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

The 18th Annual Meeting of the WOAH/FAO FMD Reference Laboratory Network

10-12th October

Hosted by: NCFAD, Winnipeg Canada

Core Members

	WOAH Reference Laboratory for FMD, Dirección de Laboratorio Animal, SENASA, Argentina Speaker: Sabrina Galdo Novo
	WOAH collaborating Centre for validation, quality assessment and quality control of diagnostic assays and vaccine testing for vesicular diseases in Europe, and FAO Reference Centre for Vesicular Diseases Sciensano, Belgium Speaker: David Lefebvre; Participant: Nick De Regge
	WOAH Reference Laboratory for FMD Botswana Vaccine Institute (BVI), Botswana Speaker: Elliot Fana
	Centro Panamericano de Fiebre Aftosa (PANAFTOSA) and PAHO /WHO and 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: Shawn Babiuk; Participants: Charles Nfon, Michele Roy
	WOAH and China National FMD Reference Laboratory, Lanzhou Veterinary Research Institute (LVRI), CAAS, People's Republic of China Speaker: Wen Dang
	WOAH FMD Reference Laboratory, French Agency for Food and, Environmental and Occupational Health & Safety (ANSES), France Speaker: Guillaume Girault; Participant: Labib Bakkali Kassimi
	FAO Reference Centre for FMD in South Asia, ICAR – Directorate of Foot-and-Mouth Disease, Indian Council for Agricultural Research, Mukteswar, Nainital (Uttarakhand), India Speaker: Rabindra Prasad Singh
	WOAH/FAO FMD Reference Laboratory, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Italy Speaker: Santina Grazioli
	WOAH Reference laboratory for FMD, Animal and Plant Quarantine Agency (APQA), Republic of Korea Speaker: Jong-Hyeon Park; Participants: Soyoon Ryoo, Sung-Han Park
	FAO FMD Reference Laboratory, Wageningen Bioveterinary Research, Lelystad, Netherlands Speaker: Aldo Dekker; Participant: Phaedra Eble
	FAO Reference Centre for FMD for Central Asia and West Eurasia and WOAH Reference Laboratory for FMD, Federal Governmental Institute, Centre for Animal Health (FGI ARRIAH), Vladimir, Russian Federation Speaker: Viktor Nikiforov
	FAO Reference Laboratory for FMD in Africa and 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
	WOAH FMD Reference Laboratory, Foreign Animal Disease Diagnostic Lab, Plum Island Animal Disease Center (PIADC), United States of America Speaker: Vivienne O'Donnel, Participant: Fawzi Mohammed

Affiliates

	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

WOAH/FAO Representatives

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

Invited Speakers

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

Vaccine Producers

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

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

TUESDAY 10th NOVEMBER 2023, DAY 1

Opening of the 18th annual meeting and adoption of the agenda

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

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

Adoption of agenda and overview of lab network - Don King

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

Brief update from WOAH – Min-Kyng Park

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

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

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

Brief Update from FAO - Samia Metwally

The FAO is looking to expand the capacity of the eight regional leading FMD laboratories (five in Africa, two in West Asia and one in the Middle East). There are changes to Reference Center Designation: (1) 5-year term instead of 4 years, (2) updated guidelines for application (these have become more stringent), (3) annual reports will now be mandatory and will be posted on website with agreement and (4) joint FAO review panel at mid-term and end of designation for extension. Upcoming events in 2024 include establishing a FAO Reference Center

Epidemiology Network and a FAO Global Conference for Animal Health/One Health Reference Centres. The presentation also briefly explained that the FAO procurement process for vaccines (including FMD vaccines) is being reviewed to enable the rapid response to events in the field and better maintain the cold chain for the vaccine products.

