Conclusions, Recommendations and Next steps

Session I: Ways to Improve Current Situation on FMD Molecular Diagnosis in the region

Conclusions:

1. The Regional Expert Group (REG) agreed to draft a regional FMD molecular diagnostic algorithm for those countries in pool 1-2 that do not have a national diagnostic algorithm.
2. The REG agreed that real-time RT-PCR methods are more sensitive than conventional RT-PCR methods, and thus are the preferred methods to be included in the initial steps of the regional diagnostic algorithm.
3. The REG agreed to use a 3-tiered approach towards molecular diagnosis for FMD, *in casu*:
    - Step 1: pan-serotype confirmatory detection of FMDV using real-time RT-PCR
    - Step 2: serotyping for O, A, Asia-1 using real-time RT-PCR
    - Step 3: sequencing of the VP-1 region for genotyping
4. The experts drafted the following molecular diagnostic algorithm for FMD in Annex 1:
5. The REG agreed to share the draft algorithm to the experts that were unable to attend the first REG (*in casu* WRLFMD)

Recommendations

On the FMD molecular diagnostic algorithm:

1. For pan-serotype diagnosis of FMD, the REG recommends to use the pan-serotype real-time RT-PCR (3D, alternatively duplex 3D and 5'UTR) for the diagnosis of FMD *(Reid 1997 and Callahan 2012).*
2. For serotyping for O, A, and Asia 1, the REG recommends to use the serotyping real-time RT-PCR for O, A and Asia 1 (Pool 1 and Pool 2) when Ct value >30 in the pan-serotype real-time RT-PCR *(REF: LVRI).*
3. For lineage-specific diagnosis, the REG recommends to use the lineage specific real-time RT-PCR s for O, A and serotype specific real-time RT-PCR for Asia 1 (Pool 1 and 2) when Ct value > 30 in the serotyping real-time RT-PCR *(REF: APQA).*

4. For sequencing of the VP-1 region for genotyping, the REG recommends to use conventional RT-PCR tests using universal VP1 primers and direct sequencing of the PCR products for genotyping *(Le 2012).*

5. For regional harmonization of real-time RT-PCR results, the REG recommends to include a standardized universal positive control in real-time RT-PCRs for detection and serotyping.

6. As an additional option for lineage-specific real-time RT-PCR, the REG recommends evaluating lineage specific real-time RT-PCR developed by WRLFMD (Pirbright), using the FMDV isolates from Gene Pool 1 and 2 to make sure the methods are applicable for the region.

On the FMDV serotyping real-time RT-PCR and sequencing work

1. AAHL, APQA and RRL-Pakchong to develop and validate new serotyping real-time RT-PCR assay for O, A, and Asia 1 (Pool 1 and 2) – in case LVRI protocols do not become available.

2. The REG recommends OIE Reference Laboratories to publish lineage specific real-time RT-PCR and genome sequencing assays, which could be applicable to the region.

3. The REG recommends OIE Reference Laboratories to consider sequencing P1/3D for developing more accurate diagnostic tests (primer sets for the serotyping and genotyping by real-time RT-PCR).

4. The REG recommends OIE Reference Laboratories to explore Full Genome Sequencing for more accurate and reliable molecular epidemiological analysis.