Appendix 3

FAO/OIE Reference Laboratory Report - FMD - April-June 2007

FMD Trends

Summary

Information collated from OIE (http://www.oie.int/wahid-prod/public.php?page=home), Promed (http://www.promedmail.org) and the FMD news service at UC Davis (http://fmd.ucdavis.edu/).

Between April and June 2007, no FMD outbreaks were officially reported in FMD-free countries that did not practice vaccination and the disease remained largely confined to traditionally infected areas. Immediate risks to Europe posed by the presence of serotypes O and A in Turkish Thrace (described in the previous report) have apparently lessened, since there have been no new outbreaks reported in this region indicating that the control measures employed there have been effective.

In the Middle East, there have continued to be reports (April, May and June) of FMD outbreaks due to serotype O in Israel in 4 provinces (Hadarim, Hazafon, Hanerkaz and Haifa). Recently, there have also been reports of FMD outbreaks that occurred earlier in the year (February 2007) in 2 separate regions in Lebanon. These affected dairy cattle in the south of the country (Aal Janoub, Hasbaya) and small ruminants in Al Baqa, Zdale suburb to the east of the country bordering Syria. The serotype causing these Lebanese outbreaks has not been established, but recent outbreaks in neighboring countries of Israel, Jordan and the Palestinian Territories remain of concern. These are the first reports of FMD outbreaks in Lebanon since 2003.

Asia: There have been new reports of FMD outbreaks in several central Asia countries. In Kazakhstan, FMD outbreaks causing deaths of cattle have been reported in the centre and west of the country (Karaganda, Qarlyk, Ulukal and Atyrau). The proximity of these outbreaks to the Russian border has led to the suspension of imports of livestock and meat products from Kazakhstan into Russia. Vaccination measures to control these outbreaks have involved 38,500 cattle and 23,500 sheep (to date). Serotype O (PanAsia lineage) has been implicated as the causative strain, although the characterisation of representative viruses from these separate regions has not yet been reported. In May and June, outbreaks affecting cattle due to serotypes O (Chuy region) and A (Bokon region) were reported in Kyrgyzstan including the Osh region adjacent to the border with Uzbekistan. In addition, there have also been unconfirmed reports of FMD outbreaks in cattle in Tajikistan District in the east of Tajikistan. Elsewhere in Asia, FMD outbreaks have continued to be reported in China; in Guangxi (affecting pigs and dairy cattle) and Guangxi (affecting cattle). In endemic areas, outbreaks of FMD have been reported in central (Ha Tinh, Phu Yen, Quang Tri, Thanh Hoa and Gia Lai provinces) and northern (Thanh Nguyen and Quang Nam) provinces of Vietnam. FMDV isolates obtained from 15 outbreaks in Quang Tri affecting predominantly cattle have been characterised as serotype Asia-1. Serotype Asia 1 was also recovered at LAH-Pirbright from a sample sent in from North Korea associated to an outbreak at the start of 2007. In the Philippines, progress has been made towards the eradication of FMD. For the past eight years, Mindanao, Visayas, Palawan and Masbate have remained free (without vaccination) from FMD.

In Africa, there have been unconfirmed reports of FMD outbreaks in Rwanda and Uganda.

