russia (Svetlana Fomina)

The Russian Federation has three zones recognized as FMD free by the OIE, comprising a large zone that is FMD-free without vaccination and two additions zones that are FMD free with vaccination (13 areas in the southwest bordering the Black and Caspian seas and the Island of Sakhalin). Application to the OIE for FMD-free status for a further 4 zones along the southern border will be made in 2022. In 2021, 131 samples from cattle and pigs with suspected FMD were tested using RT-PCR, but no FMD virus was detected. A further 444 samples collected from wildlife located in different regions for surveillance purposes yielded RT-PCR negative results. Sero-surveillance using LPBE has been undertaken in non-vaccinated populations (n=24,150 sera) to demonstrate absence of FMD circulation and the LPBE has also been used in FMD vaccination animals (n=19,879 sera) to assist in post-vaccination monitoring. In addition, the NSP ELISA has been used to test 63,706 sera from cattle during 2021. The O/ME-SA/Ind-2001e lineage has been identified as a new risk for Russia, based on the circulation of this lineage in neighbouring counties (such as Mongolia).

Pool 2: India (Rabindra Prasad Singh)

The ICAR-DFMD laboratory has moved recently from Mukteswar to Bhubaneswar. Data presented in the talk covered samples that were tested during 2020, where 215 samples were received associated with 94 serotype O outbreaks, 28 serotype A outbreaks and 9 serotype Asia-1 outbreaks in the country. During 2020, FMD sero-surveillance in India encompassed 28,284 samples which exhibited 16.2% positivity when tested by a 3AB3 NSP ELISA. New on-going surveillance for 2021 will also accommodate probang sampling in potential disease-free zones. New sequence data generated in 2020 were characterised as serotype O (O/ME-SA/Ind2001e) and Asia-1 G-IX (BD-18). The O/ME-SA/SA-2018 lineage (reported at last year's meeting) needs to be closely monitored in the region. The first detection of Asia-1/GX-IX (BD-18) in January 2020 can be interpreted as an incursion from Bangladesh or in-situ evolution within India from the prevalent Asia-1/G-VIII virus. A trivalent vaccine has been used in India since 2003 and vaccine matching is regularly carried out using monovalent BVS using 2D-VNT. Recent results show that serotypes O and Asia are well matched against respective field isolates, but the serotype A component only matches 56% of isolates collected since 2007.

Pool 3: Turkey (Can Çokçalişkan)

During 2021, 109 samples have been received from field cases of FMD in Turkey (60 typed as O and 3 FMDV-positive by RT-PCR but not serotyped), plus a further 25 samples from Iran (14 typed as O, 17 typed as A and 3 typed as Asia-1). Sero-surveillance activities in Turkey have included testing of sera (n=4596) to support the continued FMD-free (with vaccination status) in Turkish Thrace, NSP testing to support epidemiological investigations in Anatolia (n=58,279) and post vaccination monitoring. FMDV positive samples (n=35) have been sequenced to show that all the Turkish samples are serotype O within two sub-lineages (QOM-15 and ANT-10), while Iranian isolates have been characterised as O/ME-SA/PanAsia-2/ANT-10, A/ASIA/Iran-05/FAR-11 and Asia 1/Sindh-08. NB: serotype A and Asia 1 have not been detected in Turkey since 2018 and 2015, respectively. There are plans to include a new strain in the vaccine (representing an A/IRN/21 (A05-FAR-11) isolate) to compensate for the recent poor r-values generated by vaccine matching by the ŞAP Institute.

Pool 4: Kenya – East Africa (Abraham Sangula)

Ninety-eight samples were submitted from field cases of FMD in cattle during 2021 (37 typed as O, 7 as A, 1 as SAT1, 17 as SAT2). Approximately 1300 sera have been tested by VNT; where 74% were antibody positive, and approximately 200 samples have been tested using NSP ELISA with two-thirds positive for FMDV-specific antibodies. VP1 sequence data for samples submitted to the WRLFMD identified 13 serotype O, 1 A and 1 SAT1. The FMD virus lineages represented are: O/EA2, A/AFRICA/G-I, SAT1/I and SAT 2. Homologous in-vivo potency tests have been recently undertaken using the KEVEVAPI vaccine components (O K77/78, A K5/80 and SAT2 K52/84) generating >6 PD50 results for all experiments.

