WOAH/FAO
Foot-and-Mouth Disease
Reference Laboratories
Network

The 17th Annual Meeting of the WOAH/FAO FMD Reference Laboratory Network

29th November to the 1st December 2022

Core Members

	WOAH Reference Laboratory for Foot and Mouth Disease, Dirección de Laboratorio Animal, SENASA, Argentina Speaker: Sabrina Galdo; Participant: 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: Floris Breman
	WOAH Regional Reference Laboratory for Sub-Saharan Africa (RRLSSA) Botswana Vaccine Institute (BVI), Botswana Speaker: Joseph Hyera
PANAFTOSA	Centro Panamericano de Fiebre Aftosa (PANAFTOSA) and PAHO /WHO and WOAH Reference Laboratory for FMD, Brazil Speaker: Edviges Maristela Pituco
	FAO and WOAH FMD Reference Laboratory, National Centre for Foreign Animal Disease National Centres for Animal Disease, Canadian Food Inspection Agency, Canada Speaker: Charles Nfon
	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: Labib Bakkali Kassimi, Aurore Romey; Participant: Guillaume Girault
	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; Participants: Saravanan Participant: Jajati Mohapatra
	WOAH/FAO FMD Reference Laboratory, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Italy Speaker: Santina Grazioli; Participant: Maccabiana Giampietro

	WOAH Reference laboratory for Foot and Mouth Disease, Animal and Plant Quarantine Agency (QIA), Republic of Korea Speaker: Jong-Hyeon Park; Participant: Sang Ho Cha
	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 WOAH Reference Laboratory for FMD, Federal Governmental Institute, Centre for Animal Health (FGI ARRIAH), Vladimir, Russian Federation Speaker: tbc
	FAO Reference Laboratory for FMD in Africa and WOAH 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 WOAH FMD Reference Laboratory The Pirbright Institute Pirbright, United Kingdom Speakers: Donald King, Anna Ludi, Antonello Di Nardo; Participant: David Paton
	WOAH FMD Reference Laboratory, Foreign Animal Disease Diagnostic Lab, Plum Island Animal Disease Center (PIADC), United States of America Speaker: Modou Moustapha, Participant: Jamie Barnabei, Muhamed Fawzi, Robin Holland

Affiliates

	Australian Centre for Disease Preparedness (ACDP), Australia Speaker: Nagendra Singanallur
	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 Ularamu
	Şap Institute (and WELNET FMD), Ankara, Turkey Speaker: Naci Bulut; Participants: Sena Inel Turgut, Ünal Parlak, Can Çokçalışkan, Beyhan Sareyyüpoğlu
	ISRA/LNERV, Senegal Speaker: Modou Moustapha

WOAH/FAO Representatives

	The European Commission for the Control for Foot-and-Mouth Disease Speaker: Kees van Maanen; Participants: Etienne Chevanne, Fabrizio Rosso
	Food and Agriculture Organization of the United Nations Speakers: Melissa McLaws, Samia Metwally; Participant: Ibrahim WoraSalami
	WOAH – World Organisation for Animal Health Speakers: Bolortuya Purevsuren, Min-Kyung Park

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

	Lab Leader, Egypt Participant: Ahmed Refaat Ahmed Habashi
	Epi Leader, West Eurasia Participant: Satenik Kharatyan
	Lab Leader, SAARC, India Participant: Rajeev Ranjan
	PSO, South Sudan, Sudan, Palestine Participant: Kees Van Maanen

Invited Speakers

USDA	USDA, Fort Collins USA Speaker: Sarah Mielke
	FAO FMD Reference Laboratory, Wageningen Bioveterinary Research, Lelystad, Netherlands Speaker: Michiel Harmsen

Vaccine Producers

Boehringer Ingelheim	Boehringer-Ingelheim, VPH Veterinary Public Health Participants: Pascal Hudelet
Biogénesis Bagó	Biogenesis-Bago Participants: Rodolfo Bellinzoni

TUESDAY 29th NOVEMBER 2022, DAY 1

Opening of the 17th annual meeting and adoption of agenda (Don King)

Opening remarks from Dr King outlined the core activities of the Network: (1) collation/exchange of laboratory epidemiology data, (2) diagnostic test improvement and harmonization, (3) understanding FMD epidemiology and the changing patterns of risk and (4) quality of FMD vaccines

Actions from last year were reviewed.

Action	**Progress**
Action 021-01 coordinate contributions from the Network to the WOAH Code and Manual – Led by DL, CN, LB-K and SG. The harmonisation of heterologous post-vaccination responses (Paragraph "D. Vaccine matching tests" of Chapter 3.01.08 of the Manual) will be reviewed by the new working group	David is currently working to update these chapters and is currently focusing on the PCR section. It is planned that the revised text will be distributed to the Network for comments and approval.
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	Hybrid/virtual format of the Network allows for wider representation and contribution to the Annual meeting. Furthermore, additional countries were invited to the 2022 PT schemes organised by the reference laboratories.
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.	SAT 1 was not confirmed: samples from ETH tested at WRLFMD during 2022 and no viruses from the SAT 1 serotype were detected.
Action 021-04 Samples to be shipped from NVRI, Nigeria for sequence analysis as soon as possible.	Batches of Nigerian samples have been recently analysed at NCFAD and WRLFMD.
Action 021-05 WRLFMD or Sciensano to provide virus for monoclonal antibody screening to IZSLER so that the antigen ELISA kit can be upgraded.	Open – taken forward in 2022
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.	Open – this information will be placed on the website in 2022

Action 021-07 Please complete a survey to share your opinions on the EuFMD FAST Report: https//www.surveymonkey.com/r/XHVLHNS.	Completed
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.	To be discussed at this meeting and be taken forward by serology/vaccine QA/QC working group.
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, Charles, Livio, Maristela, Phaedra, Sabrina, Santina, Samia.	Will be taken forward in 2022 with Anna Ludi leading the group as she is now back from maternity leave.