In South America, there have been further FMD outbreaks of serotype O affecting cattle in Ecuador (close to the Pacific coast and further west than region affected by outbreaks earlier in the year) and in Bolivia (Santa Cruz). Across the region, vaccination programmes are continuing to control the spread of disease. In Brazil, a new initiative to vaccinate 100 million cattle and buffaloes in 14 States was started. States covered include Mato Grosso do Sul where FMD outbreaks were reported in 2006. Initial estimates (June 2007) indicate as many as 137 million cattle have been vaccinated in the country. In Argentina, >4.7 million cattle have been vaccinated in Entre Rios province neighbouring Uruguay and 0.7 million cattle have been vaccinated in Rio Negro. Details of new mass vaccination programmes have also been announced in Ecuador (3.5 million doses), Columbia (14.5 million doses) as well as Venezuela, Bolivia and Uruguay. The control of FMD in the region has led to changes in the recognized status within some South American countries. Most of the Argentine territory is now FMD-free with vaccination and the region considered FMD-free without vaccination has been extended to include Northern Patagonia. The area FMD-free with vaccination has also been extended to the region north of the Rio Negro apart from the border areas with Paraguay and Bolivia, which are being closely monitored. The state of Santa Catarina became the first FMD-free without vaccination region in Brazil. Part of the state of Pará in Brazil has been recognized FMD-free with vaccination. Further north, on the border with Ecuador, part of the Valley [Valle] and Caquetá, and Western Guainiama were declared FMD-free by the OIE. Seventy-five percent of the Colombian territory is now considered FMD-free. There are 16 million cattle in the FMD-free area.
representing 73% of the Colombian herd. The OIE has also defined a 15-km buffer zone in the common border between Argentina, Brazil, Paraguay, and Bolivia, which will not be considered FMD-free.

The WRL vaccine recommendations remain unchanged.

Results from samples received to WRL (status of samples being testing is shown in Table 1)

Middle East/Asia

FMDV serotype O
Phylogenetic analysis performed on 8 FMDV isolates from Afghanistan (collected in 2007; see Annex 2, Fig 1) and an isolate from Cambodia (collected in 2006) showed that these were all from the PanAsia lineage with circulating through the Middle East and south Asia. In addition, 3 FMDV isolates obtained from a wild cryx sampled in Saudi Arabia have been sequenced. These sequences were all identical to each other and were also characterized as belonging to the PanAsia lineage circulating in the region (see Annex 2, Figure 1). Two serotype O isolates supplied from Kyrgyzstan were also characterized and shown to be from the ME-SA topotype, but sharing closest relationship with historical viruses from Russia (from 1958 and 1976) rather than to contemporary serotype O viruses from the Middle East and Asia.

FMDV serotype A
The spread of the Iran.05 lineage of serotype A through the Middle East (Iran, Turkey and Jordan) has been described in previous reports. Three further viruses collected from Afghanistan (in 2007) were characterized as belonging to this lineage. (Annex 2, Figure 2). From South-East Asia, isolates collected from Thailand (9), Laos (2) and Cambodia (2) in 2006 have been characterized. Within countries, these isolates group together and are all characterized as members of the ASIA 1 topotype of serotype A. (Annex 2, Figure 3).

FMDV serotype Asia-1
In the previous report (Jan-Mar 2007), the first FMD outbreak since 1960 officially reported (to OIE) in North Korea was described. The causative agent was initially characterized as serotype O. However, subsequent analysis showed that sequences obtained from material sent to Pirbright were identical to an attenuated North Korean vaccine strain (data not shown). Additional field material was requested and sent to Pirbright for analysis. FMD virus subsequently isolated was characterized as Asia-1 serotype, a conclusion supported by serological testing of sera from affected animals, and sequence analysis that showed that the North Korean isolate shared highest nucleotide identity (98.3%) to Russian isolates of the Asia-1 serotype collected in 2005. (See Annex 2, Figure 4). An additional Asia-1 isolate from Kyrgyzstan (collected in 2004) was also analysed (See Annex 2, Figure 4).

Africa

FMDV serotype O
Phylogenetic analysis has been performed on 3 serotype O viruses from Africa. Two viruses from Ethiopia (collected in 2006), were closely related to other viruses from the region in the EA-3 topotypes (see Annex 2, Figure 5). A cell culture isolate from Egypt was closely related (99.2% identity) to the vaccine strain A1 Sharquis/EGY/72 (data not shown).

FMDV serotype A
Three additional isolates from the recent FMD Serotype A outbreak in Egypt were characterized. All were identical to each other and closely related to other field material received to the WRL from the outbreak. Three further African serotype A viruses (2 from Sudan and 1 from Ethiopia) were analysed and shown (Annex 2, Figure 6) to be closely related to other viruses from the regions where they were collected, within the AFRICA topotype of serotype A.

