Reference Laboratory Contract Report\textsuperscript{1}
October-December 2008

Foot-and-Mouth Disease

\textsuperscript{1} N.B. Copies of all the individual reports cited herein can be obtained from Jef Hammond, IAH-Pirbright, jef.hammond@bbsrc.ac.uk
Summary

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

ASIA

China: An outbreak of FMD was detected on 06/11/2008 in Qiangganyu, Gangu, Tianshui, Gansu Province. Typing at the National FMD Reference Lab indicted the type to be type Asia 1.

Laos: An outbreak of FMD was detected at Boten cao, Namtha, Luangnamtha Province on 07/10/2008. Typing at the National Animal Health Center Laboratory showed the causative virus to be type O.

AFRICA

Botswana: On 20/10/2008 an outbreak of FMD was detected on Newlook Farm, Kuke, Ghanzi, Ghanzi Province. Samples submitted to the Botswana Vaccine Institute were reported as type SAT 2. The farm was depopulated and the incident resolved by 03/12/2008.

Malawi: In September 2008, a suspected outbreak of FMD was reported on Kaombe Ranch, Nsanje, southern Malawi (the first since 2003). 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 contains African buffalo. These were probably the source of the 2003 outbreak. Further outbreaks were reported at Thobwa, Ngabu, Chikwawa, Southern Province (05/09/2008), Mnthumba Crush, Chikwawa, Southern Province (25/10/2008) and Mnthumba village (11/11/2008). Samples submitted to the Onderstepoort Veterinary Institute were identified as type SAT 2. A total of 79,186 cattle were vaccinated in the two affected districts of Chikwawa and Nsanje. The disease morbidity was very low. No further outbreaks have occurred and the epidemic was resolved by 03/12/2008.

South Africa: FMD virus was detected in African buffalo at Happy Lands, Maruleng, Phalaborwa, Limpopo Province on 05/12/2008, although no disease was observed. FMDV type SAT 2 was isolated at the Onderstepoort Veterinary Institute. All buffalo on the farm were immediately moved back into the Infected Zone. No cattle were kept on the affected farm or adjacent farms. The FMD free status of South Africa was not affected as the outbreak occurred in the FMD Buffer Zone. The incident was resolved by 12/12/2008.

WRL vaccine recommendations remain unchanged from the previous report (Annexe 4). However, the continued dominance of the FMDV serotype A Iran 05 strain and the poor antigenic match to A22 Iraq vaccine demonstrated against recent Turkish isolates of the A Iran 05 strain (named A-Iran-05ARD-07) necessitate further investigation of alternative vaccine strains.

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 October and December 2008 is shown in annexe 1 Table A. A record of all samples received to IAH-Pirbright (October-December 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

<table>
<thead>
<tr>
<th>Batch</th>
<th>Country</th>
<th>Serotype</th>
<th>No. of isolates</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRLFMD-2008-00028</td>
<td>Bhutan</td>
<td>O</td>
<td>2</td>
<td>Completed</td>
</tr>
<tr>
<td>WRLFMD-2008-00029</td>
<td>Botswana</td>
<td>SAT 2</td>
<td>2</td>
<td>Completed</td>
</tr>
<tr>
<td>WRLFMD-2008-00031</td>
<td>Ethiopia</td>
<td>A</td>
<td>3</td>
<td>Completed</td>
</tr>
<tr>
<td>WRLFMD-2008-00031</td>
<td>Ethiopia</td>
<td>O</td>
<td>8</td>
<td>Completed</td>
</tr>
<tr>
<td>WRLFMD-2008-00033</td>
<td>Bahrain</td>
<td>A</td>
<td>2</td>
<td>Completed</td>
</tr>
</tbody>
</table>

Total 17
Detailed genotyping results from the WRLFMD

**ASIA**

*Bahrain (type A)*
Two FMD type A viruses were isolated from samples received in November. The last known occurrence of type A in Bahrain was in 1965. Both virus isolates belonged to the A-Iran-05 lineage (ASIA topotype) (Annex 2, Figure 1).

*Bhutan (type O)*
Two type O viruses were examined and found to belong to the PanAsia-2 lineage of the ME-SA topotype, a strain currently present throughout the Middle East (Annex 2, Figure 2). They were also related to isolates found in Bhutan in 2007.

**AFRICA**

*Botswana (type SAT 2)*
Two viruses isolated from the outbreak in Ghanzi Province were examined. They were closely related to earlier virus isolates from the Maun Veterinary area to the north (Annex 2, Figure 3).

