FAO/OIE Reference Laboratory Contract Report
July-September 2008

Foot-and-Mouth Disease
Summary

There were no outbreaks officially reported in FMD-free countries that did not practice vaccination between July and September 2008.

Asia: the O-PanAsia-2 strain (ME-SA topotype) continues to dominate in the Middle East region (Pakistan, Iran, Turkey, Saudi Arabia), while in Southeast Asia (Thailand, Laos) most outbreaks appear to be of the O Mya-98 strain (SEA topotype). In the Middle East (Pakistan, Afghanistan, Iran, Turkey, Jordan, Saudi Arabia), the A-Iran-05 (ASIA topotype) has dominated for the last three years. However, since August 2007, a new sub-lineage of this strain (named A-Iran-05<sup>ARD-07</sup>) has been found in Turkey. In Southeast Asia, a local unnamed strain of type A has been circulating for a number of years, without any introductions from outside the region. No Asia 1 viruses have been submitted to the WRLFMD from recent outbreaks, but the serotype continues to circulate in parts of the P.R. of China. As India is an important reservoir of FMDV, the relationship between O, A and Asia 1 viruses circulating in India (and monitored by the Project Directorate on FMD in Mukteshwar) and those catalogued from neighbouring countries by WRLFMD needs clarification.

East Africa: In Kenya, types O, A, SAT 1 and SAT 2 continue to be isolated. In Somalia, type O viruses (EA-3 topotype) have been linked with those occurring in the Yemen Arab Republic, although ultimately viruses belonging to EA-3 probably originate in the horn of Africa.

West Africa: In Nigeria, outbreaks of type O and SAT 2 have been linked with viruses occurring in Sudan. Samples submitted from the Gabon from a suspected outbreak of FMD were negative by virus isolation and RT-PCR.

Southern Africa: FMD SAT 2 continues to cause problems in northern Botswana, eastern Namibia (Caprivi Strip) and southern Zambia. It appears that there have been multiple (n = 3) introductions into Botswana during 2007-2008. It is not clear if the origin is wildlife (African buffalo) within Botswana or from cattle/wildlife in neighbouring countries.

In September 2008, a suspected outbreak of FMD was reported on Kaombe Ranch, Nsanje, southern Malawi (the first since 2003), however, the results of laboratory testing are awaited. Tracing the origin of the infected animals indicated that some of the animals were brought in from an area close to Lengwe National Park which harbours buffaloes that were a source of the 2003 outbreak.

South America: In July-August 2008, an outbreak of FMD type A was reported on three farms in Sardinata, Norte de Santander, Colombia (first since Feb 2005).

WRL vaccine recommendations have been changed to reflect the variation in FMDV serotype A activity in the Middle East and western Asia (Annexe 4). A Iran 96 has been reduced from high to medium priority reflecting continued dominance of the A Iran 05 strain. A22 Iraq vaccine remains at high priority to cover against A Iran 05, although it has been noted that recent Turkish isolates of the A Iran 05 strain (named A-Iran-05<sup>ARD-07</sup>) showed a poor antigenic match to A22 Iraq vaccine. The SAT2 vaccine used in Botswana showed limited cross-reactivity to some recent isolates from Botswana and neighbouring countries.

Results from samples received at WRL (status of samples being tested) are shown in Table 1 and a complete list of clinical sample diagnostics made by the WRL between July and September 2008 is shown in annexe 1 Table A. A record of all samples received to IAH-Pirbright (July-September 2008) and their geographical locations are shown in annexe 1 Table B and Figure 1.

An up-to-date list and reports of FMD viruses characterised by sequencing can be found at the following website: http://www.wrlfmd.org/fmd_genotyping/2008.htm
Table 1: Status of sequencing of samples received recently to WRLFMD

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<th>Serotype</th>
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Total 116

*, received during the previous reporting period and listed as “in progress”.

Detailed genotyping results from the WRLFMD

**ASIA**

**Iran (type A)**

VP1 sequencing was completed on three type A viruses received from Iran during the previous reporting period. Two were collected in 2007 and one in 2008. They all belonged to the ASIA topotype, Iran-05 strain (Annex 2, Figure 1).