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

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

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

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

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

Brief update from EuFMD – Donal Sammin

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

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

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

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

- Summary and Introduction sections: cosmetic changes are anticipated but information will stay the same.
- Sample/Collection and transportation, to include (1) LFDs and FTA cards and (2) milk as sample matrix. The inclusion of 50% glycerol in transport media should also be defined in the text.
- Identification of the agent: add Seneca Valley virus (SVV) and highlight an improved schema for differential diagnosis of vesicular diseases.
- The section on *Virus Isolation* could benefit from improvement to: (1) suggest washing epithelial samples 2x in medium with antibiotics, (2) add supplementary cell lines (LFBKaVb6 and ZZ-R 127), (3) include transfection and electroporation of cell lines. Routine testing to confirm that cells with different passage histories maintain sensitivity to FMDV could also be included.
- Serotyping FMDV: In addition to the use of antigen ELISA, this section could explain that alternative molecular methods including VP1 sequence and topotype/lineage specific RT-PCRs provide valuable information. While the complement fixation test is not widely used, it is suggested to keep this method in the chapter.
- Nucleic acid recognition methods: It is suggested to keep the section on agarose gel-based RT-PCR assay but add in manual and (semi-automated) RNA extraction methods. There is benefit in keeping both real-time RT-PCR methods (3D and 5'UTR) since redundancy in molecular tests is useful, although the 3D assay outperforms the 5'UTR. The revised text could also outline the possibility to include an internal control to validate negative results, although these controls are not used in all laboratories. Further information could be included to outline how to interpret real-time RT-PCR data as well as other factors that define best practice for routine molecular testing.
- Molecular Epidemiology: This section should include whole genome sequencing and next generation sequencing, but these methods should not necessarily be defined as official methods.
- Serological tests: The text could be improved to mention the use of bovine standard sera for the tests as well as more details about how to read CPE in the VNT. For SPCE, information could be included to explain how "running means" can be used to monitor cross-session assay performance in the laboratory. Validation criteria for commercial NSP/SP ELISAs could be included and recommendations for the use of these tests in different geographical regions where different FMDV serotypes may be circulating.
- Vaccine selection and vaccine matching – it is not anticipated that this section will be modified during the current review of the text.
- Vaccine potency tests: Don King provided an update from a small working group (Donald King, Phaedra Eblé, Aldo Dekker and Michael Eschbaumer) to harmonise the texts of the fixed dose (PGP/PPG) and titrated dose (PD50) studies used to assess FMD vaccine potency. Harmonisation of texts that define non-protected animals in these FMD potency is urgently required and WOAH and the European Pharmacopeia have requested that Network laboratories clarify this issue since these texts are not completely aligned: viz, the PGP method outlined in the WOAH Manual (Section 5.3.2) describes unprotected animals as those that "show lesions on the feet within 7 days after inoculation", while the other three texts define non-protected animals as those that "show lesions at sites other than the tongue".

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

Nagoya Protocol – Don King

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

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

Global Review from WRLFMD – Don King

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

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

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

Russia Pool 1 - Viktor Nikiforov

Russia is officially WOAH FMD free (without vaccination) for the majority of the country with other zones free with vaccination. In May 2023, during the 90th General Session, WOAH recognised zone 5 as FMD free with vaccination. In August 2023 the Russian Federation submitted a dossier for recognition of FMD free zones with vaccination for two zones bordering Kazakhstan. During 2023, eleven samples were received from suspect cases and tested negative. In addition, LPBE was used to test 20,562 samples from non-vaccinated animals plus additional sera from vaccinated animals. A joint paper with Kazakh and Mongolian scientists has been published describing outbreaks due to O/ME-SA/Ind-2001e which concludes that new outbreaks (during 2022) are not linked to previous outbreaks with viruses circulating in Pool 1. There are new risks associated with SAT2/XIV incursion into West Asian countries and ARRIAH has written to Commonwealth of Independent States (CIS) to notify them of the risk of SAT2 incursion into their territories. ARRIAH continues to provide diagnostic reagents (including post vaccinal SAT2 serum) and carries out a proficiency testing scheme.

Abdulnaci Bulut – Pool 3: Türkiye

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

During 2023, samples have been received to the laboratory from Türkiye (O/ME-SA/PanAsia-2^{QOM-15}, O/ME-SA/PanAsia-2^{ANT-10} and SAT2/XIV), Iraq (SAT2/XIV) and Qatar (to be processed). Data suggests that SAT2/XIV may

have been circulating for more than a year in the West Asia region before being identified. Spread in Türkiye due to the initial cases of SAT2/XIV were limited due to an animal movement standstill and vaccination, but after the movement restrictions were lifted (so that animals could be released for grazing), the number outbreaks spread to east and central Anatolia. SAT2/XIV outbreaks have also been detected in West Anatolia where spread has been attributed to animal movements. These outbreaks have had impact to increase food prices in the country. Other risks in the region include A/Asia/Iran-05^{Far-11}, A/ASIA/GVII and Asia 1/Sind-08

Pool 2: India – Rabindra Prasad Singh

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

Pool 4: Ethiopia, East Africa – Daniel Gizaw

A total of 88 samples have been received to AHI, Ethiopia during the year for testing. Antigen ELISA has been used to characterise these viruses as: 0 (17), A (10), SAT 1 (6), SAT 2 (30), but no sequencing has been undertaken. SAT 1 has been detected by antigen ELISA which would represent the first time that this serotype has been detected in Ethiopia since 2007.

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

Pool 5: Nigeria – West Africa - Hussaini Ularamu

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

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

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

Pool 4: Kenya – East Africa – Abraham Sangula

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

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

Pool 1: Southeast Asia – Kingkarn Boonsuya Seeyo

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

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

New FMD outbreaks have been detected in Chungcheong Buk-do during May 2023 on 10 cattle and 1 goat farms. These outbreaks were caused by a O/ME-SA/Ind-2001e linage virus most closely related to those from Vietnam, Cambodia, Laos, Mongolia, Russia and Kazakhstan. The source of the outbreak is unknown (possibly via import of infected objects since there are many foreign workers on Korean farms) and after nine days there were no additional cases found. Since 2010 South Korea has adopted a national vaccination program for serotypes O and A, where three different vaccines from different suppliers are used. Vaccine matching suggests that the viruses causing the most recent outbreaks are matched against O/3039, O/RUS/Primorsky 2014 and O1 Campos. Local production of an FMD vaccines is also planned with vaccine candidates for O/PanAsia-2 and O/SKR/BE/2017 (this is an Ind2001 virus) being identified.