Pool 4: Ethiopia – East Africa (Daniel Gizaw)

East Africa is endemic for FMD and the disease is a major obstacle to agricultural development, livestock production and animal export. In Ethiopia, the most common serotypes are O (in the central and south-east of the country) and SAT 2 (in central and western areas). Due to COVID-19, there has been limited outbreak surveillance in the country; however, 112 samples have been tested by NAHDIC - 22 serotype O, 7 SAT1 and 24 SAT2. A further 8 samples could not be serotyped. Surveillance testing of 4695 small ruminant and bovine samples has also been undertaken, of which 849 were antibody positive and 3846 negative. Export testing has also been performed for small ruminants, where 18.1% were positive for NSP antibodies.

Action 021-03 Organise a shipment of samples to WRLFMD to further investigate the re-identification of SAT 1 in East Africa for the first time since 2007.

Pool 5: Nigeria – West Africa (Wungak Yiltawe)

There have not been many outbreaks this year but serotype O and SAT2 have been detected from the 24 suspect samples collected in the field. 8 samples were not able to be serotyped because of the antigen ELISA used but SAT1 is suspected, and these samples will be sent to WRLFMD or NCFAD, Canada for further analysis. More than 2000 samples have been received for surveillance purposes from cattle, sheep, goats and pigs where 400 samples have been identified as antibody positive. Large batches of samples have been sent recently to NCFAD, Canada and WRLFMD and results will be reported shortly.

Action 021-04 Samples to be shipped from NVRI for sequence analysis as soon as possible.

Action 021-05 WRLFMD or Sciensano to provide virus for monoclonal antibody screening to IZSLER so that the antigen ELISA kit can be upgraded.

Pool 4-6: Sub-Saharan Africa (Livio Heath)

From South Africa, forty-two clinical samples have been received during 2021, of which 2 have been serotyped as SAT 2 and 15 as SAT 3. A further 8 samples relating to illegal transborder animal movements were received from Eswatini, but no virus was detected. One SAT 2 and three SAT 3 viruses from the South African outbreaks have been sequenced. The FMD virus causing the SAT 2 outbreak in KwaZulu-Natal was closely related to an isolate collected in the north of Limpopo from 2019. The SAT 3 outbreaks are located in the same area in Limpopo that was affected during 2015. Surveillance activities using SPCE and RT-PCR have been undertaken for different purposes: (i) continued country-level survey, (ii) to support investigations into the field outbreaks of SAT 2 and SAT 3, and (iii) for animal movements in South Africa. A small number of additional samples have been tested for clients in Eswatini, Thailand, India and Zimbabwe.

Since 2015, 10 FMD outbreak events have been reported in South Africa. Buffalo are not implicated in any of the outbreaks identified in recent years and there is evidence to suggest the FMD viruses are being maintained within the domesticated cattle population, where many of the outbreaks have been characterised by mild clinical signs or sub-clinical infection. Vaccine matching using SPCE has been undertaken against the FMD vaccine strain SAR 3/04/2 that was developed 5 years ago at OVI.

Brief comments from questions:

The observation of mild clinical signs – may be related to the long-term circulation of FMD in cattle populations.

Status of locally produced vaccine? -in process of registering and hope to be in commercial use by mid-2023.

Pool 4-6: Sub Saharan Africa (Elliot Fana)

During 2021 the RRLSSA has received 49 samples collected from suspect FMD cases in 5 countries: from Namibia (serotype O and SAT2), Botswana (FMDV negative), Zambia (serotype O and SAT 2) and from Malawi (serotype SAT1). VP1 sequencing has confirmed SAT1 in samples from Malawi and O/EA-2 and SAT2 in samples from Namibia as well as serotype O/EA-2 in samples from Zambia. Cases due to O/EA-2 in Zambia and Namibia represent the recent spread of this topotype from East Africa and pose new threats for onward spread south into Botswana, Zimbabwe and South Africa. Serotype O vaccines are not widely used in Pool 6. In order to anticipate future demand for a regionally relevant serotype O vaccine, the RRLSSA has generated new vaccine matching data (using VNT) for O-Manisa and O-3039 that indicates that these two vaccine strains are antigenically matched to the field isolate from Namibia.

