Toward the control of Foot-and-Mouth disease in East Asia

Makoto Yamakawa  DVM, Ph.D.
Exotic Disease Research Station
National Institute of Animal Health (NIAH)
Foot-and-mouth Disease (FMD)

One of the most important infectious diseases for livestock industry

- FMD virus can infect many kinds of cloven-hoofed animals, such as cattle, pigs, sheep and goats and is highly contagious.
- FMD virus causes acute febrile disease with characteristic vesicular lesions in mouth, nose, mamma and foot.
- 7 distinct serotypes (O, A, C, Asia1, SAT1, SAT2, SAT3)* are identified.
- FMD causes economic damage by nutrition disorder and dyskinesia.
- FMD is a huge obstacle to the international trade of animals and animal products.

In South-East Asia (SEA) and East Asia

- **Serotypes**
  2015  O  37%  A  9%  Unidentified 54%
  2016  O  34%  A  5%  Unidentified 61%

- **Species of affected animals**
  2015  Cattle 73%  Buffalo 14%  Pig 11%  Goat 2%
  2016  Cattle 67%  Buffalo 26%  Pig  6%  Goat 1%
7 FMDV serotypes
7 endemic pools
No reported outbreaks in South America since 2013 (Venezuela)
→ An outbreak of serotype O was reported in Colombia in July 2017.
No serotype C since 2004
New FMD-free zone (without vaccination) established in northern Kazakhstan and Russia (except a new containment zone)
Significant epidemiological changes of FMD

Long-distance “trans-pool” movements from Pool 2

Pool1 including East Asia

Serotypes O (SEA(Mya-98), ME-SA(PanAsia), CATHAY) and A (ASIA(Sea-97)) are mainly prevalent. Serotype O, ME-SA topotype, Ind2001d, invaded Pool 1 from Pool 2 in 2015 (Laos, Vietnam) and spread out to Russia (2016), South Korea and China (2017).

In Jan. 2017, serotype Asia1 (ASIA (G-VIII) ) was confirmed in Myanmar.
Current status of FMD in East Asia (from 2014.1 to 2017.8)

Serotypes, Topotypes & Genotypes

**O**
- O/SEA/Mya-98
- O/ME-SA/PanAsia
- O/Cathay (China)

**A**
- A/ASIA/Sea-97
- (A/ASIA/G-VII ??)

**Russia**
- 2014.5 O Pig (7 cases)
- 2014.1 O Pig (12 cases)
- 2014.2 O Pig (11 cases)
- 2014.3 O Pig

**North Korea**
- 2014.7-8 O Pig (3 cases)
- 2014.12-2015.4 O Cattle (5 cases) Pig (180 cases)
- 2016.1-3 O Pig (21 cases)
- 2016.11 O Cattle (2 cases), 2016.12 O Cattle

**South Korea**
- 2014.1 A Cattle, 2014.2 O cattle
- 2014.2 A Cattle, 2014.9 A Cattle
- 2016.11 O Cattle (2 cases), 2016.12 O Cattle

**Hong Kong**
- 2014.1 A Cattle
- 2014.9 A Cattle
- 2014.10 A Cattle
- 2017.1 O Cattle

**Taiwan**
- 2015.4 A Cattle
- 2015.5 A Cattle

**China**
- 2014.2-3 O Pig (5 cases)
- 2014.11 O Pig
- 2014.12 Unknown Pig
- 2015.4 O Pig (2 cases)
- 2015.9 O Pig
- 2015.11-12 O Pig (3 cases)
- 2016.8 O Pig (2 cases)

**Mongolia**
- 2014.1-3 O Cattle etc., (16 cases)
- 2015.2-3 O Cattle etc., (4 cases)
- 2015.5 O Cattle
- 2015.10 O Cattle etc.,
- 2016.7 A Cattle
- 2017.1-4 O Cattle etc., (17 cases)
- 2017.7-8 O Cattle etc., (3 cases)