FMDV serotype SAT2
A single SAT2 isolate (SUD 1/2007) from Sudan has been sequenced and was most closely related to other North African viruses of the SAT2 serotype (Annex 2, Figure 7).

Table 1: Status of sequencing of samples received recently to WRLFMD

<table>
<thead>
<tr>
<th>Batch</th>
<th>Country</th>
<th>Serotype</th>
<th>No. of samples</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRLFMD.2007.00007</td>
<td>Ethiopia*</td>
<td>O</td>
<td>2</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00007</td>
<td>Ethiopia*</td>
<td>A</td>
<td>1</td>
<td>completed</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------</td>
<td>---</td>
<td>---</td>
<td>----------</td>
</tr>
<tr>
<td>WFLLFMD-2007-00008</td>
<td>Kyrgyzstan</td>
<td>Asia</td>
<td>1</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00008</td>
<td>Kyrgyzstan</td>
<td>O</td>
<td>2</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00007</td>
<td>Ethiopia*</td>
<td>O</td>
<td>2</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00005</td>
<td>Iran</td>
<td>O</td>
<td>20</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00009</td>
<td>Sudan</td>
<td>A</td>
<td>2</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00009</td>
<td>Sudan</td>
<td>SAT 2</td>
<td>1</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00010</td>
<td>Egypt</td>
<td>A</td>
<td>3</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00010</td>
<td>Egypt</td>
<td>O</td>
<td>1</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00011</td>
<td>North Korea</td>
<td>O</td>
<td>1</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00013</td>
<td>Thailand</td>
<td>A</td>
<td>10</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00013</td>
<td>Thailand</td>
<td>FMDV-GD</td>
<td>1</td>
<td>not done</td>
</tr>
<tr>
<td>WFLLFMD-2007-00014</td>
<td>Cambodia</td>
<td>A</td>
<td>2</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00014</td>
<td>Cambodia</td>
<td>O</td>
<td>1</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00014</td>
<td>Cambodia</td>
<td>FMDV-GD</td>
<td>1</td>
<td>not done</td>
</tr>
<tr>
<td>WFLLFMD-2007-00015</td>
<td>Vietnam</td>
<td>FMDV-GD</td>
<td>2</td>
<td>not done</td>
</tr>
<tr>
<td>WFLLFMD-2007-00016</td>
<td>Laos</td>
<td>A</td>
<td>3</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00017</td>
<td>Saudi Arabia</td>
<td>O</td>
<td>5</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00018</td>
<td>Afghanistan</td>
<td>A</td>
<td>3</td>
<td>completed</td>
</tr>
<tr>
<td>WFLLFMD-2007-00018</td>
<td>Afghanistan</td>
<td>O</td>
<td>8</td>
<td>completed</td>
</tr>
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<td>WFLLFMD-2007-00018</td>
<td>Afghanistan</td>
<td>FMDV-GD</td>
<td>7</td>
<td>not done</td>
</tr>
<tr>
<td>WFLLFMD-2007-00019</td>
<td>Pakistan</td>
<td>O</td>
<td>43</td>
<td>in progress</td>
</tr>
<tr>
<td>WFLLFMD-2007-00019</td>
<td>Pakistan</td>
<td>FMDV-GD</td>
<td>10</td>
<td>not done</td>
</tr>
<tr>
<td>WFLLFMD-2007-00020</td>
<td>Portugal</td>
<td>SVD</td>
<td>1</td>
<td>completed</td>
</tr>
</tbody>
</table>

* An additional 11 samples were FMDV-GD; all were RT-PCR negative using O and A VP1 primer sets.

**Vaccine matching**

Nine FMDV type O isolates (O Eth 67/05, 48/2006; O Im 34, 47, 52/2006, O Msa 11, 12/2006, O Pak 4/2006 and O San 5/2007) from Ethiopia, Iran, Mali, Pakistan and Saudi Arabia collected in 2005, 2006 and 2007 were further characterised by two-dimensional virus neutralisation test (Annex I; Table C), showing that most of these isolates were antigenically matched with O1 Manisa vaccine strains and indicating that the currently predominant type O virus can be covered by a vaccine present in many vaccine banks. Further work is required to investigate the poor match to O Pak 4/2006.

