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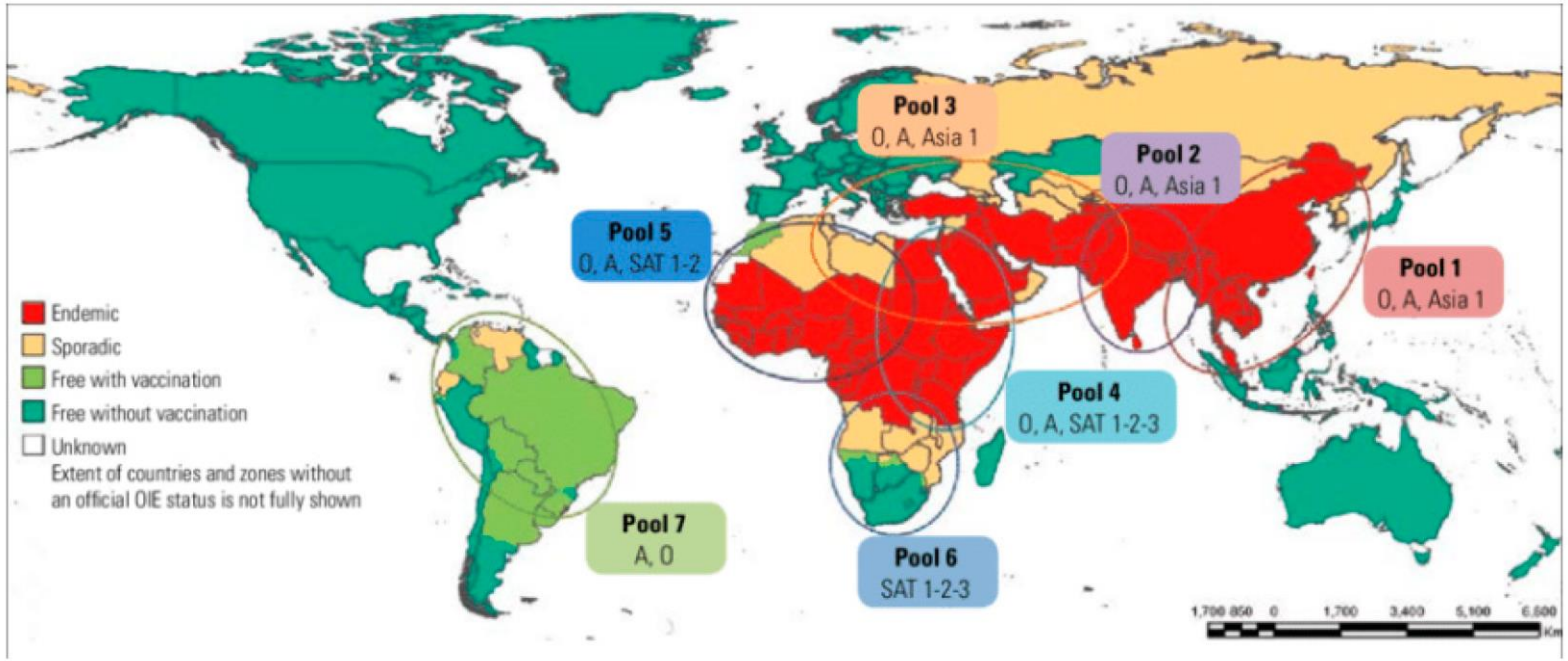
Brief introduction FMD diagnostic algorithm developing ACDP/RRL collaboration

Kingkarn Boonsuya Seeyo[3], Nalinee Hongchumpon[3],Jeeranant Chottikamporn[3], Nagendra Singanallur[1], Wilna Vosloo[1], Jianning Wang[2], Som Walker[2], Ali Al-Saabary[2], Matthew Neave[2]

1. Immunomics Team (IMM), CSIRO-Health and Biosecurity (CSIRO-H&B), Australian Centre for Disease Preparedness (ACDP), Australia
2. Diagnostics, Surveillance and Response Program (DSR), Australian Centre for Disease Preparedness (ACDP), Australia
3. Regional Reference Laboratory for FMD in South East Asia(RRL) ,NIAH ,DLD, Pakchong, Nakhonratchasima