Action 022-01 – WRLFMD to review actions from previous meetings to ensure that they have been closed.

Action 022-02 – WRLFMD to put together a short survey (excel sheet) to list kits used and to the extend they are validated to international standard. Results will be presented next year.

Update from WOAH (Min Kyung Park and Bolortuya Purevsuren)

This presentation reviewed the WOAH global status of FMD where 5 new applications from countries have been evaluated during the 2021-2022 evaluation cycle. Out of the 5 applications, 2 received a positive outcome, which include a new zone recognised as FMD-free with vaccination in Russia and the endorsement of the official control programme in Botswana, which focuses on the northern part of the country that does not have an official FMD-free status. However, FMD outbreaks in Botswana during August 2022 have led to suspension of Zone 6b, which was previously free from FMD without vaccination. Elsewhere, FMD outbreaks in Kazakhstan (zone 5) has led to suspension of the FMD-free status in the zones of the northern part of the country, due to the outbreak in zone 5 and due to implementation of vaccination since June 2022 in the neighbouring zones 1 to 4 previously having and FMD-free status without vaccination. FMD outbreaks which started in April have led to the suspension of FMD-free status in Indonesia.

The presentation also outlined changes to the WOAH Terrestrial Code and Manual, where the latest version of the FMD Chapter (potentially proposed for adoption in May 2023) includes the following changes:

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. Harmonisation of requirements for official recognition and maintenance of official FMD-free status and endorsement of official control programme by WOAH
3. Elaborated provisions regarding the establishment of a protection zone in face of threat
4. Recommendations for importation of fresh meat of small ruminants from FMD-infected countries/zone

Changes to the Terrestrial Manual Chapter 3.1.8 on FMD adopted in 2022 included: (1) addition of SVV infection to list of vesicular diseases that cannot be differentiated from FMD and (2) amendment of Table 1 test methods available for FMD diagnostics to qualify that it is essential to perform a confirmatory test (Ag-ELISA or RT-PCR) when virus isolation positive results are obtained.

Dr Purevsuren provided a brief update on the GF-TADs FMD Working Group including the work of the SEACFMD Campaign in Southeast Asia which is current under review.

Update from FAO (Samia Metwally)

This update from the FAO reviewed the FAO-WOAH global FMD control strategy and the work of the regional epidemiology and laboratory networks to combat zoonotic transboundary animal diseases. During the presentation, Dr Metwally requested support from the Network laboratories to review a new document that provides practical surveillance guidelines that are tailored for endemic countries at different/lower stages of the PCP.

Action 022-03 – Network has agreed to review this document.

Global and regional epi and lab networks for zoonotic TADs and emerging pathogen threats have new objectives including preparation of a global action plan to strengthen cooperation, collaboration and information sharing in Africa, Middle East and West Eurasia. FMD Roadmap meetings have been recently conducted for West, Central and East Africa (http://www.gf-tads.org/fmd/events/en/). Country level changes to PCP status were also presented where Georgia has advanced to stage 3, Jordan, Saudi Arabia and Tanzania have advanced to stage 2 and Gambia, Mali and Nigeria have advanced to stage 1.

A new initiative from the FAO and WOAH aims to compile a body of evidence that FMD Serotype C is no longer circulating in livestock populations. A taskforce has been established with members from FAO, WOAH and WRLFMD with 2 phases (1) gathering evidence and measuring risk and (2) reducing risk and maintaining preparedness.

EuFMD update (Kees van Maanen)

Dr van Maanen provided an overview of the work of EuFMD including recent achievements to improve training infrastructure, and national lab capacity for FAST diagnosis. The EuFMD supports the work of the WRLFMD and has also recently contributed to enhanced global surveillance via funding to ANSES and IZSLER for sample collection, shipment, data analysis, as well as PT schemes. Small scale vaccine immunogenicity studies have been recently completed in Jordan, Palestine and Uganda where the results will be published shortly. Research projects supported under the 10th Fund for Applied Research (FAR) call included: (1) emergency vaccination against FAST disease in disease free countries, (2) enhancing laboratories capacity for FAST diseases, (3) evaluating vaccination approaches strategies and vaccine types for FAST diseases, and (4) digital support tools for optimization of surveillance and other control activities for FAST diseases.

Other topics for discussion:

Nagoya Protocol – impacts and actions (All)

As discussed in previous closed sessions of the Network, the implementation of the Nagoya Protocol (https://www.cbd.int/abs/about/) continues to have relevance for international reference laboratories. It was noted by the Network that Nagoya is already having an impact on our work as the protocol needs to be addressed in any formal MTAs that cover shipping of materials between laboratories. The recent presentation by Dr Hudelet at the EuFMD OS22 highlighted important

issues relating to the Protocol on the development of new vaccine strains – that are essential for the control of FMD.

