Regional Meeting for OIE Reference Centers for Asia and the Pacific
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Wilai Linchongsubongkoch

Regional Reference Laboratory for FMD in South East Asia
National Institute of Animal Health
Department of Livestock Development, Thailand
1. TOR of OIE Reference Laboratory

2. Diagnostic service for Foot and mouth disease (FMD) and laboratory capacity

3. FMD status

4. Strain characterization (vaccine matching and nucleotide sequencing)

5. OIE Reference Laboratory network and SEACFMD Laboratory network for FMD

6. FMD Research Collaboration/training
1. Term of Reference (TOR)
OIE Reference Laboratory for Foot and Mucous Disease (FMD)

- To use, promote and disseminate diagnostic methods validated according to OIE Standards;

- To provide diagnostic testing facilities, scientific and technical advise on disease control measures to OIE Member Countries.

- To provide scientific and technical training for OIE Member country

- To establish and maintain the laboratory network among the OIE Reference Laboratories and national laboratories in the region

- To organize inter-laboratory proficiency testing or proficiency testing (PT) with laboratories to ensure equivalence of results and maintain the system of Quality Assurance (QA), biosafety and biosecurity for the pathogen.

- To produce and develop reference material in accordance with OIE standard, to store and distribute the biological products or reagents use in diagnosis to laboratories
2. Diagnostic service for FMD and laboratory capacity

Antigen detection

* ELISA typing
* Virus isolation
  - Pri. lamb kidney cell
  - BHK 21 Cell line
* RT-PCR

Antibody detection

* LP ELISA Test
* NSP test

Strain characterization

* Vaccine matching (r-value)
* Genomic variation by sequencing (phylogenetic tree)
Standard diagnostic methods (Accredited by ISO17025:2005);

1. Antigen detection; specimen is infected tissue, tongue epithelium, vesicular fluid or others

   1.1 ELISA typing,

1.2 Virus isolation:  
   Pri. Lamb kidney cell 
   BHK21 cell line

1.3 RT-PCR : (conventional or real time PCR)
2. Antibody detection; specimen is mainly serum

2.1 Liquid phase blocking ELISA (LP ELISA):
To detect antibody titer to FMDV type O, A, Asia1 (structural protein)

2.2 NSP Test:
To detect antibody to non-structural protein for differentiation between vaccinated and infected animal
3. Strain differentiation;

3.1 Antigenic variation or vaccine matching:

Objective: To determine serological relationship between virus vaccine train and field outbreak strain for seed vaccine selection.

Diagnostic service provided by OIE Reference Laboratory/2

- Complement Fixation (CF) Test
- Virus Neutralization (VN) Test
- Liquid phase blocking ELISA (LP ELISA)

**Vaccine matching test**

**Initial ELISA typing**

**Virus isolation to get high viral titer**

**Antigen titration to select an optimal dilution**

- \( r \) -value = \[
\begin{align*}
\text{Serum titer against heterologous field strain} \\
&= \frac{\text{Serum titer against homologous vaccine strain}}{\text{Reference vaccine strain}}
\end{align*}
\]

- \( r = 0-0.19 \): highly significant serological variation from reference vaccine strain.
- \( r = 0.20-0.39 \): significant difference from reference vaccine strain, but protection may be satisfactory if using a sufficiently potent vaccine.
- \( r = 0.40-1.0 \): not significantly different from reference vaccine strain.
3.2 Genomic variation (sequencing): VP1 sequencing

Objective: to analyze genetic relationship among virus vaccine strain and virus field outbreak strains for tracing back to original virus causing outbreak in the field.