**Pakistan (types O & A)**

VP1 sequencing of 6 type O viruses isolated from samples received from Pakistan during the last reporting period was completed. They represented viruses collected in 2007. All belonged to the ME-SA topotype, PanAsia-2 lineage (Annex 2, Figure 2). Those within the PanAsia-2 lineage fell on multiple sub-lineages, some of which included representatives from other countries, indicating a complex epidemiological situation.

A single type A virus (PAK/73/2007), collected in 2007, was unrelated to A-Iran-05-like viruses previously found in Pakistan and fell on a distinct lineage most closely related to viruses from Iran and Pakistan in 2000-2003 (Annex 2, Figure 3). PAK/73/2007 also contained a type O virus. This was distinct from other type O’s in this batch, being a member of the Pak-98 lineage (not shown).
Saudi Arabia (type O)

VP1 sequencing was completed on one type O virus received from Saudi Arabia which belonged to the ME-SA topotype, PanAsia-2 lineage. This virus was closely related to Saudi Arabian viruses received earlier in the year (Annex 2, Figure 2).

Turkey (types O & A)

Nine type O and 23 type A viruses, collected in 2008, were received during the previous reporting period. Complete VP1 sequences of three of the type O and seven of the type A viruses were provided by the FMDI-Ankara and the remainder were sequenced in the WRLFMD. All the type O viruses belonged to the ME-SA topotype, PanAsia-2 lineage (Annex 2, Figure 4), while all the type A viruses belonged to a sub-lineage of Iran-05 (called Iran-05MD-07), which is unique to Turkey (Annex 2, Figure 1).

Thailand (types O & A)

Ten type O viruses received from Thailand (collected in 2007) all belonged to the SEA topotype, Mya-98 strain, although they fell on a number of different sub-lineages (Annex 2, Figure 5).

Two type A viruses, again collected in 2007, belonged to the ASIA topotype and a lineage found throughout Southeast Asia. However, each virus was distinct and related to viruses from Malaysia, isolated in the same year (Annex 2, Figure 6).

Lao PDR (type O)

Five type O viruses from Laos, collected in 2007 and 2008, belonged to the SEA topotype, Mya-98 strain and were closely related to the viruses from Thailand, falling on two of the sub-lineages (Annex 2, Figure 5).

Central Asia (type Asia 1)

Six complete VP1 sequences of type Asia 1 viruses were received from ARRIAH; three were from Tajikistan from samples collected in 2003, one was from Uzbekistan (2003) and two from Kyrgyzstan (2004). All were closely related to each other and to other contemporaneous viruses from the region (Annex 2, Figure 7).

AFRICA

Kenya (types O, A, SAT 1 & SAT 2)

Eleven type O viruses (collected in 2007 and 2008) belonged to the EA-2 topotype, but fell on three distinct lineages each also having examples of Kenyan viruses isolated in 2004 and/or 2005 (Annex 2, Figure 8).

Two type A viruses (collected in 2008) belonged to the AFRICA topotype and were most closely related to earlier Kenyan viruses from 2005-2006 (Annex 2, Figure 9).

Two type SAT 1 viruses (collected in 2006) belonged to the NWZ topotype and were closely related to previously-received Kenyan viruses from 2005-2006 (Annex 2, Figure 10).

Fifteen type SAT 2 viruses (collected in 2007) all closely related to each other and were most closely related to viruses from Tanzania in 2004 (Annex 2, Figure 11).

Somalia (type O)

Three type O viruses, collected in 2007, belonged to the EA-3 topotype and were most closely related to viruses from the Yemen Arab Republic collected between 2003 and 2006 (Annex 2, Figure 8).

Nigeria (types O and SAT 2)

A single type O virus, collected in 2007, belonged to the EA-3 topotype and was most closely related to viruses from Sudan (2004-2005) (Annex 2, Figure 8).

Nine type SAT 2 viruses (one collected in 2007 and eight in 2008) were most closely related to viruses from Sudan (2007), Niger (2005), Libya (2003) and Cameroon (2005) (Annex 2, Figure 11).
Botswana (type SAT 2)

Four type SAT 2 viruses, collected from cattle in two different locations in 2008, fell into two distinct lineages. SAT2/BOT/12/2008 and SAT2/BOT/13/2008, from Mohembo West, Tshethana, were most closely related to a virus (SAT2/NAM/304/98) isolated from an African buffalo in the West Caprivi Game Reserve, Namibia in 1998 (Annex 2, Figure 12). SAT2/BOT/14/2008 and SAT2/BOT/15/2008, collected in Satau Crush, Khundu, were most closely related to viruses isolated from cattle in Namibia (Caprivi Strip) and Zambia (Kazungula) in 2007-2008. Both sets of viruses were distinct from earlier viruses from the Maun area in 2007-2008. This suggests that there were three introductions of different SAT 2 viruses into Botswana in 2007-2008 (Annex 2, Figure 12).

