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

Summary report from the **9th OIE/FAO FMD Laboratory Network Meeting**
Brescia, Italy: 26th – 27th November 2014

Day 1:

- An introductory welcome and overview of IZSLER was provided by Dr Giorgio Varisco (Technical Director of IZSLER)
- Global situation for FMD (Data from WRLFMD, presented by Dr Don King)
  During 2014 (to date), 554 sample submissions have been received from 24 countries. Isolates generated from these samples are mainly serotype O and represent 5/7 FMDV serotypes (serotype C has not been detected since 2004 and serotype SAT 3 has not been detected at WRLFMD during 2014). Together with data provided from laboratories in the OIE/FAO FMD Network, this information is used to monitor the global distribution of FMDV, and to provide early intelligence about emerging lineages that may pose new threats. The recent spread of the O/ME-SA/Ind-2001 lineage is a tangible example of the important role played by laboratory data, and these FMD outbreaks in two countries in North Africa (Tunisia and Algeria) that were previous FMD-free (with vaccination) highlight the contribution of the Network to detect the spread of FMD. Opportunities to further increase the scope and impact of the Network activities were introduced (specific topics to be discussed later in the breakout sessions).

Summary of regional and country updates

- Southeast Asia (from RRL-Pakchong presented by Dr Somjai Kamolsiripichaiporn).
  The RRL-Pakchong provides laboratory support and capacity for pool 1 which includes countries that are either FMD endemic or FMD-free (without vaccination). During 2014, FMD samples have been received and characterised from Cambodia, Lao PR and Thailand. VP1 sequence data provides evidence for the circulation of a new lineage within serotype A (sub-lineage of A/ASIA/Sea-97), while serotype O sequences are all O/SEA/Mya-98. Dr Kamolsiripichaiporn summarized vaccine matching data for locally produced vaccine strains and international vaccines (data via WRLFMD) against representative field viruses. An overview of the results from an annual PTS organized by RRL-Pakchong was also presented.

- China and East Asia (from LVRI, Lanzhou presented by Dr Jijun He)
  Recent results for clinical samples received from PR China (n=27, from 5 outbreaks) and DPR Korea (n=6) were presented. Samples from China have been characterized as either serotype O or serotype A, while samples from DPR Korea were serotype O (porcine origin) or comprised mixed serotypes O and A (cattle origin). No serotype Asia-1 viruses have been detected in China since 2009. Sequences for serotypes O (O/SEA/Mya-98) and A (A/ASIA/Sea-97) appear to represent new FMD virus lineages in China. As part of on-going active surveillance in China, addition samples (3045 OPF from cattle, sheep and goats, and 1120 LB from pigs) have been screened by real-time RT-PCR, and ~9,000 sera have been tested by LPBE and 3ABC ELISA for SP and NSP antibodies. Results from this active surveillance programme have identified

additional FMD virus and antibody cases indicative of wider circulation in a number of Chinese provinces.

- Southern Africa (from ARC-OVI, South Africa presented by Dr Rahana Dwarka)
  In addition to specimens from South Africa, ARC-OVI has recently received samples from 5 other countries in Southern Africa (Botswana, Mozambique, Namibia, Swaziland and Zimbabwe). Sequence data collected during 2014 from outbreaks in Bushbuckridge (adjacent to the Kruger National Park, in a discrete location from previous outbreaks in 2012) were reported, showing that the causative FMD viruses are from the SAT 2 serotype (topotype I). There is evidence that some of these outbreaks have occurred in cattle receiving vaccine (at 3 PD50). Other SAT 2 outbreaks have occurred in Maputo, Mozambique. *In-vitro* cross-reactivity data has indicated that SAT2/ZIM/7/83 does not provide adequate cross-reaction to the majority of field strains in southern Africa, whereas SAT2/ZIM/14/90 and SAT2/SAR/3/04 cross-react more broadly to a panel of 28 SAT2 field isolates.