**Publication of data to the scientific community and the industry**

FMD papers published in the reporting period from the Pirbright Laboratory (Pirbright authors underlined):


### TABLE B: Summary of samples collected in received to IAH (April – June 2007)

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of samples</th>
<th>Virus isolation in cell culture/ELISA</th>
<th>RT-PCR for FMD (or SVD) virus (where appropriate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FMD virus serotypes</td>
<td>SVD virus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O</td>
<td>A</td>
</tr>
<tr>
<td>AFGHANISTAN</td>
<td>45</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>CAMBODIA</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>EGYPT</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>LAOS</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MALTA</td>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NORTH KOREA</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>PAKISTAN</td>
<td>58</td>
<td>43</td>
<td>-</td>
</tr>
<tr>
<td>PORTUGAL**</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SAUDI</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>ARABIA</td>
<td>4</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>SUDAN</td>
<td>12</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>THAILAND</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VIETNAM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>152</td>
<td>59</td>
<td>23</td>
</tr>
</tbody>
</table>

* Institute for Animal Health, Pirbright Laboratory, Woking, Surrey GU24 0NF

** FMD virus serotype identified following virus isolation in cell culture and antigen detection ELISA

- FMD (or SVD) virus serotype identified following virus isolation in cell culture and antigen detection ELISA
- FMD foot-and-mouth disease
- SVD swine vesicular disease
- NVD no FMD, SVD or vesicular stomatitis virus detected
- RT-PCR reverse transcription polymerase chain reaction for FMD (or SVD) viral genome
- ** samples from Portugal submitted for SVDV characterisation

NPF, 12 July 2007

### TABLE C: Antigenic characterisation of FMD field isolates by matching with vaccine strains by ELISA and/or VNT - r Value data from 1st April to 30th June 2007

<table>
<thead>
<tr>
<th>r1 Values by 2dmVNT</th>
<th>O Manna</th>
</tr>
</thead>
<tbody>
<tr>
<td>O Eth 67/2005</td>
<td>&gt;0.93</td>
</tr>
<tr>
<td>O Eth 48/2006</td>
<td>&gt;0.93</td>
</tr>
<tr>
<td>O Im 34/2006</td>
<td>0.64</td>
</tr>
<tr>
<td>O Im 47/2006</td>
<td>&gt;0.69</td>
</tr>
<tr>
<td>O Im 52/2006</td>
<td>0.64</td>
</tr>
<tr>
<td>O M 11/2006</td>
<td>&gt;0.75</td>
</tr>
<tr>
<td>O M 17/2006</td>
<td>0.66</td>
</tr>
<tr>
<td>O Pak 4/2006</td>
<td>&lt;0.13</td>
</tr>
<tr>
<td>O San 5/2007</td>
<td>&lt;0.94</td>
</tr>
</tbody>
</table>

**Interpretation of r1 values**

- In the case of VNT:
  - \( r_1 \geq 0.3 \): Suggests that there is a close relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection.
  - \( r_1 < 0.3 \): Suggests that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect.
Annex 2: Phylogenetic analysis of characterised FMDV isolates:

Fig 1: Serotype O FMDV from ASIA (Afghanistan and Saudi Arabia are highlighted)

Report on FMDV O from Afghanistan and Saudi Arabia in 2007

Software: MEGA 3.1
No. of Taxa: 160
Data File: m4avg1megdb/fmdvo/AFG32007a.meg
Data Title: O/AFG/2007
Data Type: Nucleotide (Coding)
Analysis: Phylogeny reconstruction
Tree Inference: Neighbour-Joining
Method: Neighbor-Joining
Phylogeny Test and options: Bootstrap (1000 replicates; seed=64238)
Include Sites: 1st+2nd+3rd+Noncoding
Omits/missing Data: Pairwise Deletion
Codon Positions: 1st+2nd+3rd+Noncoding
Substitution Model: Nucleotide: Kimura 2-parameter
Substitutions to include: C: Transitions + Transversions
Pattern among lineages: Same (Homogeneous)
Rates among sites: Uniform rates
No. of Sites: 558
No of bootstrap reps: 1000
Only Bootstrap Values of 70% and above are shown