Namibia (type SAT 2)

Three type SAT 2 viruses from cattle in the Caprivi Strip were closely related to an isolate received earlier in 2008 and to Namibian viruses from 2007. Other viruses in this group were from Zambia in 2007-2008 and Botswana in 2008 (Annex 2, Figure 12).

Zambia (type SAT 1)

Five type SAT 1 viruses were isolated from samples from Zambia (Southern Region). Their VP1 sequences belonged to the North-west Zimbabwe (NWZ) topotype and were most closely related to those of viruses previous found in Zambia earlier in 2008 (Annex 2, Figure 13).

Vaccine matching

Three FMDV type A isolates (A TUR 24/2007; A TUR 7 and 11/2008) from Turkey collected in 2007 and 2008 and four FMDV type SAT1 isolates (SAT1 BOT 20 and 22/2006; and SAT1 ZAM 9 and 13/2008) from Botswana and Zambia collected in 2006 and 2008 were further characterised by two dimensional virus neutralisation test (see Annex 1; TABLE C). The results showed that A TUR 24/2007 and A TUR 11/2008 were antigenically matched with A22 Iraq 24/64 and A 5925, respectively while A TUR 7/2008 failed to match with A 5925. SAT1 BOT 20/2006 and 22/2006 were antigenically close to SAT1 RHO 12/78 while the other two isolates SAT1 ZAM 9/2008 and 13/2008 were not.

Thirteen FMD type SAT 2 isolates (Sat2 Nig 2 and 7/07; Sat2 Nmb 1, 2 and 4/08; Sat2 Bot 6, 11 and 12/08; Sat2 Ken 2/08; Sat2 Ken 7, 9 and 16/07 and Sat2 Eth 2/07) from Nigeria, Namibia, Botswana, Kenya and Ethiopia have been characterised by two dimensional VNT and/or LPBE. The results showed that SAT2 Nmb 2 and 4/2008 were antigenically matched with all of SAT2 K52/84, SAT2 K65/82 and SAT2 ZIM 11/89 vaccine strains. SAT2 Nig 2/2007 and SAT2 BOT 11/08 were antigenically close to SAT2 Eritrea and SAT2 K65/82, respectively. SAT2 BOT 12/08 were matched with both SAT2 Eritrea and SAT2 K65/82. The remaining viruses were not close to SAT2 ZIM 7/83 and/or SAT2 Eritrea. (Annex 1; TABLE C).
### Table A: Summary of clinical sample diagnostics made by the WRL between July and September 2008

<table>
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<tr>
<th>Country</th>
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**TOTAL : 38**
FMD(V) foot-and-mouth disease (virus)
GD genome detected
VI/ELISA FMDV serotype identified following virus isolation in cell culture and antigen ELISA
RT-PCR reverse transcription polymerase chain reaction on epithelial suspension for FMD viral genome
NVD no foot-and-mouth disease, swine vesicular disease or vesicular stomatitis virus detected
NK not known

TABLE B: Summary of samples collected and received to IAH-Pirbright (July-September 2008)

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<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>
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<td>NAMIBIA</td>
<td>3</td>
<td>- - - - 3 - - - -</td>
<td>3 - -</td>
</tr>
<tr>
<td>THAILAND</td>
<td>15</td>
<td>10 2 - - - - - - - - - - - - - -</td>
<td>3 14 1 -</td>
</tr>
<tr>
<td>ZAMBIA</td>
<td>5</td>
<td>- - - - 5 - - - -</td>
<td>4 - -</td>
</tr>
<tr>
<td>TOTAL</td>
<td>38</td>
<td>15 2 - - - - - - - 9</td>
<td>31 7 -</td>
</tr>
</tbody>
</table>