Pool 7: South America (Maristela Pituco)

No suspect FMD samples have been received during 2021; however, testing has been performed for differential diagnosis of other endemic diseases (VS, SVV, bluetongue and poxvirus infection). A collaborative research project with NCFAD, Canada has completed the complete genome sequencing of 95 historical South American serotype O and A strains collected during 1950-2018. Together with other data, these sequences demonstrate that the North Andean region is an isolated ecosystem, and there is no evidence that FMD viruses (from serotype O or A) from this region have spread to other parts of South America.

With the exception of Venezuela, other countries in the South American region remain FMD-free (representing 95.9% of the total cattle population). PANAFTOSA is working with Venezuela to improve surveillance and to strengthen vaccination strategies to improve immunity. Proficiency schemes were organised for 22 laboratories from 17 countries this year to strengthen laboratory quality.

Question relating to differential diagnosis – what is the presence of Seneca Valley Virus infection in the pig population? For the COSALFA 2020, two countries reported the detection of Seneca Valley Virus as part of routine surveillance for FMD (and its differential diagnoses): Brazil with 86 farms affected and Colombia with 9 farms. In total, 1884 suspicions of FMD were investigated and ruled out by the countries of the Region in 2020. *https://www.paho.org/sites/default/files/informe_situacionpaises-2020-borrador_1.pdf*

Pool 7: South America (Sabrina Galdo)

Twenty-two samples relating to FMD 6 suspect cases have been received this year, all were negative for FMDV. Surveillance of more than 9,000 samples has been carried out to confirm absence of virus circulation and all were FMDV negative. Sequencing of 19 type O and 4 type A FMD viruses has been undertaken as part of a collaboration with Vietnam and 1 type O and 3 type A as part of a project with South Korea. Vaccine matching tests demonstrate good antigenic match for a range of serotype O field isolates (from O/CATHAY, O/ME-SA/Ind-2001, O/ME-SA/PanAsia and O/SEA/Mya-98 lineages) against the O1 CAMPOS vaccine strain, but a poor match for monovalent A24 Cruzeiro for the strains circulating in South Korea and Vietnam. However, the trivalent vaccine O1 CAMPOS/A24 Cruzeiro/A Argentina 2001 provided better results for these serotype A viruses. Three in-vivo potency studies have been undertaken using the trivalent vaccine (O1 Campos, A24 Cruzeiro, A/Argentine 2001) with challenge by serotype O isolates from South Korea which all yielded with good results (all >6PD50).

The only risk currently identified is in relation to trans-pool movements in other regions so evaluating the optimisation of antigen bank is a priority.

Action 021-06 Potency tests are expensive with ethical constraints and are being undertaken by a number of partner organisations. From a network perspective, we should consider how we can coordinate these studies and ensure that data are rapidly communicated to support FMD control initiatives. WRLFMD to explore whether this information can be included as a new webpage on the Network website.

Update from SCIENSANO (David Lefebvre)

No suspect cases but 10 samples from 4 herds were received by Sciensano for exclusion diagnostics - no FMD virus detected. Further samples were received for import, export drug testing and vaccine control, all were FMDV negative. Participation in three proficiency testing schemes from ANSES, TPI and BVI this year gives the opportunity in the absence of samples to keep sequencing knowledge up to date. No vaccine matching performed this year, but small-scale training is provided to students.

WEDNESDAY 24th NOVEMBER 2021, DAY 2

Update from CSIRO (Wilna Vosloo)

FMD activities include two research collaborations with FLI, Germany. The first project investigated the safe transport of infected epithelium samples, and whether FMDV will be inactivated using commercially available buffers and if virus can then be recovered for further investigation. McIlvane buffer was shown to be the simplest, cheapest and most effective option for on-farm inactivation and

all buffers preserved RNA for at least 48 hours. The second project aimed to understand FMD virus survival confirming that temperature, rather than humidity impacts upon FMDV survival. FMD viruses were rapidly inactivated at temperatures > 20°C but could survive for up to 10 days at 10°C. A further project (with MSD) has adopted a systems-immunology approach to study early immune response to FMD vaccines in pigs by comparing intramuscular vaccine with intradermal vaccines administered using an IDAL device. This presentation also highlighted a number of in vivo studies that have been conducted recently to assess FMD vaccine performance (see Action 021-06).