Pool 1: East Asia and China – Wen Dang

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

Pool 7: South America - PANAFTOSA – Edviges Maristela Pituco

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

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

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

Pool 7: South America SENASA – Sabrina Galdo Novo

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

Update from Sciensano – David Lefebvre

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

Update from IZSLER – Santina Grazioli

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

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

Update from ANSES - Guillaume Girault

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

Update from FADDL – Vivienne O'Donnell

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

is ongoing). Reagents have been supplied as part of a PTS for FMD and SVA (this included positive controls), six training courses on foreign animal disease diagnostics were also carried out.

Update from WBVR – Aldo Dekker

Fifty-six samples were tested by RT-PCR for absence of FMD. Sera was tested by VNT, but this was mostly for PT samples and export/import of animals. Ten strains were also sequenced to check the nanopore sequencing protocol. PGP and PD_{50} studies were carried out using homologous strains. Reference sera calibrated to protection has been made and is available for O Manisa (more sera are planned). The mapping of 146S VHS using XLMS has also been published (https://pubmed.ncbi.nlm.nih.gov/36699337/) These VHHs could be used as a semi quantitative diagnostic test to assess the quality of FMD vaccines by comparing 146S and 12S antigens. Efforts are being made to produce 12S ELISA kits (stable plates coated with VHH M3).

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

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

Update from NCFAD – Shawn Babiuk

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

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

There are 4 modules on the openFMD website:

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

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

Nomenclature Steering Group Updates

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

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

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

The FMD Network Data Management spreadsheet used for the Annual Report will now include three pieces of information: clinical/suspected cases, surveillance and sequencing. The new excel datasheet which will be sent out this year and has an information section on how to fill it in. This is not meant to add more work as the spreadsheet mirrors what is already included in the annual reports to WOAH.

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

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

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

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

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

Table 1: Conjectured relative prevalence of circulating FMD viral lineages in each Pool.

Lineage	Southeast/ Central / East Asia [Pool 1]	South Asia [Pool 2]	West Eurasia & Near East [Pool 3]	North Africa	Eastern Africa [Pool 4]	West / Central Africa [Pool 5]	Southern Africa [Pool 6]	South America [Pool 7]
O ME-SA PanAsia-2			30					
O ME-SA PanAsia	10							
O SEA Mya-98	**21.5**							
O ME-SA Ind2001	**40**	76[1]	5.5[1]	0				
O EA or O WA			1.5	60	53.5	69	16	
O EURO-SA								**90**
O CATHAY	10.5							
A ASIA Sea-97	**18**							
A ASIA Iran-05	0		28					
A ASIA G-VII		20	5					
A AFRICA				30	17	15		

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

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

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

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

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

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

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

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

This project undertaken by USDA has analysed two datasets – one from the WOAH/FAO FMD Ref Lab Network and one from the WRLFMD (data from 1942-2021 comprising 21,909 samples of which 176 were serotype C). Using Bayesian methods, data from the Network were grouped by pool at three scales, (i) stratified by year and country, (ii) stratified by country, and (iii) unstratified. Two measures were used to support or refute claims of serotype C extinction, the detection probability (the probability that FMD virus serotype C will be detected if present, given a prevalence threshold), and the detection capability (the lowest prevalence value that can be detected by the system at a determined detection probability). The results from this analyses suggest that the 95% detection probability standard is met, (i) at prevalence thresholds (PT) of 1, 2, and 5% in all endemic pools when the data is unstratified, (ii) at the 1% PT in a few countries but in most countries (except RVP West/Central Africa) at the 5% PT when data is stratified by year, and (iii) only in a few countries and years when data is stratified by year or country. The detection capability reflects trends found in the detection probability, and

highlights countries with the largest sample submissions. These results provide important information regarding the evidence that serotype C is extinct.

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

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

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

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

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

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

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

New methods to directly assess 146S content – Toby Tuthill

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

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

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

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

Doing things differently – Don King

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

costs of these technologies. In the short term, greater development and use of lineage-specific RT-PCRs could help laboratories in endemic countries to identify FMD viruses causing outbreaks and triage samples for sequencing analyses (https://foot-and-mouth.org/science/lineage-specific-pcr).

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

Genomics and Bioinformatics at CFIA NCFAD – Oliver Lung

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

Any other business:

Drs Min-Kyung Park, Samia Metwally and Donal Samin reinforced the important work that is undertaken by this Network. Delegates were sorry to hear that Dr Monique Eloit (WOAH) and Dr Keith Sumption (FAO) will retire very shortly. The meeting delegates expressed their appreciation for the continued support and guidance provided to the Network over many years and passed on their best wishes to Monique and Keith for their well-earned retirement. The meeting concluded with a vote of thanks to colleagues at CFIA-NCFAD, Winnipeg, Canada (Charles Nfon, Shawn Babiuk, Michele Roy and colleagues) for hosting the meeting.

Close of meeting.