VI/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

Figure 1. Geographical locations of clinical sample diagnostics made by the WRL between July and September 2008
<table>
<thead>
<tr>
<th>WRL Ref Number</th>
<th>O Manisa</th>
<th>A22 Irq</th>
<th>A Tur 4/06</th>
<th>A 5925</th>
</tr>
</thead>
<tbody>
<tr>
<td>O TUR 4/2008</td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O TUR 10/2008</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O TUR 26/2008</td>
<td>0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O TUR 30/2008</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A TUR 1/2008</td>
<td></td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A TUR 6/2008</td>
<td></td>
<td></td>
<td>&gt;0.83</td>
<td></td>
</tr>
<tr>
<td>A TUR 7/2008</td>
<td>0.11</td>
<td></td>
<td>&gt;0.97</td>
<td>0.28</td>
</tr>
<tr>
<td>A TUR 8/2008</td>
<td></td>
<td></td>
<td>&gt;1.0</td>
<td></td>
</tr>
<tr>
<td>A TUR 11/2008</td>
<td>0.17</td>
<td></td>
<td>0.91</td>
<td>0.34</td>
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<tr>
<td>A TUR 24/2007</td>
<td></td>
<td>0.67</td>
<td></td>
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<td>A TUR 28/2008</td>
<td>0.19</td>
<td></td>
<td></td>
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<tr>
<td>A TUR 32/2008</td>
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<td>&lt;0.16</td>
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<td></td>
</tr>
<tr>
<td>A TUR 33/2008</td>
<td>0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Interpretation of $r_1$ 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.

In the case of ELISA:

$r_1 = 0.4-1.0$. 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.

$r_1 = 0.2-0.39$. Suggests that the field isolate is antigenically related to the vaccine strain. The vaccine strain might be suitable for use if no closer match can be found provided that a potent vaccine is used and animals are preferably immunised more than once.

$r_1 < 0.2$. Suggests that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect.

<table>
<thead>
<tr>
<th>Field Isolate</th>
<th>$r_1$ Values by 2dmVNT</th>
<th>$r_1$ Values by LPBE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sat2 Zim 7/83</td>
<td>Sat2 Eritrea</td>
</tr>
<tr>
<td>Sat1 Bot 20/2006</td>
<td>0.37</td>
<td>0.43</td>
</tr>
<tr>
<td>Sat1 Bot 22/2006</td>
<td>0.37</td>
<td>0.43</td>
</tr>
<tr>
<td>Sat1 Zam 9/2008</td>
<td>0.17</td>
<td>0.43</td>
</tr>
<tr>
<td>Sat1 Zam 13/2008</td>
<td>0.22</td>
<td>0.43</td>
</tr>
</tbody>
</table>
Annex 2: Phylogenetic analysis of characterised FMDV isolates

Software: MEGA 4.0
No. of Taxa: 172
Data File: n:\levim\meg\mvd\Val\TUR2008b.meg
Data Type: Nucleotide (Coding)
Analysis: Phylogeny reconstruction
Tree Inference: ==============================
->Method: Neighbor-Joining
->Phylogeny Test and options: Bootstrap (1000 replicates; seed=64238)
Include Sites: 645
->Gaps/Missing Data: Pairwise Deletion
->Codon Positions: 1st+2nd+3rd+Noncoding
Substitution 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
*, not a WRLFMD Ref. No.

N.J. Knowles, K. Ebert & J. Wadsworth, 11 August 2008

Fig. 1. FMDV type A in Iran in 2008.
Fig. 2. FMDV type O in Pakistan (2007) and Saudi Arabia (2008).
Fig. 3. FMDV type A in Pakistan (2007).
Fig. 4. FMDV type O in Turkey in 2008.
Fig. 5. FMDV type O in Thailand (2007) and Laos (2007-2008)
Software: MEGA 4.0
No. of Taxa: 122
Data File: n:evd/meg/dbfmdv/Tha/TAI2007a.meg
Data Title: Thailand 2007
Data Type: Nucleotide (Coding)
Analysis: Phylogeny reconstruction
Tree Inference:  
-> Method: Neighbor-Joining
-> Phylogeny Test and options: Bootstrap (1000 replicates; seed=64238)
Include 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: 642
No. of Bootstrap Reps = 1000
Only bootstrap values of 70% and above are shown
* not a WRLFMD Ref. No.