Update from ANSES (Souheyla Benfrid)

During 2021, samples have been tested from Burkina Faso, Niger and Mauritius. Samples from Burkina Faso comprised 12 LFDs collected during 2020, from which serotype SAT 2 FMD viruses were detected (sequenced as SAT 2/VII/Lib-12), while samples from Niger included 7 LFDs and 59 swabs which yielded 15 serotype A and SAT 2 viruses (sequenced as A/AFRICA/G-IV and SAT2/VII, respectively). An outbreak in Mauritius (Rodrigues Island) this year resulted in the receipt of two Epi and 16 blood samples and the detection of 2 serotype O samples; sera from this outbreak were received for NSP and ELISA testing, 4 were NSP and O positive (unvaccinated) and 31 were type O positive by NSP negative (17 of which were vaccinated and 14 unvaccinated). Sequencing confirmed the reappearance of O/ME-SA/Ind-2001e in Mauritius.

Other activities include the validation of a new Triplex one-step real time RT-PCR for detection of FMDV and ongoing revision experiments for a Duplex one-step rtRT-PCR for detection of viruses from the A/AFRICA/G-IV lineage. ANSES organised an FMD Proficiency Testing Scheme for 42 laboratories from 40 countries.

Update from IZSLER (Santina Grazioli)

No clinical samples have been received this year.

IZSLER has produced and distributed 409 ready-to-use ELISA kits to 37 countries which represents an 80% decline based on previous years; this reduction in requests may be primarily linked to shipping issues this year. Two mulitplex lateral flow devices have been developed in collaboration with the University of Turin for identification and serotyping of FMDV in the field (One for EurAsian serotypes and one that detects PanFMDV, SAT 1, SAT 2). Validation using clinical samples gave diagnostic sensitivity results to antigen ELISA testing. Extensive field validation on clinical samples is now needed.

IZSLER has participated in an interlaboratory study (coordinated by WRLFMD) that aims to harmonise and calibrate VNT methods used for post vaccination monitoring. IZSLER provided support for the AgResults Foot and Mouth Disease Challenge Project focusing on the antigen profiling of 67 FMDV isolates for use as candidate reference antigens from Pool 4 of the EA region. Furthermore, a field trial to assess the effectiveness of multivalent FMD vaccines (O, A and Asia 1) used in Transcaucasian countries has been performed where 180 sera from Azerbaijan have been tested – where early results suggest that the vaccine is not expected to induce protective responses in the vaccinated population, even after a second administration of the vaccine.

Update from NCFAD (Charles Nfon)

This presentation provided an overview of data generated from an on-going collaboration with NVRI, Nigeria. A large batch of tissue samples (n=177) have been sent to Canada from which serotype O (n=103), serotype A (n=22) and SAT 2(n=24) have been detected. Sequence data confirm the presence of FMD viruses from the O/EA-3, A/AFRICA/G-IV and SAT 2/VII lineages. For serological surveillance,

1060 samples from multiple states in Nigeria have been received to add knowledge to the seroprevalence of FMD, 708 are still to be tested with ~85% positivity identified so far.

Update from FADDL/APHIS, Plum Island (Robin Holland)

Diagnostic testing is maintained for vesicular diseases and 1,738 samples have been tested for FMD by RT-PCR, the majority of which have been submitted for swine vesicular investigations in the US (many of which are associated with Seneca virus A infection). All FMDV testing as part of the passive surveillance programme in the US has yielded negative results. Capability for sequencing FMDV is maintained although none has been performed in 2021. A desktop exercise to plan for the event of an FMD outbreak has recently been undertaken.

Introduction from WBVR, The Netherlands (Phaedra Eble)

This presentation provided a brief history of WBVR and introduced the research team and the laboratory tests that are all ISO17025 accredited (RT-PCR, VI, Ag ELISA, VNT, Ab ELISA and NSP ELISA). A lot of epidemiological/transmission studies have been carried out to quantify the effect of emergency vaccination on dampening down an epidemic and this information is used in Dutch contingency plans, EU regulations and OIE standards.

Contract research for third parties involves animal experiments to test FMD Vaccines, including efficacy experiments, serological tests and challenge experiments in small and large animals. In the last five years 90 studies have been undertaken and this has resulted in a very valuable serum collection. As an FAO Lab, it is anticipated that WBVR can assist with post-vaccination monitoring, vaccine quality control, sharing information on the relationship between homologous and heterologous potency and also contribute llama FMDV-specific antibodies from a large collection.