- Southern Africa (from BVI, Botswana presented by Dr George Matlho)
  Results for samples collected from 5 countries (Botswana, Mozambique, Namibia, Zimbabwe and Mali) were presented. Within Botswana FMD outbreaks due to SAT1 (topotype I –WZ) have been reported in Ngamiland in June and October 2014. Samples collected from Zimbabwe in 2010 and 2014 represented SAT 2 topotypes I and II respectively; and Mozambique SAT 2 topotype I. All isolates for SAT 1 virus showed good match to currently used local vaccine strains (SAT 105 and SAT 109) and SAT 2 viruses against SAT251 as determined by 2D-VNT method. A new SAT 2035 vaccine strain has been adopted specifically for Ngamiland region of Botswana and has been used since 2013. FMDV-specific SAT 2 sequences were detected in samples from Mali by PCR, although no isolation was made due to poor preservation of samples during shipment to BVI. Results from BVI were confirmed by WRLFMD and CODA-CERVA. In conclusion, SAT1 and SAT 2 virus are predominant in both cattle and buffalo, while SAT 3 had the lowest significance in the region. All countries which submitted samples for vaccine matching were able to control outbreaks in cattle with the use of the locally produced vaccine.

- Nigeria (from NVRI, Vom presented by Dr David Ehizibolo with Dr Kris De Clercq from CODA-CERVA)
  A brief summary of the laboratory diagnostic capacity at NVRI was presented. During 2014, a panel of 37 representative samples from 8 Nigerian states has been sent to the WRLFMD for analysis (work funded by EuFMD). Results were presented showing FMD virus isolates recovered from these samples were from serotypes O, A and SAT 2. Additional tissue and probang samples have been tested by real-time RT-PCR at CODA-CERVA (as part of an OIE Twinning project), while NSP serosurveys conducted in Nigeria during 2014 have shown that only 11% (n=360) of camel sera and 1.5% (n=800) of pig sera were positive for FMDV.

- Ethiopia (from NAHDIC presented by Dr Hagos Asgedom)
  A recent study has shown that approximately one-third of the districts in Ethiopia are affected by FMD outbreaks on an annual basis with the highest incidence occurring in central Ethiopia/Addis Ababa. Tissue, swab and tissue samples (n=2035) have been tested this year for the presence of FMDV and FMD virus-specific antibodies. Of the FMD virus and antibody positive samples, serotype O was most frequently detected: the remainder of samples collected during 2014 being serotype A. Locally produced vaccine (from the National Veterinary Institute: NVI) for serotypes O, A and SAT 2 is available, although the numbers of doses are insufficient (and too expensive) for wide-scale use.

- Kenya (from FMD Laboratory, Embakasi presented by Dr Abraham Sangula)
  Results for specimens (n=175) received to the laboratory during 2014 from Kenya were presented. FMD virus positive samples comprised serotypes O (n=81), A (n=3), SAT 1 (n=15) and SAT 2 (n=21) and sequence analysis of these isolates was assisted by DTU, Denmark. Dr Sangula also provided an overview of the Eastern Africa FMD Laboratory Network (EALN-FMD) that encompasses 12 countries in the region. The objectives of the EALN-FMD are (1) to improve the quality of FMD laboratory assays in Eastern Africa, (2) to complement activities of each individual country in fulfilling the objectives of the Progressive Control Pathway for FMD (PCP-FMD), (3) to understand global and regional FMDV circulation, and (4) undertake research and make recommendations on labs, vaccines and FMD control. Based on data from the Network,

conjectured epidemiological patterns indicate that cases due to serotype O are increasing in Kenya, Uganda and South Sudan, while serotypes A, SAT 1 and SAT 2 are more prevalent to the countries in the south of the region (including Tanzania).

- Asia (from FGBI-ARRIAH presented by Dr Alexsei Scherbakov)
  During 2014, FGBI-ARRIAH has tested samples (n=282) collected from FMD outbreaks in the Russian Federation, Mongolia and Kyrgyzstan. VP1 (and selected complete genome) sequence data highlight putative epidemiological links with other countries in East Asia and Southeast Asia for the O/SEA/Mya-98, O/ME-SA/PanAsia and A/ASIA/Sea-97 lineages. Outbreaks (serotypes O and A) in Russia have occurred at three locations along the southern border with Mongolia and China. A 30km buffer zone has been established along the southern border where all ruminants (cattle and sheep) are vaccinated. A representative field isolate for the O/ME-SA/PanAsia lineage generated positive antigenic matching data against 4 vaccines (O-Manisa, O/PanAsia-2, O/RUS/2010 and O/PanAsia), while an O/SEA/Mya-98 isolate was only positively matched against O-Manisa and O/RUS/2010. A recent serotype A isolate (A/Zabaikalsky/14) did not match with A22 550 and only generated borderline data for two other vaccines (A/Turkey/06 and A/Iran/05) indicating that a new vaccine may be required.