*Ethiopia (types O and A)*
Eight FMD type O viruses were sequenced and compared to previously isolated viruses. They were all closely related to each other and to some from Ethiopia in 2007 (Annex 2, Figure 4). Three type A viruses were related to an Ethiopian isolate from 2007 and to viruses from Kenya (2005) and Egypt (2006) (Annex 2, Figure 5).

**Vaccine matching**
Two FMDV type O isolates (O Bhu 2/2008 and O Bhu 3/2008) from Bhutan collected in 2008 and one FMDV type SAT1 isolate (SAT1 BOT 22/2006) from Botswana collected in 2006 were further characterised by two dimensional virus neutralisation test and/or liquid phase blocking ELISA (see Annex 1; TABLE C). The results showed that both isolates from Bhutan were antigenically close to all of O BFS 1860, O Ind R2/75 and O Kaufbeuren vaccine strains and were also matched with O1 Manisa. SAT1 BOT 22/2006 was antigenically matched with both SAT1 RHO 12/78 and SAT1 BOT 1/68.
Annex 1.

Table A: Summary of clinical sample diagnostics made by the WRL between October and December 2008

<table>
<thead>
<tr>
<th>Country</th>
<th>WRL for FMD Sample Identification</th>
<th>Animal</th>
<th>Date of Collection</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAHRAIN</td>
<td>BAR 4/2008 Cattle 17.11.08</td>
<td>NVD</td>
<td>VI/ELISA</td>
<td>Negative NVD</td>
</tr>
<tr>
<td></td>
<td>BAR 5/2008 Cattle 17.11.08</td>
<td>NVD</td>
<td>RT-PCR</td>
<td>Positive FMDV GD</td>
</tr>
<tr>
<td></td>
<td>BAR 6/2008 Cattle 24.11.08</td>
<td>A</td>
<td></td>
<td>Positive A</td>
</tr>
<tr>
<td></td>
<td>BAR 7/2008 Cattle 24.11.08</td>
<td>A</td>
<td></td>
<td>Positive A</td>
</tr>
<tr>
<td>BOTSWANA</td>
<td>BOT 16/2008 Cattle 04.10.08</td>
<td>SAT 2</td>
<td></td>
<td>Positive SAT 2</td>
</tr>
<tr>
<td></td>
<td>BOT 17/2008 Cattle 04.10.08</td>
<td>NVD</td>
<td>RT-PCR</td>
<td>Positive FMDV GD</td>
</tr>
<tr>
<td></td>
<td>BOT 18/2008 Cattle 04.10.08</td>
<td>SAT 2</td>
<td></td>
<td>Positive SAT 2</td>
</tr>
<tr>
<td>BHUTAN</td>
<td>BHU 1/2008 Cattle 12.09.08</td>
<td>NVD</td>
<td>VI/ELISA</td>
<td>Positive FMDV GD</td>
</tr>
<tr>
<td></td>
<td>BHU 2/2008 Cattle 12.09.08</td>
<td>O</td>
<td></td>
<td>Positive O</td>
</tr>
<tr>
<td></td>
<td>BHU 3/2008 Cattle 12.09.08</td>
<td>O</td>
<td></td>
<td>Positive O</td>
</tr>
<tr>
<td>ETHIOPIA</td>
<td>ETH 7/2008 Cattle 08.01.08</td>
<td>A</td>
<td>VI/ELISA</td>
<td>Positive A</td>
</tr>
<tr>
<td></td>
<td>ETH 8/2008 Cattle 08.01.08</td>
<td>A</td>
<td></td>
<td>Positive A</td>
</tr>
<tr>
<td></td>
<td>ETH 9/2008 Cattle 01.08.08</td>
<td>A</td>
<td></td>
<td>Positive A</td>
</tr>
<tr>
<td></td>
<td>ETH 10/2008 Cattle 26.08.08</td>
<td>NVD</td>
<td>VI/ELISA</td>
<td>Positive FMDV GD</td>
</tr>
<tr>
<td></td>
<td>ETH 11/2008 Cattle 26.08.08</td>
<td>NVD</td>
<td>RT-PCR</td>
<td>Positive FMDV GD</td>
</tr>
<tr>
<td></td>
<td>ETH 12/2008 Cattle 26.08.08</td>
<td>NVD</td>
<td></td>
<td>Positive FMDV GD</td>
</tr>
<tr>
<td></td>
<td>ETH 13/2008 Cattle 19.10.08</td>
<td>O</td>
<td></td>
<td>Positive O</td>
</tr>
<tr>
<td></td>
<td>ETH 14/2008 Cattle 19.10.08</td>
<td>NVD</td>
<td>VI/ELISA</td>
<td>Negative NVD</td>
</tr>
<tr>
<td></td>
<td>ETH 15/2008 Cattle 19.10.08</td>
<td>O</td>
<td></td>
<td>Positive O</td>
</tr>
<tr>
<td></td>
<td>ETH 16/2008 Cattle 19.10.08</td>
<td>NVD</td>
<td>VI/ELISA</td>
<td>Negative NVD</td>
</tr>
<tr>
<td></td>
<td>ETH 17/2008 Cattle 19.10.08</td>
<td>NVD</td>
<td></td>
<td>Negative NVD</td>
</tr>
<tr>
<td></td>
<td>ETH 18/2008 Cattle 19.10.08</td>
<td>NVD</td>
<td></td>
<td>Negative NVD</td>
</tr>
<tr>
<td></td>
<td>ETH 19/2008 Cattle 19.10.08</td>
<td>O</td>
<td></td>
<td>Positive O</td>
</tr>
<tr>
<td></td>
<td>ETH 20/2008 Cattle 19.10.08</td>
<td>O</td>
<td></td>
<td>Positive O</td>
</tr>
<tr>
<td></td>
<td>ETH 21/2008 Cattle 19.10.08</td>
<td>O</td>
<td></td>
<td>Positive O</td>
</tr>
<tr>
<td></td>
<td>ETH 22/2008 Cattle 19.10.08</td>
<td>NVD</td>
<td>VI/ELISA</td>
<td>Positive FMDV GD</td>
</tr>
<tr>
<td></td>
<td>ETH 23/2008 Cattle 21.10.08</td>
<td>O</td>
<td></td>
<td>Positive O</td>
</tr>
<tr>
<td></td>
<td>ETH 24/2008 Cattle 21.10.08</td>
<td>O</td>
<td></td>
<td>Positive O</td>
</tr>
<tr>
<td></td>
<td>ETH 25/2008 Cattle 21.10.08</td>
<td>O</td>
<td></td>
<td>Positive O</td>
</tr>
<tr>
<td></td>
<td>ETH 26/2008 Cattle 01.11.08</td>
<td>NVD</td>
<td>VI/ELISA</td>
<td>Negative NVD</td>
</tr>
<tr>
<td>MALAYSIA</td>
<td>MAY 1/2008 Cattle 26.03.08</td>
<td>NVD</td>
<td>VI/ELISA</td>
<td>Positive FMDV GD</td>
</tr>
<tr>
<td></td>
<td>MAY 2/2008 Cattle 29.05.08</td>
<td>NVD</td>
<td></td>
<td>Positive FMDV GD</td>
</tr>
<tr>
<td></td>
<td>MAY 3/2008 Cattle 18.06.08</td>
<td>NVD</td>
<td></td>
<td>Positive FMDV GD</td>
</tr>
</tbody>
</table>