Discussion: Tools to disseminate and display FMD data

Update on FMDV genotyping dashboard (Antonello Di Nardo/Uli Mueller)

The Network of OIE and FAO Reference Laboratories provides critical surveillance information about the circulation of FMDV lineages. This presentation highlighted new tools that have been recently developed by WRLFMD to disseminate FMDV sequence data and provide tools for viral genotyping. The core capability for these tools centre on a new FMDV sequence database (www.fmdbase.org) which displays and allows for downloading of publicly available (n=12,146) FMDV sequences. Unpublished FMDV sequence data can also be retrieved (n=16,764) from the database. A new initiative in partnership with Epi-Interactive (New Zealand) and funded by EuFMD is developing a FMDV Genotyping dashboard to allow users to generate custom phylogenetic trees and reports for inputted sequences. A prototype version of this tool was demonstrated by Dr Mueller.

Update on FMD Surveillance dashboard (Melissa McLaws/Shankar Yadav)

This second presentation provided an overview of new dashboard tools that have been established to help present FMD data to the wider community. This work builds on the quarterly reports from WRLFMD/EuFMD to (i) visualise FMD surveillance information, (ii) track progress along the PCP, (iii) identify gaps (i.e., monitoring of specific lineages) and (iv) promote and facilitate the sharing of data within the regional roadmaps. Three prototype dashboards have been established using Tableau Public (free software) for the display of (i) PCP information, (ii) FMD surveillance information and (iii) FMD vaccination data. It is planned that these systems will be interoperable with the Network website and other systems such as EMPRES-i. These systems were demonstrated, and feedback is requested - contact: melissa.mclaws@fao.org.

Action 021-07 Please complete a survey to share your opinions on the EuFMD FAST Report: https//www.surveymonkey.com/r/XHVLHNS.

Discussion:

[1] *Will the dashboards accommodate different FMDV lineages?* – MM: Yes, this is certainly an ambition of these tools – AdN: Scope to establish a single portal that uses the epidemiological/sequence data and connects to systems such as NextStrain (https://nextstrain.org/)

[2] What type of data will be included in the FMD vaccine dashboards? - MM: Initially this could accommodate vaccination questionnaires used at the regional roadmap meetings – but it is also imagined that vaccine matching data will also be very valuable.

Discussion: Vaccine Selection and post vaccination

Priorities for collaborative vaccine matching studies within the Network (Aldo Dekker)

The presentation reviewed approaches used to select FMD vaccines and predict their ability to protect animals from disease in the field, including vaccine matching (r1-value determination) and heterologous testing by VNT (where access to the vaccine strain is not required). The use of these methods can be complicated by relationship between VN titres and levels of protection which is not always the same within an FMDV serotype. Specific weaknesses of current approaches include (i) inherent variability of VNT methods in different laboratories and differences between results generated by VNT and LPBE, (ii) the use of pooled bovine vaccinal sera (BVS) that does not provide adequate information on variance of the calculated r1-values, (iii) uncertainty and variability in the homologous potency of vaccines that are used to generate the BVS, and (iv) the complication of defining vaccine performance when multivalent vaccines are used. Immediate priorities include collection of standard reference sera for FMD vaccines (from standardized conditions) and the collection of more data from PVM studies. It was also suggested that better precision in r-values might be achieved by using five separate BVS (as opposed to pooled BVS) perhaps using a 1D assay format.

Alternative tests for vaccine evaluation (Amin Asfor)

An on-going collaborative OIE Twinning project with AU-PANVAC and WRLFMD aims to establish new immunoassay formats to evaluate the quality of FMD vaccines used in East Africa. New assays including total IgG, isotype and avidity formats have adapted approaches that have been successfully used in South America. Compared to VNT, these assay formats have the benefit that they do not require cell culture facilities and can potentially be used in low-containment laboratories. Sera from FLI (Brehm et al., 2008) has been used evaluate candidate assays; where VNT was found to be the best indicator of protection after vaccination. In this preliminary study, all ELISA formats provided a poorer indication of protection and feedback from other laboratories is sought to see whether similar results have been generated with these assays.

Question: Does recombinant integrin used in these assays differentiate between 146S and 12S FMDV components? – AA: No this is not the case – and the presence of 12S particles is an important confounder with these assays.