- South Asia (presentation provided by Drs Saravanan Subramaniam and Bramhadev Pattnaik from PD-FMD)
  Unfortunately no-one from PD-FMD could attend the meeting. However, Drs Pattnaik and Subramaniam kindly provided a presentation that summarized the recent activities of PD-FMD. In India and Nepal, FMDV serotype O continues to dominate and has accounted for 97.5% of the total specimen submissions during the past two years; of these 145/146 samples sequenced were from the O/ME-SA/Ind-2001 lineage (sub-lineage d) which has displaced the O/ME-SA/PanAsia lineage across the Indian sub-continent. A summary data for Indian vaccine strains shows that they continue to be matched against field strains from India (% of matched strains (2012-2014): 86.0%, 28.1% and 73.5% for O/INDR2/1975, A/IND40/2000 and Asia1/IND63/1972, respectively).

- North Africa (from IZSLER presented by Dr Emi Brocchi)
  Dr Brocchi provided an overview of the important contribution made by IZSLER to provide diagnostic and technical training assistance during the recent FMD O/ME-SA/Ind-2001 outbreaks in North African countries (Libya, Tunisia and Algeria). In addition to testing of clinical samples, IZSLER has also undertaken serological studies: in Libya (>3000 tests) to detect SP and NSP antibodies; and in Tunisia to demonstrate the potential of the O-BFS vaccine to generate a booster response in cattle and in sheep (following previous vaccination with O-Manisa/O-TUN/99). ISZLER (in partnership with WRLFMD) has supplied a range of ELISA test kits for the detection and characterization of FMD virus antigen and FMD virus-specific antibodies to ~20 countries (in Asia, Africa and FMD-free countries) during 2014.

- South America (from PANAFTOSA presented by Dr Rossana Allende, and from SENASA presented by Dr Eduardo Maradei)
  No clinical cases of FMD have been reported in 2014 across the entire South American continent, and it is now >35 months since the last FMD outbreak. At the OIE meeting in May 2014, the FMD free zones (with and without vaccination) were extended in a northerly direction to include a larger part of the South American continent. A focus at PANAFTOSA has been to provide laboratory support to the serological surveys that are underway in Ecuador where 3,649 samples have been tested for PVM purposes and > 20,000 sera have been screened using the 3ABC (I-ELISA)/EITB to attempt to detect circulating FMDV. During 2014 training courses have been offered by PANAFTOSA in laboratory diagnostics (cell culture, PCR, ELISA/EITB) and biorisk management, while SENASA has provided training to AU-PANVAC, Ethiopia and to laboratories in Vietnam and Cambodia. Dr Maradei highlighted two recent publications describing the antigenic characterization of FMD viruses from South America and the results from cross-protection studies.

- Turkey (from SAP-Ankara presented by Dr Naci Bulut)
  FMD continues to circulate in Turkey, although the number of outbreaks has declined sharply since July 2013, particularly since spring 2014. During this year, SAP has tested 270 samples from clinical cases in the country. This material generated isolates from 3 FMD serotypes O (n=

100), A (n=91) and Asia-1 (n=24). The predominant lineages currently circulating in Turkey are O/ME-SA/PanAsia-2 (FAR-09), A/ASIA/Iran-05 (SIS-10) with sporadic cases due to Asia-1 (Sindh-08 lineage). In addition, >100,000 sera from Turkey have been tested for a variety of purposes including post-outbreak sero-monitoring, active surveillance surrounding the Kurban festival, and assessment of vaccine performance in the field. Dr Bulut also briefly outlined the activities of the West Eurasia Laboratory Network (WELNET FMD), including FMD surveillance activities in other countries such as Pakistan, Iran and Kyrgyzstan.