**Xinjiang Uygur**
- 2016.11 O Cattle
- 2017.1 O Cattle
- 2017.2 O Cattle
- 2017.4 O Cattle
- 2017.4 A Cattle (2 cases)

**Tibet**
- 2014.1 A Cattle
- 2014.9 A Cattle
- 2014.10 A Cattle
- 2017.1 O Cattle
- 2016.3 O Pig
- 2017.3 O Cattle
- 2017.5 O Pig
- 2014.2-3 O Pig (5 cases)
- 2014.11 O Pig
- 2014.12 Unknown Pig
- 2015.4 O Pig (2 cases)
- 2015.9 O Pig
- 2015.11-12 O Pig (3 cases)
- 2016.8 O Pig (2 cases)

**Tibet**
- 2015.1 A Cattle
- 2015.5 A Pig
- 2016.4 A Pig
- 2014.11 O Pig
- 2015.4 A Cattle
- 2015.5 A Cattle
- 2014.4 O Cattle
- 2016.11 O Pig
- 2015.1 A Cattle
- 2015.5 A Pig
- 2017.2 O cattle (8 cases) A Cattle

**Tibet**
- 2014.7-8 O Pig (3 cases)
- 2014.12-2015.4 O Cattle (5 cases) Pig (180 cases)
- 2016.1-3 O Pig (21 cases)
- 2016.11 O Cattle (2 cases), 2016.12 O Cattle

**Xinjiang Uygur**
- 2014.5 O Pig (7 cases)
History of FMD Outbreaks in Japan

- FMD occurred only twice in 100 years
- Good animal quarantine system
- Geographical advantage as islands

<table>
<thead>
<tr>
<th>Year</th>
<th>Animal species</th>
<th>Place (prefectures)</th>
<th>No. of slaughtered animals</th>
<th>Serotype/topotype of the virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1900-1908</td>
<td>cattle</td>
<td>18 prefectures</td>
<td>4,051</td>
<td>unknown</td>
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<td>2000</td>
<td>cattle</td>
<td>Miyazaki, Hokkaido</td>
<td>740</td>
<td>O/ME-SA topotype PanAsia lineage</td>
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<tr>
<td>2010</td>
<td>cattle, pigs, goats, sheep</td>
<td>Miyazaki</td>
<td>297,808 (including vaccinated animals)</td>
<td>O/SEA topotype Mya-98 lineage</td>
</tr>
</tbody>
</table>

Pathogenicity and infectivity of the 2000 strains seemed to be weaker than those of the 2010 strain.
Recent outbreaks of FMD in East Asia

• FMDV has spread rapidly and widely beyond the ‘Pool’.
• FMD outbreaks are predominantly caused by FMDV serotype O.
• Two topotypes, South-East Asia (SEA) and Middle East – South Asia (ME-SA) are mainly prevalent.
• SEA topotype (Mya-98 lineage) is widespread in South-East Asia and East Asia (Pool 1).
• A new strain belonging ME-SA topotype, Ind2001d, invaded Pool 1 from Pool 2 in 2015 (Laos, Vietnam) and spread out to Russia (2016), South Korea and China (2017).
• FMD outbreaks due to serotype A (ASIA, Sea-97) have been sporadically observed in recent years. We have to prepare for incursion of A/ASIA/G-VII (an another new threat for Pool 1).

(Serotype Asia 1 (ASIA/G-VIII) newly appeared in Myanmar in January, 2017.)
Vaccination is one of the control measures of FMD. Efficacy of commercial vaccines against field strains is evaluated by serological tests (in vitro) and animal experiments (in vivo). Vaccine evaluation only starts with vaccine matching. Pilot studies and field studies are important to demonstrate the vaccines are efficacious.