Regional reference antigens for heterologous testing: an update (Don King)

In many FMD endemic regions, vaccines can be sourced from a wide range of different suppliers (containing different vaccine strains and formulated at different potencies). Quality assessment of FMD vaccines often focuses only on the homologous responses (according to the OIE Manual), while performance of the vaccine in the field is dependent upon heterologous antibody responses that can

protect against the virus strains that are circulating. Vaccine matching remains as an important tool but does not assess the responses of multivalent vaccines and is limited by access to commercial live vaccine strains and relevant BVS. In 2019, the Network proposed to support heterologous testing of final formulated vaccine products (as supplied to customers) using regionally relevant reference FMDV antigens. This heterologous system has been recently developed for FMD viruses circulating in East Africa (http://www.wrlfmd.org/node/2096/) where 16 FMD virus reference antigens (from 4 serotypes) have been used. In partnership with IZSLER, the WRLFMD has generated antigenic profiles for these viruses using VNT and binding of monoclonal antibodies. This simple system can be used to directly compare post-vaccination responses from different vaccines (even after booster vaccination) and has the benefit that access to the vaccine strains from the companies is not required. In order to further develop these tools, it is recommended that the Network partners work to identify candidate antigens that might be used (and made widely available) in each of the endemic pools and support work to collect representative sera collected after vaccination with a wide range of different vaccines. Although further work is required to define serological cut-offs and to address inter-laboratory variability of the VNT, the approach is broadly supported by the SCAD committee of the OIE and may be suitable for inclusion into the OIE Manual at a future point.

Questions: FAO is requesting that vaccine companies supply relevant sera (at small volumes) that can be used to help standardise testing in the countries where the vaccine is used.

Action 021-08: FAO will also explore whether larger volumes of post-vaccination sera might be supplied to Network laboratories for use as reference sera.

Action 021-09: It is proposed that the Network re-establish a working-group to cover vaccine selection and quality and the harmonisation of heterologous post-vaccination responses. Proposed members include: Aldo, Amin, Charles, Livio, Maristela, Phaedra, Sabrina, Santina, Samia (+ Anna Ludi when she returns from maternity leave).

Global and Regional FMD Risks: Review of regional and FMDV lineage distribution information and maps in Annual Report

Conjectured relative prevalence of circulating FMD viral lineages in each Pool*. For each of the regions, data represent the relative importance of each viral lineage [prevalence score estimated as a percentage (%) of total FMD cases that occur in domesticated hosts].

* - these are viral ecosystems not necessarily FAO or OIE recognised regions

Lineage	Southeast / Central / East Asia [Pool 1]	South Asia [Pool 2]	West Eurasia & Middle 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			35					
O ME-SA PanAsia	10							
O SEA Mya-98	33							
O ME-SA Ind2001	20 (40)	80 (need to accommodate SA-2018) - 6 ↑	7	10 (2) ↓				
O EA or O WA			3	55	55 (+0.5) ↑	70 (65) O-EA-3>O/WA ↓	16 (O/EA-2) ↑	
O EURO-SA								80 (90) ↑
O CATHAY	10.5							
A ASIA Sea-97	26 (18) ↓							
A ASIA Iran-05	0		27 (22) ↑					
A ASIA G-VII		16 (10) ↓	15 (10) ↓					
A AFRICA				25 (33) ↑	22	15 (17) ↑		
A EURO-SA								20 (10) ↓
Asia-1	0.5 (0) ↓	4	12.5					
SAT 1				0	8	3	27 (16) ↓	
SAT 2			0.5	10	14	10 (15) ↑	57 (52) ↓	
SAT 3					1 (0.5) ↓		16	

↑↓ Proposed new changes (new values in)

Recent epidemiological changes that were discussed at the meeting include:

- Increasing dominance of the O/ME-SA/Ind-2001e lineage in Southeast and East Asia (Pool 1)
- Continued absence of serotype Asia 1 in Pool 1
- Presence of a new lineage called O/ME-SA/SA-2018 in Pool 2
- Decreasing threats posed by A/ASIA/G-VII in Pool 3 (although this lineage is still present)
- Situation in North Africa where O/ME-SA/Ind-2001d has not been detected for >5 year
- Emergence of the O/EA-2 topotype in Pool 6 (Zambia and Namibia)

It is also proposed that the FMD viral lineages listed in this table will be modified to separate the African serotype O and A topotypes/clades (pending).