- European laboratory activities (from ANSES, presented by Dr Labib Bakkali Kassimi)
  This presentation summarized recent work undertaken by ANSES to characterise FMD field viruses from West Africa (Benin, Ghana, Nigeria and Cameroon) and Pakistan. In addition, ANSES are actively involved in the facilitation of training and technology transfer to a number of West African countries (particularly those that are French-speaking) and in 2015 will lead a new project to genetically and antigenically characterise FMDV strains collected from Tunisia. Research projects at ANSES have recently developed a new real-time RT-PCR (which includes an internal control) to detect FMDV RNA, novel RT-PCR assays to type FMD viruses in West Africa, and have evaluated simple ways to transport FMD virus positive samples to reference laboratories.

- European laboratory activities (from DTU-Lindholm, presented by Dr Graham Belsham)
  DTU have recently participated in a DANIDA funded project to investigate the epidemiology of FMD in Uganda that has highlighted the presence of 5 FMDV serotypes (O, A, SAT 1, SAT 2 and SAT 3) in the last 2-3 years. The SAT 3 virus was detected in a long-horn Ankole calf translocated to the buffalo/cattle interface. These studies defining the genetic diversity of FMD viruses from Uganda highlight the impracticalities of the current topotype definitions for some SAT viruses (specific point covered later in the meeting and by the proposed working group).

- North American laboratory activities (from FADDL, USDA presented by Dr Hernando Duque)
  In addition to domestic submissions from suspect FMD cases in the USA, during 2014 FADDL has received samples from Jordan (n=6) that were all FMD negative. Dr Duque provided a brief overview of the North American Foot-and-Mouth Disease Vaccine Bank (NAFMDVB) that tests and stores commercial FMDV vaccines for use in the USA, Canada and Mexico. Results from recent in-vivo cross-protection studies using three monovalent FMD emergency vaccines (A22 Iraq, A/Arg/2001 and A/May/97) and subsequent challenge by contact at 7 days post vaccination (dpv) with A22 Iraq virus were presented. Protection achieved with these vaccines was 80%, 0% and 40% respectively. In a follow up study 37.5% of the bovines vaccinated with the A Malaysia vaccine were protected against a needle challenge with A22 Iraq virus at 7 dpv.

- North American laboratory activities (from NCFAD, Canada presented by Dr Charles Nfon)
  Seventy domestic submissions from suspect FMD cases in Canada were received at the NCFAD in 2014 and all tested negative. The NCFAD also provided material and coordinated proficiency testing for the Canadian Animal Health Surveillance Network laboratoriesAn overview was provided of two collaborative FMDV projects involving NCFAD and (i) Ministry of Primary Industries, New Zealand (diagnostic test methods in red deer), and (ii) CSIRO, Australia (early vaccine protection in sheep).

- Australian laboratory activities (from AAHL, presented by Dr Wilna Vosloo)
  AAHL coordinates a national PT exercise for laboratories in the Australian LEADDR network. A research project has focused on the antigenic properties of serotype A viruses from Southeast Asia (undertaken in collaboration with RRL-Pakchong) using the LPBE test.

Topics for breakout groups:

- Enhancing the global OIE/FAO FMD Laboratory Network
- A new framework for European/African FMDV surveillance
- Priorities for laboratory capacity and training

Day 2:

Summary of points and recommendations from the breakout sessions:

Opportunities to **Enhance the Network** were discussed including whether we should learn from (i) best practice, (ii) network organization and (iii) mechanisms used for communication employed by other OIE/FAO Networks such as OFFLU. The opinion of the delegates was that the specific governance of OFFLU was not appropriate for the OIE/FAO FMD Laboratory Network, particularly since GFRA already exists as a successful research network for FMD. However, the organization of OFFLU into specific thematic working groups is an idea that could be explored, and it was suggested that the core OIE/FAO FMD Network Partners could consider the constitution of the Network as a topic for discussion at next year's meeting (in a closed session). For example, the Network currently focuses on laboratory outputs which would benefit from formal interaction with epidemiologists and research on the socio/economic impacts of FMD. Another example of a possible gap in the current Network could be addressed by establishing a **new framework for European/African FMD surveillance**. This type of collaborative group could seek OIE support, and would provide a formal recognition to legitimize sample collection, testing and reporting in under-sampled endemic pools in Africa for those laboratories within Europe that do not have an official OIE or FAO status. However, the constitution of such a group should endeavor to be inclusive and (where necessary) should aim to engage with other members of the Network (such as those laboratories outside of Europe and Africa). Together, these activities should aim to provide strength to the regional lab networks to address gaps and priorities for **laboratory capacity and training** and to facilitate the recognition of "Leading Regional Laboratories".