4. Production of diagnostic reagent: provide to member country
3. Status of FMD showing distribution of regional virus pools

Source: WRLFMD, Pirbright, UK
FMD Status in South East Asia (Pool 1)

* Serotype O
  - SEA topotypes (Mya98)

* Serotype A
  - ASIA topotype

Serotype Asia 1: ASIA, Gr. VI
  - (no outbreak in this region since 2008)

- FMD free country: Philippine, Brunei, Indonesia (1986)
- Malaysia: Free in Sabah and Salawak

Source: Dr. Ronello Abila, OIE-SRR
3. FMD status in 2016

3.1 Antigen ELISA typing/ RT-PCR

Diagnostic result by ELISA typing in 2016 (Thailand)

- O: 25.60%
- A: 20.20%
- NVD: 54.20%

Diagnostic result by rRT-PCR in 2016 (Thailand)

- Pos: 8.70%
- Neg: 91.30%

<table>
<thead>
<tr>
<th>Country</th>
<th>total</th>
<th>Results of antigen ELISA typing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serotype O</td>
</tr>
<tr>
<td>Thailand</td>
<td>253</td>
<td>137</td>
</tr>
<tr>
<td>Lao PDR</td>
<td>47*</td>
<td>-</td>
</tr>
<tr>
<td>Myanmar</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>322</td>
<td>156</td>
</tr>
</tbody>
</table>

Lao PDR*: Due to less volume of initial fluid and bad quality of specimen were received, ELISA typing and rRT-PCR were negative all
4. Strain characterization

4.1 Summary of vaccine matching of type O in 2016

<table>
<thead>
<tr>
<th></th>
<th>O/189/87 Thai vaccine strain r-value range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Thailand</td>
<td>23</td>
</tr>
<tr>
<td>Lao PDR</td>
<td>47*</td>
</tr>
<tr>
<td>Myanmar</td>
<td>22*</td>
</tr>
</tbody>
</table>

*VI could not be done, due to less virus obtained and bacterial contamination in samples.
### 4.2 Summary of vaccine matching of type A in 2016

<table>
<thead>
<tr>
<th>Country</th>
<th>n</th>
<th>A/Lopburi/2012</th>
<th>A/Sakolnakorn/97</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-0.19  2.0-0.39  0.40-10</td>
<td>0-0.19  2.0-0.39  0.40-1.0</td>
</tr>
<tr>
<td>Thailand</td>
<td>15</td>
<td>4    7     4</td>
<td>-    -    15</td>
</tr>
<tr>
<td>Lao PDR</td>
<td>ND</td>
<td>ND    ND    ND</td>
<td></td>
</tr>
<tr>
<td>Myanmar</td>
<td>ND</td>
<td>ND    ND    ND</td>
<td></td>
</tr>
</tbody>
</table>

Vaccine matching of FMDV type A in Thailand during 2012-2016, compare between A/Lopburi/2012 and A/Sakolnakorn/97 vaccine strain

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![Figure 2 r-value of FMDV type A/Lopburi in Thailand during 2012-2016](image1)

![Figure 6. r-value of FMDV type A/Sakolnakorn in Thailand during 2012-2016](image2)
### 4.3 Summary of samples sequenced in 2016:

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>n</th>
<th>Sequencing results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serotype O/topotype</td>
</tr>
<tr>
<td>Thailand (n=95)</td>
<td>VP1</td>
<td>SEA topotype, Mya-98 = 64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ME-SA topotype Ind2001d = 6</td>
</tr>
<tr>
<td>Lao PDR (n=2)</td>
<td>VP1</td>
<td>SEA topotype, Mya-98 = 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myanmar (n=7)</td>
<td>VP1</td>
<td>SEA topotype, Mya-98 = 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ME-SA topotype, Ind2001d = 2</td>
</tr>
</tbody>
</table>
Phylogenetic tree FMDV type O from Thailand, Lao and Myanmar during 2015-2016

Source: WRLFMD, UK
Phylogenetic tree
FMDV type A 2015-2016

A/TAI/2016

Sea-97

Topotype: ASIA
Sea-97 strain
Changes in epidemiological patterns?

- The epidemiological status of FMD in SEA region:
  For type O, there were mostly SEA topotype, Myn98 lineage. Interestingly, it was found that O/ME-SA/Ind2001d were found in Myanmar in June 2016 and Thailand in September 2016.
  (For Lao PDR and Vietnam had found since 2015)

- For type A viruses in Thailand were ASIA topotype, Sea-97 str.