**In vitro evaluation by serological tests** *(Virus neutralization test, Liquid phase blocking ELISA)*

\[
r1 \text{ value} = \frac{\text{Antibody titer of vaccinal serum against field isolate (heterologous)}}{\text{Antibody titer of vaccinal serum against vaccine strain (homologous)}}
\]

**VNT (Virus neutralization test)**
- r1-value 0.3 cut-off
- Not Matched: r1-value is <0.28
- Borderline: r1-value is between 0.28 and 0.32
- Matched: r1-value is >0.32

Vaccine matching is measuring the antigenic similarity between a field isolate and vaccine strains. ‘r1 values’ are calculated by comparing the cross-reactivity of a vaccinal serum, a field isolate and vaccine strain. ‘r1 values’ of 0.3 and above are efficacious to protect.
**Vaccine matching for O/ME-SA/Ind-2001d**

1st case was reported in
Russian Federation: Cattle, November 2016
Close to the Chinese border
Republic of Korea: Cattle, February 2017
PR of China (Xinjiang Province): Cattle, January 2017

**O/ME-SA/Ind-2001d lineage**
(A new FMDV lineage has entered SEA and East Asia)
Good evidence from *in vivo* studies and field studies that vaccines provide appropriate heterologous responses

<table>
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<tr>
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<td>0.63</td>
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<td>SAU/7/2013</td>
<td>0.54</td>
<td>0.32</td>
<td>1.15</td>
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<td>SAU/1/2014</td>
<td>0.28</td>
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<td>0.69</td>
<td>0.39</td>
<td>0.89</td>
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<td>SAU/7/2016</td>
<td>0.32</td>
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<td>0.48</td>
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<td>SR1/1/2013</td>
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<td>SR1/28/2014</td>
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<td>0.55</td>
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<td>0.87</td>
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<td>VIT/8/2015</td>
<td>0.71</td>
<td>0.58</td>
<td>0.55</td>
</tr>
<tr>
<td>VIT/20/2016</td>
<td>0.66</td>
<td>0.56</td>
<td>0.52</td>
</tr>
</tbody>
</table>

VNT (Virus neutralization test) r1-value 0.3 cut-off

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23rd meeting of the OIE Sub-Commission for foot and mouth disease in South-East Asia, China and Mongolia
Siem Reap, Cambodia, 9-10 March 2017
New serotype A in West EurAsia (A/ASIA/G-VII)
Initial reports: September 2015
Saudi Arabia, Turkey, Iran, Armenia
Originating from the Indian sub-continent (Pool 2)

VNT (Virus neutralization test)  r1-value 0.3 cut-off

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<td>nd</td>
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<td>A-IND-40-2000</td>
<td>0.26</td>
<td>nd</td>
<td>0.03</td>
<td>0.24</td>
<td>nd</td>
</tr>
</tbody>
</table>

A/ASIA/G-VII lineage (a threat for East Asia)
Poor in vitro match to many commercial vaccines of serotype A
Current gap and vulnerability for emergency vaccination in FMD-free countries
Vaccine trial: A22 -2/7 protected, A/May/97 -5/7 protected (preliminary data)
Regional FMD epidemiologic situation is dynamic and complex
• Monitoring of FMD outbreaks
• Genetic and antigenic characterization of field strains are constantly needed for improving and developing prevention and control measures of FMD (vaccination strategy, containment and so on...).

Transboundary transmission of FMD is associated with cross-border movements and trade of live animals and animal products
• Biosecurity measures in importing and exporting procedures should be enhanced.

Early diagnosis is essential for containment and elimination of exotic FMD viruses
• Diagnostic methods should be improved and developed.

-More rapidly and accurately !-
Diagnostic research on FMD in NIAH, Japan
:For contribution to FMD control
Development of antigen detection systems using monoclonal antibodies

Monoclonal antibodies facilitate
- identification of FMDV and discrimination of serotype of field strains.
- antigenic characterization of the isolates for vaccine matching
We have developed antigen-detection sandwich ELISA method using monoclonal antibodies against FMDV. Our ELISA is more sensitive than the international standard method of indirect sandwich ELISA. Now we can detect all serotypes of FMD virus and discriminate each serotype!
We have made an anti-FMDV monoclonal antibody reacting with all serotypes and seven serotype-specific monoclonal antibodies. We are now trying to develop lateral flow antigen detection system (immune-chromatography) for detecting viral antigens and for identifying serotypes simply and rapidly in collaboration with private companies.