Network partners agree to prepare a position paper with input from the vaccine companies to clearly outline the difficulties that the Nagoya Protocol is causing. Two forms of the document are imagined: (i) a formal review that might be submitted for peer-reviewed publications and (ii) a short summary document to summarise the main issues that might be circulated by WOAH and FAO to CVOs and other government officials. Dr Metwally mentioned that the FAO works with both ministers and CVOs and a summary of the impact could be distributed to them. It was also noted that Nagoya is not just an issue for FMD but also other disease such as ASFV.

Action 22-04 – WRLFMD to draft a Nagoya position paper (and short summary) with contribution from the vaccine companies.

Nomenclature Group Update (Antonello Di Nardo)

Following discussion at last year's Network meeting, a survey was distributed to the Network partners to gauge opinions on the nomenclature that should be recommended for FMDV positive samples and isolates (with 15 responses). Five items were considered important in naming the viruses: serotype, country, year, sequential number of registry and reference laboratory. Three options were presented: (i) continue with the current system where there is inconsistent naming of viruses by different laboratories but perhaps include a laboratory code in the sample ID (ii) follow the results of the survey and use a format that follows Serotype/Country/Sequential no/year lab and (C) using a centralised system to assign sequential names for FMDV samples collected by the Network but this approach would require coordination and resources to maintain such a system, and would also mean that names would be disconnected from the local LIMS used in each of the labs . After discussion, it was agreed that the simplest option would be (ii) and the Network working group will now work to prepare a formal recommendation that accommodates these points.

Action 22-13 – Should the management section on the first day be confidential? Next year there will be no management session. If you disagree, please contact WRLFMD.

Action 22-14 – The working groups: Nomenclature, Serology/vaccine QA/QC, Manual and Code working group could be put on website

The meeting was opened with a welcome address from Dr Matthijn de Boer on behalf of Prof. dr. Annamarie Rebel (Director of Wageningen Bioveterinary Research). The delegates expressed thanks to the meeting hosts, WBVR, and to WOAH, FAO and EuFMD for supporting the meeting. A vote of thanks was also given to Jacqueline Wijbenga at WBVR, and Sarah Belgrave and Julie Maryan at WRLFMD for assistance with the meeting logistics. Sponsorship for the meeting was obtained from Biogenesis Bago, Boehringer Ingelheim and MSD Animal Health.

Update from WRLFMD (Don King)

During 2022, 252 samples from 12 countries have been tested at the WRLFMD. This is in addition to genotyping reports where only sequences were submitted and analysed. These sample numbers are lower than in 2021 and the probably reflect the increased costs and logistics associated with international shipments. Other Network partners confirmed similar observations – which indicate that costs and logistics to receive samples into the Network are more challenging post-COVID.

The presentation focussed on headline epidemiological events that have occurred during 2022:

- A new clade within O/ME-SA/PANASIA-2^{ANT-10} has caused outbreaks in Eastern Mediterranean countries (Jordan, Palestine and Israel). These FMD viruses are most closely related to those found in Pakistan and UAE and this lineage appears to have become more dominant than sub-lineage O/ME-SA/PANASIA-2^{QOM-15} that was previously found in this location.
- There continues to be increased dominance of O/ME-SA/Ind-2001e over other serotype O lineages. For example, in SEACFMD countries there were previously four lineages of serotype O; however, since 2020/2021 only O/ME-SA/2001e has been detected. Indonesia, which has previously been free from FMD (since 1990) has reported its first FMD cases duef O/ME-SA/Ind-2001e. This is a difficult outbreak to control because there are 17.7 million head of cattle in addition to an even larger population of small ruminants. It is anticipated that Indonesia will use vaccine from different manufacturers and will require support from the Network to monitor the performance of these vaccines.
- For Pools 2 and 3, an emerging lineage called O/ME-SA/SA-2018 has been detected in India, Sri Lanka and UAE. There is an increased number of reports for this lineage; however, vaccine matching suggests there is good antigenic match.
- FMD outbreaks in the Maghreb in North Africa have been due to the O/EA-3 topotype and sequence data shared within the Network shows that this is a new introduction that is distinct to cases that occurred in 2018.
- For Southern Africa (Pool 6), the O/EA-2 topotype continues to cause more outbreaks and has now been reported in Zambezi, Namibia, Malawi and Mozambique. Together with cases in Zambia (2018-2021) this is the first detection of serotype O in southern Africa for ~20yrs. These findings are important because serotype O vaccines are not widely used in the region.
- Published reports of FMD cases in Egypt have been characterised as O/EURO-SA and A/EURO-SA: South American viruses. These unexpected outbreaks need to be monitored closely since there is potential for onward spread in North Africa and the Eastern Mediterranean

Pool 1: Southeast Asia (Kingkarn Boonsuya Seeyo)

An increasing number of samples are characterised as from the lineage O/ME-SA/Ind-2001e. Training of scientists from other regional labs is continuing, and this includes biosafety training activities. Research activities include validation of ELISA (specificity and sensitivity). Also, studies are

ongoing looking at the antigenic comparison of O/ME-SA/2001 and the locally produced vaccine strains; since there is discussion to switch to using a tailored O/ME-SA/2001 vaccine strain.