❖ Topic

- ❑ FMDV molecular epidemiology studies and development of a serotyping real-time PCR tailor-made for viruses circulating in Southeast Asia



❖ The aims of the present proposal are as follows:

- ❖ *Prepare cDNA from the FMDV genome (RNA) isolated from samples submitted to RRLFMD.*
- ❖ *Establish methodologies to sequence the complete capsid coding P1 region of FMDV originating in SEA*
- ❖ *Develop and validate serotype-specific real-time RT-PCR (ssRT-qPCR) assay (s) for use across SEA*

❑ *Development of serotype-specific real-time RT-PCR (ssRT-qPCR) assay for serotyping FMD viruses originating in Southeast Asia (SEA) and application of nucleotide sequencing and phylogenetic analysis to study the molecular epidemiology of FMD in SEA.*

❖ Responsible organisations:

I. *Immunomics Team (IMM), CSIRO-Health and Biosecurity (CSIRO-H&B), Australian Centre for Disease Preparedness (ACDP), Australia*

II. *Diagnostics, Surveillance and Response Program (DSR), Australian Centre for Disease Preparedness (ACDP), Australia*

III. *FMD Laboratory (Regional Reference Laboratory for FMD in South East Asia; (RRLFMD), Pakchong, Thailand*

❖ Importance History:

Laboratory confirmation of clinical cases of foot-and-mouth disease (FMD)

Important for vaccine selection.

The time needed to perform these assays could delay the use of appropriate vaccines.

Lack of pool-specific reagents for performing Ag-ELISA could result in low sensitivity of the assays, a significant issue for the national laboratories primarily confirming FMD serotypes in Southeast Asia (SEA).

- *To develop a dependable, sensitive, and specific serotype-specific real-time RT-PCR assay (ssRT-qPCR), it is essential to design primers and probes in gene regions that show genetic homogeneity to one serotype but sufficient heterogeneity to the other serotypes*

❖ Scope of the project

Goal

✓ Finished

✓ On going

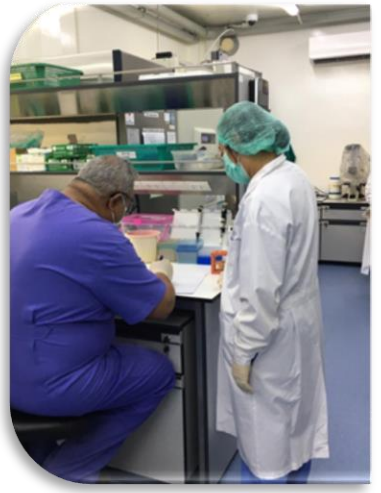
1. Extract total RNA directly from clinical tissues samples or viruses grown on primary cells of known serotypes (approx. 50 serotype O and 50 serotypes A samples)
2. Prepare cDNA using suitable kits
3. Transfer the cDNA to ACDP *(Considering to do at RRLFMD)*
4. Evaluate ssRT-qPCR assays y developed by The Pirbright Institute and other assays available
5. Amplify and sequence the complete capsid coding P1 region of the FMDV genome using suitable sequencing methods
6. Evaluate and re-design primers and probe sets for serotypes O and A based on ssRT-qPCR assays if required
7. Transfer the methodology to FMD-P and validate the ssRT-qPCR assays on field samples for the diagnostic characteristics (specificity and sensitivity)
8. Deploy the ssRT-qPCR assays for routine use in FMD-P and other national laboratories in SEA

❑ Output:

- ❑Serotype-specific real-time RT-PCR assays for SEA.
- ❑Molecular epidemiology studies using P1 sequences.

❑ Outcome:

- ❑Fully validated serotype-specific real-time RT-PCR assay(s) for direct serotyping of FMDV isolates in SEA.
- ❑A better understanding of the genetic variability and evolution of FMDV in SEA.



Thank you for your attention