**PLoS ONE, 2015 10(8): e0134931**

Immune-chromatography kit for detecting viral antigen (final version): a case of detection of SAT2
Mechanism of FMDV antigen detection and serotyping kit using silver amplification immunochromatography system
How to use lateral flow antigen detection system (Immunochromatography kit)

Collect samples from the lesions of FMDV infected animals

Apply to the device (Now improving!)

Silver amplification immunochromatography (No Sensitization apparatus !!)
Detection and serotyping of FMDV using clinical samples obtained from cattle experimentally infected with Turkish strain of serotype O: O/TUR/ 5/2009 (ME-SA topotype)

Confirmation of utility of our immunochromatography system.
Genomic analysis of viral genes using next generation sequencer

Genomic sequencing will contribute to:
- Confirm serotype
- Clarify possible region of origin
- Understand epidemiological relation among FMD-affected farms
- Find broad relatedness to vaccine strains

NGS provides high level information!
We have isolated many strains of FMD virus from clinical samples of 292 cases in the 2010 epidemic in Japan.

The L-fragment genes (approx. 7.8kb) of 104 strains were amplified by RT-PCR and sequenced by using a next generation sequencer.

Nucleotide sequences of 2010 isolates showed more than 99.5% identity to the sequence of initial isolate obtained from the first case of the epidemic without any genetic deletion or insertion.

These results indicated that a single strain of FMD virus was introduced from overseas and its nucleotide sequence has changed gradually during the epidemic.
Technical support & Collaboration (NIAH)

OIE twinning project on FMD and other transboundary animal diseases between Mongolia (State Central Veterinary Laboratory) and Japan (Exotic Disease Research Station, NIAH) (2016-2018)

Research collaboration on FMD between Regional Reference Laboratory for FMD in South East Asia, Thailand and Exotic Research station of NIAH, Japan from 2015.
Technical support in the fight against FMD and TADs for Mongolia through the OIE: 2016-2018

NIAH provides technical support in the fight against FMD and other transboundary animal diseases (TADs) for Mongolia through OIE. FMD is one of the most feared livestock diseases: it is highly infectious and a serious threat to the economic value of livestock. The NIAH, which is the only institution providing definite diagnosis of FMD in Japan, has been designated as a collaborating center of the OIE. The NIAH decided to provide technical support to the State Central Veterinary Laboratory (SCVL) in Mongolia to improve the diagnostic techniques for FMD and other TADs through the twinning project, which has been approved by the OIE.
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Collaboration between RRL of FMD-Thailand and NIAH-Japan

Exotic Research Station of NIAH-Japan is collaborating with Regional Reference Laboratory for FMD in South East Asia Thailand (RRL) on research and improvement of diagnosis techniques for FMD.

1st FMD scientific meeting of both laboratories have been held in RRL in November, 2015. This meeting will be held biyearly (2nd meeting will be held in this autumn in Tokyo).

Training of molecular diagnosis for RRL staff and full genome sequencing of the Thai strains of FMDV using next generation sequencer were conducted in NIAH-Japan (March, 2016).

Experimental infection of the Thai strain of FMDV serotype A isolated in 2015 using cows and pigs in NIAH-Japan (August-September, 2016).

Clinical samples were used for pathological training of RRL staff in NIAH-Japan (April, 2017).
1. Sharing disease and scientific information
2. Early notification of the FMD outbreak to OIE Member countries in the region and OIE headquarters
3. Strengthen the border control to prevent FMD virus entry
4. Promotion of collaborative projects among FMD laboratories in the region (exchange of researchers and materials)
5. Technical supports to South East Asian countries for diagnosis of FMD
6. Financial supports to provide FMD vaccines to South East Asian countries

We need cooperation and collaboration with not only East Asian countries but also SEA countries to control FMD.