Pool 1: East Asia and China (Wen Dang)

Three serotype O lineages of FMDV have been detected since 2020: O/SEA/Mya-98, O/CATHAY and O/ME-SA/Ind-2001, while the last case of serotype A was seen in 2019. During 2022, there has been only one official FMD outbreak reported in China due to O/CATHAY although surveillance has identified positive samples comprising O/SEA/Mya-98 (n=3), O/ME-SA/Ind-2001e (n=12) as well as O/CATHAY (n=24). It is believed that O/Mya-98 is circulating in pigs however no clinical cases have been reported since 2021. The total number of clinical cases is decreasing which could be due to a decrease in illegal animal movement from southeast Asia due to the COVID pandemic.

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

No FMD outbreaks have been reported in South Korea. Samples have been collected for quarantine inspection: RT-PCR (385) and SP ELISA (501,112) and NSP ELISA (505,641). In 2022, three different commercial vaccines were used in cattle and pigs in the country. The sero-positivity using the SP ELISA is increasing, with 97.9% cattle having FMDV-specific antibodies. Testing has continued for NSP antibodies; however, none have been detected during this year. Samples which were typed as O/ME-SA/Ind-2001e have been collected from Vietnam and Cambodia as part of research collaborations. Serotype O vaccine matching was performed on four samples: Laos (2), Vietnam (1) and Cambodia (1) where antigenic match was seen against O-3039 and O/SKR/BE/2017. For A/ASIA/SEA-97 two samples from Cambodia were tested with one being matched against A/SKR/YC/2017. A/ASIA/G-VII (strains received from WRLFMD) did not match against A/SKR/YC/2017. Small scale heterologous *in-vivo* challenge studies using challenge strains of O PanAsia, A Sea-97 and A Iran-05 have been recently carried out. Encouraging data was presented for vaccines from different manufacturers (Boehringer Ingelheim, Biogenesis Bago and ARRIAH).

Question: What is the testing algorithm used for NSP surveillance? Two ELISA kits are used as well as the EITP in very some instances which allow false positives to be excluded.

Pool 1: Russia (tbc)

In December 2021, there was an FMD outbreak in Orenburg Oblast due to a O/ME-SA/Ind-2001e lineage virus. Samples have been tested from Russia (152), Kazakhstan (2) and Mongolia (3); which all represent closely related viruses in the O/ME-SA/Ind-2001e lineage. Vaccine matching for three of these strains confirms an antigenic match to O/Kazakhstan/2010 (O/Pan Asia 2) and O/Zabaikalsky 2016 (O/Ind-2001). 40,000+ serological samples have been submitted from Russia as part of surveillance, and additionally cattle and buffalo serological samples from Pakistan have also been tested for VNT and LPBE SP. During 2022, Russia has supplied kits to Belarus, Bangladesh and Russia.

Pool 2: India (Rabindra Prasad Singh)

Serotype O, A and Asia 1 FMD viruses were detected in 2021 and 2022. Lineage O/ME-SA/Ind-2001d has not been seen since 2018 and appears to have been replaced with O/ME-SA/Ind-2001e. After only being identified in a few states in 2019/2020, the emerging O/ME-SA/SA-2018 lineage has now been detected in many Indian states and there is almost an equal number of Ind-2001e and O/SA-2018 outbreaks. In addition, FMD viruses related to O/ME-SA/PanAsia have been detected Jammu and Kashmir. For serotype A, the dominant lineage is G-18/non-deletion/2019. For Asia 1, it is Group IX, which was first reported in Bangladesh. Overall, there has been an increase in number of

outbreaks (almost 8-fold) compared to last year and there has been an increased in number of outbreaks due to serotype A. The percent match for vaccine matching against serotype A is 40%, with a 100% match for serotype O and Asia 1. The laboratory has recommended to change the serotype A vaccine strain used in the country. The laboratory also carried out within country serological PTS with 10 laboratories.

Action 22-05 – ICAR-DFMD to send sequences of PanAsia related isolates from Jammu and Kashmir to WRLFMD.

Pool 3: Turkey (Naci Bulut)

Only serotype O FMD outbreaks have been reported in Turkey during 2022. The Thrace region remains free from FMD with the used of vaccination since 2010. The laboratory has tested 148 samples from the Anatolia region which were from 86 field outbreaks. Most samples have been characterised as O/ME-SA/PanAsia-2^{QOM-15} and the remainder are O/ME-SA/PanAsia-2^{ANT-10}. Vaccine matching suggests that there is a good match with the locally produced vaccines. No serotype A viruses have been detected since 2018 and no Asia 1 since 2015. Sera is routinely collected 30 days post-vaccination. Under this scheme, over 107,000 samples have been tested by LPBE and NSP ELISA. There has been a sharp decline in prevalence by NSP in young animals (4-11months) which correlates with a decrease in the number of outbreaks in Anatolia. Elsewhere in Pool 3, for Pakistan, Iran, and Afghanistan there is very low vaccination coverage achieved (less than 50% coverage). There is a big gap on the sample submission to regional lab as no regional submissions have been received to the Şap Institute during this period. During 2022, three potency *in-vivo* studies have been performed with new potential vaccine strains. A study has also been carried out to investigate the duration of immunity conferred by the Şap Institute FMD vaccine. The best results were seen when a 6PD_{50} vaccine was used with a booster and no maternal antibodies were present.