Opinion from the core members (OIE and FAO Reference Laboratories) indicated that it might be possible to invite "associate" members to the Network, although an agreement (LoU or MTA) may be required to properly document the roles and responsibilities of these new laboratories. Furthermore, the Network should ensure that the meeting does not get too large since this might be a detriment to the exchange of real-time data between the core participants which currently works very well.

Actions:

- Individual core members were invited to consider proposals regarding the composition of the Network and the format of the meeting - provide feedback to the secretariat (see workplan for 2015 – below).

- ANSES will prepare a document to outline a collaborative network for FMD in Africa (interested parties should contact Dr Labib Bakkali Kassimi or Dr Stéphan Zientara at ANSES.

Antigenic Characterisation of FMD virus field isolates

This session was guided by three presentations from Drs Kris De Clercq (CODA-CERVA), Anna Ludi (WRLFMD) and Aldo Dekker (CVI-Lelystad) who introduced the approaches currently used to define the antigenic properties of FMD virus isolates, and the limitations of using these methods for vaccine selection. The goal of achieving equivalence of results between different methods (such as VNT and LBPE) and different laboratories is constrained by the inherent variability of the tests used. Furthermore, there is sometimes confusion in endemic settings regarding how to interpret vaccine matching reports provided by Network laboratories (such as WRLFMD) that focus on the emergency use of vaccines in FMD-free settings.

In the short term, it was agreed:

[1] that the following standardized method should be employed to generate BVS for vaccine matching studies (particularly those that are used to recommend vaccine antigens for FMD-free without vaccination regions):

- Monovalent single vaccine
- Adjuvant (use commercial formulated product)
- > 3PD50 or >6PD_{50} (nature of product should be defined) or >=80% PGP
- 21-28 days post vaccination
- No Boost
- Pool of five cattle with individual titres mid-range (i.e. no low responders (may need to define criteria for exclusion)

[2] Where possible, vaccine matching reports should include details of the individual titres
[3] To establish a Network working group to recommend practical approaches that can be used for vaccine selection in endemic and FMD-free with vaccination settings (see Workplan 2015 – below).

Virus isolate and lineage nomenclature (presentation by Dr Don King – WRLFMD)
In view of the increasing amount of data that is shared between laboratories, this presentation outlined the motivation to establish a standardized nomenclature for FMD samples (covering viral isolates and sequences), FMD viral topotypes and lineages, that might also include vaccines and antisera. It was agreed that that these issues would be addressed by a working group to be established during 2015 (see Work Plan below).

Sequence databases (update provided by Dr Don King – WRLFMD)
The concept of the "Open-FMD" system was introduced last year by Dr Filip Claes from FAO and was briefly summarized in this year's presentation from FAO by Dr Metwally. At the recent 3rd Global Conference of OIE Reference laboratories (Incheon, South Korea), the OIE made a recommendation that OIE Reference centres "*contribute to the design of the future OIE Platform for the collection and management of genomic sequences in animal health, in particular when notifying positive diagnostic results to the OIE, to be used within the WAHIS mechanism*". In the context of FMD, WRLFMD have discussed these two approaches with OIE and FAO in an attempt to harmonize the efforts as well as reduce redundancy between the two possible systems. As these systems develop it will be critical to ensure that real-time links are maintained between the different data sources, and that tools are provided to the different Network laboratories to allow them to analyse data and generate reports.

Update from OIE (presented by Dr Joseph Domenech)
Dr Domenech's talk covered the OIE's activities to support FMD control and eradication, and an update on FMD GF TADs Working Group activities. The talk included a brief summary of the OIE Reference Laboratories, regional activities, and OIE standards (horizontal generic approaches, and vertical disease specific chapters) relating to the control of livestock diseases. The GF TADs working group is collecting country data regarding PCP status, and is assisting countries to prepare national FMD control programmes (in partnership with the activities of the FAO – see below).