- FMD virus distribution were changed during 2015 -2016

- The major factors of the epidemiology are from animal movement along the border
5. FMD Laboratory network connection

- ANSES, France
- Onderstepoort, South Africa
- LVRI, China
- QIA, Korea-

Global level
1st OIE REF Labnet meeting has established in 2006

Regional level
1st SEAFMD Labnet meeting has established in 2005 up to the present
Objective: Laboratory Network (regional and global)

• Rapid diagnosis of FMD viruses
  o Early detection and confirmation of FMD virus serotypes
  o Provide recommendation on vaccine strain selection for implementation of control measure

• Strengthen surveillance
  o Molecular epidemiology
  o Mapping on the evolution of FMDV serotypes

• Enhance the laboratory capacity building on FMD diagnosis
  o Exchange visits of experts
  o Regular training programs

• Harmonization of diagnostic protocols

• Quality assurance system
  - To organise PT programme or inter-laboratory comparison testing

• To advocate submission of samples for diagnosis or confirmation to reference laboratories
5.1 Inter-laboratory comparison or proficiency test organized by RRL

Inter-laboratory comparison round 4/2015-2016 organized by RRLFMD, Pakchong, Thailand

Participating lab: 17 labs:
- 9 FMD laboratories within Thailand
- 8 SEAFMD laboratories, Cambodia, Lao PDR, Philippines, Malaysia, Myanmar, Vietnam_ Hanoi, Vietnam_ Ho Chi Minh, Singapore, Thailand

1. ELISA Reagents providing:
- Antigen detection and antibody detection for FMDV serotype O, A and Asia1
- Rabbit trapping antibody for O, A and Asia1
- Guinea pig detection antibody for O, A and Asia1
- Control inactivated antigen for O, A and Asia1
- Control serum for C++, C+ and C- for O, A and Asia1

2. Unknown samples:
Unknown serum = 5 samples for LP ELISA and NSP test
Unknown virus = 5 samples for antigen typing test

3. Additional document:
Questionnaires, tracing sheet, record forms
SOP if necessary for some lab
EXAMPLE: ANALYSIS OF INTER-LAB RESULTS ROUND 4/2015-2016

Figure 1: ELISA typing for serotype identification of FMDV type O, A and Asia1

Figure 2: Overview of antigen typing result from each laboratory. Cut off OD > 0.20 defined as positive

Figure 3: Overview ROC data of OD antigen control C of FMDV type O, A and Asia1

Figure 4: Overview ROC data of OD antigen control C of FMDV type O, A and Asia1 per serotype and laboratory

Figure 5: Overview ROC data of OD antigen control C of FMDV type O, A and Asia1

Figure 6: Overview ROC data of OD antigen control C of FMDV type O, A and Asia1 per serotype and laboratory

Modified Youden plot

Figure 7: Type O; Plasmode sample 1 and 4

Figure 8: Type A; Plasmode sample 1 and 3

Figure 9: Type Asia1; Plasmode sample 1 and 4

Figure 10: Mean LP ELISA titer of serum sample no 1-5 and reference data of type O, A and Asia1

Result of NSP testing using commercial kits, 3ABC NSP and 3B NSP kit

<table>
<thead>
<tr>
<th>Kit</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>3ABC NSPs test</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>3B NSPs test</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>
## Conclusion