Pool 4: Kenya – East Africa (Abraham Sangula)

In Kenya there have been reports of FMD outbreaks due to serotype O (n=13), serotype SAT 1 (n=4) and serotype SAT 2 (n=14). SAT 2 is becoming more dominant in the country, which is a change from past years where serotype O was dominant. However, additional samples (n=49) have not yet been serotyped and all samples collected during 2022 need to be sequenced. Surveillance carried out for suspect outbreaks includes VNTs and NSP ELISAs. Vaccine matching for serotype O (by VNT) shows a match which OK77/78. Four homologous *in-vivo* potency studies have taken place with >6 PD_{50} values.

Action 22-06 – Send the latest batch of samples to WRLFMD.

Pool 4: Ethiopia – East Africa (Daniel Gizaw)

The name of the laboratory has changed to the Animal Health Institute (AHI), to combine responsibility for national animal health diagnostics with the National control and eradication of tsetse fly and trypanosomosis. Outbreaks for FMD have been reported in most of the country where the following serotypes have been identified: O (n=67), A (n=33) and SAT 2 (n=22). Based on sequencing completed by WRLFMD, the current lineages circulating are: A/AFRICA/G-IV, O/EA-3, O/EA-4, SAT 2 XIV, SAT 2 XIII and SAT 2 VII^{Lib-12}. Serotype SAT 1 was last seen in 2007.

Pool 5: Nigeria – West Africa (Hussaini Ularamu)

Recent samples tested by NVRI comprise serotype O (n=10), serotype A (n=5) and serotype SAT 2 (n=5). SAT 1 has not been seen since 2015. Surveillance samples have also been submitted for 3ABC NSP ELISA; approximately 50% of sera were positive. A new risk to the area is an increase in the number of animal movements from the Central African Republic. ISO17025 accreditation has been obtained for NSP ELISA.

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

This presentation covered the recent FMD outbreaks that have been reported in South Africa. The SAT 2 outbreaks have been going on for almost two years; with 119 outbreaks in KwaZulu-Natal caused by viruses that are closely related to those recovered from cases in 2021. SAT 3 outbreaks have occurred in Limpolo and the central provinces of North-West, Gauteng, Mpumalnga and Free State. A total of 1,175 clinical samples have been tested by OVI, with 11 samples typing as SAT 2 and 55 typing as SAT 3. Currently South Africa is using BVI vaccine, where the original plan was not to deploy vaccines outside of the buffer zone; however, vaccination outside of this zone has now started. Buffalo have not been implicated in all outbreaks and anecdotal evidence suggests that the viruses are being maintained within the cattle population. Since illegal animal movements have been implicated in some of the outbreaks, a new identification system for cattle is being implemented.

Surveillance has results in the testing of >200,000 samples for SPCE and >7,000 samples for NSP. For tracking outbreaks, SP-ELISA has been implemented as the first test followed by NSP ELISA. OVI has also performed serological testing in support of regional surveillance and trade purposes for samples from: Eswatini, Malawi, Lesotho, Mozambique and Zimbabwe.

Discussion – at this stage vaccination is only a single vaccine dose is administered since the policy is vaccination to slaughter. However, this may change to vaccinating animals twice.

The outbreaks are mostly in cattle but have also been seen in small ruminants (particularly sheep). There are some reports of FMDV circulation in buffalo, but this is only for serotype SAT 2 in the North of the country. Wildlife has not been tested since they are not considered important maintenance hosts.

Pool 4-6: Sub Saharan Africa (Joseph Hyera)

Submissions have been received from five countries with the following lineages: O/EA-2 found in Malawi and Mozambique and SAT 2 topotype 2 in Botswana, Zimbabwe, Mozambique and Namibia. Serological surveillance took place in Malawi, Mozambique and Botswana. Recent data highlights that the O/EA-2 topotype has moved from pool 4 to pool 6 and is currently considered as a high threat. Vaccine matching suggests that O1 Manisa is a good match for representative O/EA-2 field isolates.

Pool 5: Update Senegal (Modou Moustapha)

There have been no submissions from suspect FMD cases during 2022 because field veterinarians are on strike and will not send samples for laboratory testing.

Pool 7: South America (Sabrina Galdo)

During 2022, suspect FMD samples (n=18) were submitted to SENASA but no FMD virus was detected and these were diagnosed as poxvirus cases. Active surveillance occurred for 4,000 samples, with 134 positives on the 3ABC ELISA which were all subsequently negative on EITB (i.e., false positives). Ninety samples were tested by VIAA antigen AGID for export; these were all negative. Samples from Pirbright were sequenced and these will later be used for vaccine matching.

Serotype C3 Indaial is currently included in the vaccine formulation as it is believed that additional virus strains in the formulation may improve the performance of high potency monovalent FMD vaccines (see: Di Giacomo et al., 2022). A decision on whether to continue to use serotype C has not yet been decided. Two potency studies (PD_{50}) using trivalent vaccine and challenging with homologous strains have occurred; in both cases the results were above $6PD_{50}$.

Pool 7: South America (Edviges Maristela Pituco)

In 2022, no samples of suspected FMD were received. Support has been given to Chile to identify Seneca virus A (manuscript has been published) where the incursion of the virus appears to be from the south and not connected to the USA. Venezuela is greatest concern for vesicular disease; between 2011-2017 seventy-two epithelial tissues were sent and these were all VSV.