Update from FAO (presented by Dr Samia Metwally)
In addition to describing the FAO's activities during 2014 (including an update about "Open FMD"), this presentation also reviewed the implementation of the PCP-FMD since 2012. Within the endemic pools gaps still remain in West Africa where many countries are still at PCP stage 0, and in East Africa where the majority of countries are still at PCP stage 1. However, it is encouraging that the PCP-FMD approach and reinforcement of veterinary services within countries are gradually gaining acceptance, and now ~60 countries are engaged in in this programme in several of the FMD endemic pools. This presentation also briefly reviewed FAO missions to DPR Korea, Uganda, Tunisia (an OIE mission in collaboration with EuFMD and with FAO's participation), Algeria and Egypt.

Update from EuFMD (presented by Dr Kees van Maanen)
An overview of the activities of EuFMD was provided by Dr van Maanen including the top-level objectives to (1) improve readiness for FMD crisis management by Members, (2) reduce the risk to Members of an FMD incursion from the neighborhood, and (3) promote the global FMD control strategy. EuFMD will fund small applied research projects and current have a call for proposals.

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

Draft Work Plan for 2015

1: Scope of the OIE/FAO FMD Laboratory Network:

- With assistance from OIE and FAO, the network will obtain and analyse samples from under-sampled endemic pools
- Network partners will provide a central resource of expertise and advice regarding FMD control, vaccines and diagnostics
- The Network will continue to explore (and support) tools for real-time sharing of Laboratory data generated within the Network, and by OIE and FAO
- Core OIE and FAO Network partners to consider the organization of the Network and opportunities to make it a more inclusive network to maximize data collected from the field
- Core Network partners to review MoU documentation outlining the establishment and coordination of the network

2: Virus nomenclature:

- Establish a **Network Working Group** to address isolate, strain and topotype nomenclature and to provide recommendations about coherent naming of FMD viruses.
  Initial priorities for working group:
  [1] To propose common nomenclature to be used to describe samples and sequences (FMDV positive [and FMDV negative?] specimens)
  [2] To define topotype nomenclature for SAT serotypes (including nucleotide sequence cut-offs for different serotypes)
  [3] To explore formal approaches (such as establishing a standing Network sub-group committee) to oversee the naming of new FMD viral lineages
  Proposed Members: *Nick Knowles, Wilna Vosloo, Fuat Ozyoruk, Alexi Scherbakov, Rahana Dwarka, representative from PDFMD (tbc).*

3: FMD vaccines and recommendations for vaccine matching:

- Network partners will provide feedback and support for the OIE/FAO PVM guidelines
- Establish a **Network Working Group** to explore vaccine recommendations for endemic and FMD-free (with vaccination) settings
  Initial priorities for working group:
  [1] Review data from previous PT exercise with a view to publishing this data
  [2] Plan a further practical study that can be used to harmonise in-vitro vaccine matching methods (VNT and LPBE) used in different laboratories within the Network.
  [3] Explore whether alternative serological approaches are more appropriate for vaccine matching recommendations in endemic settings where multivalent vaccines provided by local or international suppliers are employed. If so, the group should consider developing standardized laboratory methods for this purpose that can be rolled-out to members within the Network.
  Proposed Members: *Kris De Clercq, Emi Brocchi, Anna Ludi, Rosanna Allende, George Matlho, China (tbc), PDFMD (tbc), Kees van Maanen (observer, tbc).*

4: Communication:

- WRLFMD to coordinate the preparation of an Annual Report
  Agreed timelines for preparation of 2014 report: - Network partners to provide feedback on pools they work closely with. Network members to provide an update to WRLFMD for report (include data for November and December 2014)
  - Final summaries: January 2015
  - Draft Report: February 2015
  - Report Published: March 2015
- WRLFMD to organise an Annual meeting (location to be agreed after discussion with OIE and FAO) – will be at the end of the year
  Agreed that (where possible) this should be hosted by a member lab of the network
- Proposal to enhance real-time exchange of data between partners, possibly in each of the pools – communicate new virus strains in real-time or other information; or quarterly conference call; link with EUFMD update monthly report (calendar to have specific times to write/edit for each lab). However, this will not require another report.

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

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