N.J. Knowles & J. Wadsworth, 27 June 2007
Report on FMDV A sequences from Afghanistan in 2007

Software: MEGA 3.1
No. of Taxa: 125
Data File: /levd/neg/meg/db/fmdv/a/AFG02007a.meg
Data Title: A/AFG/2007
Data Type: Nucleotide (Coding)
Analysis: Phylogeny reconstruction
Tree Inference: =============================
Method: Neighbor-Joining
Phylogenetic Test and options: Bootstrap (1000 replicates; seed=64238)
Inclued Sites: =============================
Gaps/Missing Data: Pairwise Deletion
Codon Positions: 1st+2nd+3rd+Noncoding
Substitution Model: ==========================
Model: Nucleotide: Kimura 2-parameter
Substitutions to Include: d: Transitions + Transversions
Pattern among Lineages: Same (Homogeneous)
Rates among sites: Uniform rates
No. of Sites: 645
No Of Bootstrap Reps = 1000
Only bootstrap values of 70% and above are shown

N.J. Knowles & J. Wadsworth, 25 June 2007
Report on FMDV A from Thailand, Cambodia and Laos in 2006

Software: MEGA 3.1
No. of Taxa : 118
Data File: n/a
Data Title: Thailand, Cambodia, Laos 2006
Data Type: Nucleotide (Coding)
Analysis: Phylogeny reconstruction
Tree Inference: Neighbor-Joining
Phylogeny Test and options: Bootstrap (1000 replicates; seed=84843)
Include Sites: ______________________________
Gaps/Missing Data: Pairwise Deletion
Codon Positions: 1st+2nd+3rd+Noncoding
Substitution Model: ______________________________
Mode: Nucleotide: Kimura 2-parameter
Substitutions to Include: d: Transitions + Transversions
Pattern among Lineages: Same (Homogeneous)
Rates among sites: Uniform rates
No. of Sites: 842
No Of Bootstrap Reps = 1000
Only bootstrap values of 70% and above are shown

N.J. Knowles & J. Wassborn, 13 June 2007

Fig 3 Serotype A from SE Asia
Report on FMDV Asia 1 from North Korea in 2007 and Kyrgyzstan in 2004

N.J. Knowles & J. Wardsworth, 11 July 2007
Fig 5: FMDV O from Ethiopia

Report on FMDV O in Ethiopia in 2006

Software: MEGA 3.1
No. of taxa: 120
Data File: n:id/megalign/fmdv.oETH20068.maf
Data Title: ETH 2006
Data Type: Nucleotide (Coding)
Analysis: Phylogeny reconstruction
Tree inference: -----------------------------------------------
Method: Neighbor-Joining
Phylogeny Test and options: Bootstrap (1000 replicates; seed=4238)
Include Sites: -----------------------------------------------
Gap/Missing Data: Non informative
Codon Positions: 1st+2nd+3rd+Noncoding
Substitution Model: -----------------------------------------------
Rates: Homogeneous
Rho: 1
Substitutions to include: 6Transitions + Transversions
Pattern among Lineages: Same (homogeneous)
Rates among sites: Uniform rate
No. of Sites: 542
No. of bootstrap replicates = 1000
Only bootstrap values of 75% or more are shown
* Not a WRLFMD Ref No.

N.J. Knowles & J. Wadsworth. 11 May 2007

[Diagram showing the phylogenetic relationships among FMDV O strains from Ethiopia in 2006]

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Fig 6: FMDV A from Ethiopia and Sudan

Report on FMDV A in Ethiopia in 2000 and Sudan in 2006

N.J. Knowles & J. Wadsworth, 10 May 2007
Fig 7: FMDV SAT2 from Sudan

Report on FMDV in Sudan in 2007

N.J. Knowles & J. Wadsworth, 10 May 2007