The outbreak of the FMDV serotype O/EURO-SA in Egypt has been characterised and the closest sequence is O/Auauca/Colombia/2017 although currently there are no outbreaks in Colombia. The serotype A found in Egypt has a closest genetic relative to isolates collected in Venezuela. Phylogenetic trees were presented highlighting a evolutionary gap in sampling that could represent virus replication in Venezuela or Egypt.

Discussion: Seneca Valley vaccines are being developed. These are promising and show good performance. For vesicular stomatitis, vaccines against the New Jersey serotype already exist but are restricted to the Andean region.

Action 22-07 – The risk assessment, which concludes that serotype C poses an insignificant risk, will be shared with the network.

Update from SCIENSANO (David Lefebvre)

No FMD suspect cases have been received. A WOAH Twinning project between SCIENSANO and the National Veterinary Laboratory in Burundi has been accepted. This project will start when funding becomes available (date to be confirmed).

Update from ANSES (Labib Bakkali-Kassimi)

Samples received by ANSES from Tunisia were characterised as serotype O/EA-3 and represent a new incursion of this topotype into North Africa. Additional samples were received from Niger (serotype O/EA-3, A/G-IV), Mali (SAT 2/VII) and Oman (O/ME-SA/SA-2018, O/ME-SA/Ind-2001e, A/Africa/G-I, O/EA-3, and O/ME-SA/PanASIA-$2^{Ant\text{-}10}$). ANSES have also carried out NSP surveillance for Niger and Oman. As the EURL for FMD, ANSES organises a PTS that is distributed to 46 laboratories (40 countries).

Discussion: The A/AFRICA/G-I strains in Oman are closely related to isolates from the Bahrain quarantine station (originally from East Africa) and it is not clear how the virus entered Oman. It

could be that two different incursions have occurred from East Africa. Suggestion was made to make contact with the laboratories in Eritrea, and Somalia to seek further samples for characterisation.

Update from IZSLER (Santina Grazioli)

No clinical samples for field cases of FMD have been received.

During 2022, there has been close collaboration with EuFMD to carry out post-vaccination trials and improve laboratory preparedness. No in-person training has been carried out; however virtual training to update field laboratories in Northwest Syria has occurred. Five percent of samples in Syria were negative for both SP and NSP; all others (app. 1200 samples) were positive for FMD antibodies. Twenty sera from Mauritania were tested, and all were FMDV negative. Compared to 2021, supply volumes for the IZSLER kits has increased, but the levels are still not back to pre-COVID pandemic levels. The requests for kits are mostly for SP-ELISA type O. The current kits don't recognize SAT 3 but work to find new monoclonal is underway and can be anticipated to replace serotype C in the kit. IZSLER hosts the reagent bank for improved emergency diagnostic response in Southeast European countries (includes PCR reagents).

Discussion: For the LFDs the panFMD detects serotype C. There is no market to make an LFD that just detects serotype C. Lateral flow for SAT 3 is more important as panFMD covers serotype C. LFD works well on clinical sign (epithelial or fluid); however, sensitivity is lower than PCR.

Action 22-08 – The network should keep track of the LFDS that are being developed and validated.

Update from FADDL/APHIS, Plum Island (Muzafar Makhdoomi)

During 2022, FADDL has tested 2,537 suspect samples but none were positive for FMD. No vaccine matching has taken place but carried out one PD_{50} (serotype O homologous challenge). Currently there are two vaccine banks managed by FADDL: the North American Foot and Mouth Disease Vaccine Bank (NAFMDVB, Canada and USA) and the National Vaccine and Veterinary Countermeasures Bank (NAVVCB). The Foreign Animal Disease Diagnostic Course has been held this year, which may be supplemented with virtual training content in the future. The laboratory carried out a site visit to Columbia to improve biosecurity, surveillance and movement control. During mid to end of 2024, all FMD activities will move to NBAF in Kansas; however, there will be duplication of capacity at both sites for a certain amount of time. The laboratory would also like to host the FMD Network Meeting at NBAF once they have moved.

Discussion – If possible, in the future, it would be great to include results from endemic areas (from the research group).

Introduction from WBVR, The Netherlands (Aldo Dekker)

No suspicious samples were submitted to WBVR during 2022. The laboratory has carried out one *in-vivo* potency studies for commercial reasons – with confidential results. WBVR have also carried out a prime/boost multivalent study for WRLFMD (as part of the on-going twinning project with AU-PANVAC). In regard to standardizing serology, sera are available from potency tests for various strains however there are limitation on how these could be shared (due to commercial restrictions). The lab is working with IZSLER to look at standardising sera that could be used to calibrate the results for ELISA.

Discussion: Should animals be kept separately after challenge to prevent transmission of FMD between the study animals? Currently there is no legal document to stipulate this and there are ethical difficulties in housing animals separately.

Action 22-09 – WBR (Aldo Dekker) to review animal studies and prepare a report highlighting the evidence for keeping animals separate after infection.

Update from NCFAD (Charles Nfon)

During 2022, samples have been submitted from Canada and Ghana. The samples from Ghana were characterised as belonging to the O/EA-3 topotype. The vaccine matching test is up and running and the laboratory would like to harmonise their methods with other Network laboratories. NCFAD currently have an ongoing project looking at a machine-learning approach to predict vaccine matching results using only sequence data – initial results for serotype O and A indicate that the tool can predict vaccine matching status for up to 90% of field isolates.