N.J. Knowles, K. Ebert & J. Wadsworth, 16 September 2008

Fig. 6. FMDV type A in Thailand in 2007.
Fig. 7. FMDV type Asia 1 in Central Asia in 2003-2004.
Fig. 8. FMDV type O in Kenya (2007-2008), Somalia (2007) and Nigeria (2007).
Fig. 9. FMDV type A in Kenya in 2008.

Software: MEGA 4.0
No. of Taxa: 119
Data File: n:\evd\meg\db\fmdv\a\KEN2008a.meg
Data Title: Kenya 2008
Data Type: Nucleotide (Coding)
Analysis: Phylogeny reconstruction
Tree Inference: 

- Method: Neighbor-Joining
- Phylogeny Test and options: Bootstrap (1000 replicates; seed=64238)
- Gaps/Missing Data: Pairwise Deletion
- Codon Positions: 1st+2nd+3rd+Noncoding
- Model: Nucleotide: Kimura 2-parameter
- Substitutions to Include: d: Transitions + Transversions
- Pattern among Lineages: Same (Homogeneous)
- Rates among sites: Uniform rates

No. of Sites: 642
No Of Bootstrap Reps = 1000
Only bootstrap values of 70% and above are shown

*, not a WRLFMD Ref. No.

N.J. Knowles, K. Ebert & J. Wadsworth, 28 July 2008
Fig. 10. FMDV type SAT 1 in Kenya in 2006.
Fig. 11. FMDV type SAT 2 in Kenya and Nigeria in 2007-2008.
Fig. 12. FMDV type SAT 2 in Botswana and Namibia in 2008.

Software: MEGA 4.0
Data File : n:\evd\meg\db\fmdv\sat2\NMB2008b.meg
Data Title : SAT2 2008
Data Type : Nucleotide (Coding)
Analysis : Phylogeny reconstruction
Tree Inference : Neighbor-Joining
 Phylogeny Test and options : Bootstrap (1000 replicates; seed=64238)
 Method : Neighbor-Joining
 Phylogeny reconstruction
 Data Type : Nucleotide (Coding)
 Data Title : SAT2 2008
 Data File : n:\evd\meg\db\fmdv\sat2\NMB2008b.meg
 No. of Taxa : 136
 No Of Bootstrap Reps = 1000
 Only bootstrap values of 70% and above are shown
 *, not a WRLFMD Ref. No.

N.J. Knowles, K. Ebert & J. Wadsworth, 14 September 2008

No. of Sites : 648
->Rates among sites : Uniform rates
->Pattern among Lineages : Same (Homogeneous)
->Substitutions to Include : d: Transitions + Transversions
->Model : Nucleotide: Kimura 2-parameter
->Codon Positions : 1st+2nd+3rd+Noncoding
->Gaps/Missing Data : Pairwise Deletion
->Include Sites :
 Phylogeny Test and options : Bootstrap (1000 replicates; seed=64238)
 Method : Neighbor-Joining
 Phylogeny reconstruction
 Data Type : Nucleotide (Coding)
 Data Title : SAT2 2008
 Data File : n:\evd\meg\db\fmdv\sat2\NMB2008b.meg
 No. of Taxa : 136
 No Of Bootstrap Reps = 1000
 Only bootstrap values of 70% and above are shown
 *, not a WRLFMD Ref. No.
Fig. 13. FMDV type SAT 1 in Zambia in 2008.
Annex 3. Recent FMD Publications cited by PubMed


Annex 4. RECOMMENDATIONS FROM THE WRL ON FMD VIRUS STRAINS TO BE INCLUDED IN FMDV ANTIGEN BANKS – September 2008

High Priority

O Manisa (covers panasian topotype)
O BFS or Campos
A24 Cruzeiro
Asia 1 Shamir
A22 Iraq
SAT 2 Saudi Arabia (or equivalent)
(not in order of importance)

Medium Priority

A Eritrea
A Iran ‘96
SAT 2 Zimbabwe
A Iran 87 or A Saudi Arabia 23/86 (or equivalent)
SAT 1 South Africa
A Malaysia 97 (or Thai equivalent such as A/NPT/TAI/86)
A Argentina 2001
O Taiwan 97 (pig-adapted strain or Philippine equivalent)
A Iran ‘99
(not in order of importance)

Low Priority

A15 Bangkok related strain
A87 Argentina related strain
C Noville
SAT 2 Kenya
SAT 1 Kenya
SAT 3 Zimbabwe
A Kenya
(not in order of importance)