<table>
<thead>
<tr>
<th>Trouble shooting</th>
<th>Cause/factors</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control panel out of acceptance limit</td>
<td>Working dilution is not appropriate or wrong dilution</td>
<td>Re-titration of regents</td>
</tr>
<tr>
<td>2. High OD that occurred in test sample and the antigen control of the antigen typing</td>
<td>The technique for preparation of the antigen solution for each serotype.</td>
<td>Need more training and more experience for standardize all techniques</td>
</tr>
<tr>
<td></td>
<td>- Poor technique in making serial dilution, buffer preparation, checking pH of buffer, etc.</td>
<td>- Need checking the pH condition of all reagents</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Check quality of purified water</td>
</tr>
<tr>
<td>3. Irregular results or technician error</td>
<td>- Some laboratories do not set up the Internal Quality Control (IQC) in the assay system</td>
<td>- Implement the IQC in test plate and regular monitoring</td>
</tr>
<tr>
<td></td>
<td>- Essential equipment never done for calibration or verification</td>
<td>- Calibration of equipment annually</td>
</tr>
<tr>
<td>4. Less diagnostic knowledge in laboratory testing both on theoretically and practically</td>
<td>- Less experience in practicing and training regularly in scope of responsibility</td>
<td>- Planning for annual training both within or out side the country</td>
</tr>
</tbody>
</table>
6. Training and technology transfer in 2016

1. FMD antigen detection training course during 4-8 July 2016
   Participant from 9 Veterinary Research and Development Centers (VRDC) within Thailand and
   National Institute of Animal Health

2. Dr. Valerie Mioulet, Head of Virology Section, WRLFMD
   Institute, UK. visit RRL for investigation of testing Cambodia
   samples, 27 June –1st July 2016, OIE support.
3. AAHL, Australia: Dr. Singanallur, Nagendrakumar, Research collaborative work on vaccine matching. 29th July-11 August 2016

4. Dr. Sandar Lwin, Myanmar; Laboratory Training for FMD Diagnostics Capacity during 24th October 2016 - 13rd January 2017, under IAEA support
5. Follow up the research collaborative work and scientific discussion
Dr. Kenichi Sakamoto, NIAH Director, Japan  , 18 May 2016

6. FAO LMT and BLMT visit and assessment the biosafety system of the BSL3 Lab and staff 10 May 2016

7. Dr. Kriistina Boyd, Scientific visit and implement the PACS to RRL staff, 10 October 2016
## 6. Collaborative Projects

<table>
<thead>
<tr>
<th>Collaborators</th>
<th>Collaboration Project</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Institute of Animal Health (NIAH), Kodaira, Japan</td>
<td>1) Collaborating projects on Technology transfer on sequencing and immunohistochemistry</td>
<td>- Two trainee from RRL were trained in NIAH, Japan for FMDV whole genome sequencing</td>
</tr>
<tr>
<td></td>
<td>2) FMDV Whole Genome Sequencing</td>
<td>- Scientific information on molecular epidemiology of FMDV and genomic variation information</td>
</tr>
<tr>
<td></td>
<td>3) The recovery of FMD virus in probang (O/P) samples in positive NSP animals</td>
<td>- Information for FMD carrier animals to support the epidemiology and FMD control in Thailand</td>
</tr>
<tr>
<td>Department of National Park Wildlife and Plants Conservation, Thailand</td>
<td>Sero-surveillance study in Wild life (One Health approach)</td>
<td>Information of FMDV serology in wildlife animals, zoo and national parks</td>
</tr>
<tr>
<td>World Reference Laboratory (WRL), Pirbright Institute, UK</td>
<td>Development of molecular serotyping of foot and mouth disease virus by real time RT-PCR</td>
<td>Rapid and new technology for FMDV genotyping in field specimens</td>
</tr>
<tr>
<td>Animal and Plant Quarantine Agency (QIA), Republic of Korea</td>
<td>1. Validation of developed rapid diagnostic kit for FMD serotype</td>
<td>1. New diagnostic test kit for rapid test to be applied in FMD control measures</td>
</tr>
<tr>
<td></td>
<td>2. Technology transfer to QIA staff on vaccine matching technique</td>
<td>2. Update and sharing information on FMD antigenic variation in the region</td>
</tr>
</tbody>
</table>
Guideline:

For packing and dispatch of sample or biological material via international airline following the biosafety and biosecurity principle
Thank you for your attention

Acknowledgements

RRL staff;
- Kingkarn Boonsuya Seeyo
- Amornrat Shoonnasart
- Sahawat Ungvanichaban
- Romphruek Udon
- Alongkorn Panthumart
- Janya Samanit
- Sopha Singkleebuth