Update from CSIRO (Nagendrakumar Singanallur Balasubramanian)

No suspect cases were submitted to CSIRO. Coordinated projects include a regional Southeast Asia proficiency testing program, which includes 8 specific pathogens (FMD is one of these). This includes 23 countries: South Asia (6), Southeast Asia (10), East Asia (6) and Pacific (1). Publications from the laboratory include the validation of assay (specifically on sensitivity/specificity) and system-based approach to assess host response.

General topics for discussion

Review of Serotype C project (Sarah Mielke)

Serotypes C FMD viruses have not been detected anywhere in the world since 2004 and a previous publication co-authored by the Network has proposed that this serotype may now be extinct. This presentation described the results from a study conducted by USDA to understand whether data from the Network can be used to substantiate a claim of serotype C extinction. The analysis uses surveillance data from the WRLFMD (1942-2021) and the Network (2012-2020) for regional pools 1-6 to build a framework to analyse the data grouped by pool at three levels of data summary, (a) by year and country, (b) combined over years for each country, and (c) combined over years and countries within a pool. This work supports claims of freedom from FMD serotype C at different scales with varying degrees of certainty by combining data with explicit epidemiological assumptions. Assumptions were (1) risk of introduction for serotype C is equal across years and (2) outbreak data in key countries represents the entire RVP. Three detection prevalence thresholds were used (1%, 2% and 5%) where the analysis tested the 95% probability of detecting serotype C. Using an unstratified approach (year and country) results support serotype extinction in all 6 regional pools, but when looking specific countries (not year) the evidence become less well supported where many countries do not reach 95% probability (at the different prevalence thresholds). The purpose of the talk was to present these data to the Network and to request for feedback on the assumptions that were made, and to review the specific data for the endemic pools.

Action 22-10 –Share document with network – with the request that Network partners provide feedback - at least for one endemic setting

Opportunities for enhanced surveillance using LFDs - review/feedback on inactivation protocol (Aurore Romey)

It is widely recognised that global surveillance of FMD could be enhanced by using lateral flow devices (LFDs) to collect FMDV genomic material that can be further analysed when these devices are sent to a laboratory. Shipping FMDV-positive LFDs requires careful consideration to ensure that biosafety risks are minimised. From previous studies it has been found that LFDs soaked in 0.2%

citric acid for 15 minutes inactivated FMDV, but diagnostic assays can still be carried out. For further validation this approach, LFDs and inactivation protocols were implemented in the field (Nigeria, Turkey and Pakistan). Virus could not be isolated using cell culture after citric acid inactivation; however, infectious virus could be recovered via transfection in some of these samples (using ZZR cells). *In vivo* studies at FLI show that RNA can be infectious when injection but not by non-invasive exposure. A protocol to describe this method has been approved by EuFMD STC. To ensure application of inactivation protocol in the field three actions could be implemented: (1) certificate stamped and signed by the veterinary services (2) sending directly the tube containing LFD in the citric acid solution and (3) adding a pH indicator on the LFD.

Discussion: In certain countries (Italy, UK and USA), full length RNA is considered an infectious pathogen and therefore shipments will still need to come under IATA regulations. However, shipping of these LFDs without dry ice is still a huge advantage and cost saving. It was agreed that the Network should provide opinion on the inactivation protocol and highlight any local/national restrictions that might have impacts on the international shipment of LFDs.

Action 22-10 - ANSES to prepare a survey that will summarise the different national restrictions and opinions on the specific controls that should be adopted to minimise the risk of sending LFD shipments

Thursday 1st December 2022, DAY 3

General topics for discussion (cont.):

FMD Vaccine Quality Control

Alternative tools for FMD vaccine QA/QC (Michiel Harmsen)

This presentation summarised the use of llama heavy-chain antibodies (VHHs) to directly assess the integrity of FMDV capsids using reagents that recognise epitopes that are present on 146S and 12S components. This direct immunoassay approach addresses the ethical and costs concerns associated with FMD vaccine batch testing via immunisation studies animals. A range of VHHs are now available for FMDV which offer (1) low limit of detection, (2) higher throughput (3) serotype and often strain specific and (4) and ability to assess the quality of VLP vaccines as well as conventional vaccines. Some of these serotype-specific reagents are broadly reactive while others are highly strain specific and therefore a different VHH may be required for each vaccine. The presentation also highlighted VHHs that could be used to discriminate between 75S (empty particles, no RNA) and 146S. The current format of these tests uses a double antibody sandwich (DAS) ELISA and the use of heat treatment can used to qualify 12S and 146S components. Beyond their use of vaccine QC, the VHHs could also be used for epitope mapping. In the future, it is anticipated that secondary phage libraries will be used to improve strain recognition.

Heterologous cut-offs and inter-lab variability (Anna Ludi)

This talk described results from a recently published collaborative paper involving Network partners: "Predicting cross-protection against foot-and-mouth disease virus strains by serology after vaccination" (Gubbins, 2022). The aim of this paper is to understand the correlation between heterologous *in-vivo* potency studies and virus neutralisation tests. Using data generated from 17 vaccine heterologous potency studies, the average heterologous neutralising antibody titre associated with 75% protection ranged from 1.17 to 2.46 $\log_{10}$. Although it was not possible to

define a common threshold, it was noted that the data were more consistent if data from two outlier O Manisa studies was removed. Further cross-protection data are needed to understand the factors that underpin this variability and to develop more robust antibody thresholds.

The presentation also described results from a project funded by EuFMD which studied the reproducibility of the FMD VNT in European laboratories. Variable results were observed when the same sera were tested against the same viruses in different FMD reference laboratories. Harmonisation of methodology and re-calibration of results based on the use of reference sera will be explored to try to reduce these differences.

Action 22-11 – The Network Working Group on vaccines/serology will look at standardised sera, as well as other options to calibrate and harmonise the data across laboratories.

Do FMD vaccines cover regional risks? (Don King)

FMD vaccines provided from different suppliers often contain different vaccine strains (with different potencies and formulation). Vaccine matching studies undertaken by Network partners assess the antigenic suitability of vaccine strains to cover genetically diverse field strains. However, the extent of vaccine matching is very limited due to difficulties to access BVS and vaccine viruses and therefore many FMD vaccines used in endemic settings are usually not tested. The Network meeting in 2019 endorsed the concept of reference antigen panels that might be used to test vaccines by assessment of heterologous VNT titres. This presentation summarised work from the WRLFMD where an antigen panel has been implemented for Eastern Africa as part of a twinning project with AU-PANVAC. This approach can be used to highlights poor quality vaccines and gaps where current vaccines do not cover key FMDV lineages in the area.

PRAGMATIST for antigen bank managers has now been developed (Ludi et al., 2022). Work is now underway to modify this tool for endemic settings using an approach that will adopt regionally relevant reference antigens.

Discussion: The Network should agree on virus reference antigens for the global pools and share these viruses within the Network. Work to continue to collect representative post-vaccination sera is also a priority.

Informatic tools for FMD

Updates on FMD Dashboard (Antonello Di Nardo)

WRLFMD (with support from EuFMD) has developed a range of dashboards for FMD. The system currently hosts 3 applications: FMDbase, FMDtype and PRAGMATIST. These will be pulled together into an openFMD App in 2023. In 2023, a FMD Surveillance Dashboard will be developed, to present data on FMD outbreaks (contemporary and historical) with the possibility to link to this other tools.

Action 22-12 Feedback needed on the nomenclature options 1, 2 or 3

SEACFMD dashboard (Bolortuya Purevsuren)

A demonstration of the SEACFMD dashboard was provided which shows information on FMD outbreaks in Southeast Asia (per year, etc). The system has different streams that can be adjusted to fit the background of the user (example policy maker, animal worker, etc.), and also displays upcoming events and a toolbox that includes communication material (in local languages).

Review of regional and FMDV lineage distribution information (Notes for Annual Report)

Suggested changes per serotype are highlighted below:

Serotype O

- For O/EA-3 include Gulf States of the Middle East on the map
- Add ME-SA/2018
- Show movement of O/EA-3 out of Africa

Serotype A

- Add A/AFRICA-GIV

Changes per pools are highlighted below:

Pool 1

- Ind-2001e two new arrows: (1) Indonesia story (risk area further east/south), (2) Mongolia into Kazakhstan and Russia
- Antigenic variability within serotype A has led to switch in vaccine stain for Packchong
- Asia 1 is absent from the region

Pool 2

- Increased number of outbreaks caused by O/SA-2018 (40%) vs Ind-2001e (60%)
- Risk of O/SA-2018 moving into Southeast-Asia
- Highlight spread to pool 3
- Group IX of Asia 1 is causing recent outbreaks
- 95% of outbreak are from serotype O; however, A also recently detected.

Pool 3

- G-VII not detected by any laboratory since 2018; should importance be decreased?
- A IRN-05 has had a switch in antigenicity with a decrease in A IRN-05$^{FAR\ 13}$ cases and an increase in A IRN-05$^{FAR\ 11}$
- Potential introduction of East African viruses into the region (serotype O and A through trade with Gulf States).
- Asia 1 circulating in Pakistan (60% serotype Asia 1 but the last time more balanced between the 3 serotypes). We may see an increase in Asia 1 outbreaks as the current immunity will be low due to only a few outbreaks being seen
- Possibility of South American strains in Eastern Mediterranean countries from Egypt (serotype O and A)

Pool 4/5

- Serotype O is predominant serotype followed by SAT 2 then A (serotype O - 50%, serotype A - 25%, serotype SAT 2 - 25%)
- O-EA-3 is dominant over O-WA (2015/2017 last detected)
- O/EA-2 big arrow into Southern Africa – has a potential route
- SAT 1 south of Nairobi
- SAT 1 topotype X – geographic restricted and little evidence it is circulating
- SAT 2 could potentially spread North
- For serotype A, A/Africa-G-IV is the most dominant
- Gap in surveillance in West and Central Africa also towards the East (difficulties collecting the samples and shipment)

Pool 6a

- Increase risk of O/EA-2
- Risk of SAT 2 and SAT 3; Botswana has a different lineage of SAT 2
- SAT 2 outbreak appears to be spreading from domestic animal to domestic animal rather than involvement of buffalo

Pool 7

- Serotype O and A in Egypt at the same time, may help to give light with what is happening in Venezuela (serotype O and A). No new risk for pool 7.

General Comments

- Gap that we don't have coordinates for the WRL data – excel sheet will help to collect more of this data.
- Maybe these maps should go on website