TOTAL : 33

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

<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 SVD virus NVD</td>
<td>Positive Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O  A  C  SAT 1  SAT 2  SAT 3  SAT  Asia</td>
<td></td>
</tr>
<tr>
<td>BAHRAIN</td>
<td>4</td>
<td>2 - - - - - - 2 3 1</td>
<td>3 1</td>
</tr>
<tr>
<td>BHUTAN</td>
<td>3</td>
<td>2 - - - - - - 1 3 -</td>
<td>3 -</td>
</tr>
<tr>
<td>BOTSWANA</td>
<td>3</td>
<td>- - - - 2 - - - 3</td>
<td>3 -</td>
</tr>
<tr>
<td>ETHIOPIA</td>
<td>20</td>
<td>8 3 - - - - - - 9 15 5</td>
<td>15 5</td>
</tr>
<tr>
<td>MALAYSIA</td>
<td>3</td>
<td>- - - - - - - - 3 3</td>
<td>3 -</td>
</tr>
<tr>
<td>TOTAL</td>
<td>33</td>
<td>10 5 - 2 - - - 16 27 6</td>
<td>27 6</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 October and December 2008
**TABLE C:** Antigenic characterisation of FMD field isolates by matching with vaccine strains by VNT and/or LPBE – r1 value data from 1st October to 31st December 2008

<table>
<thead>
<tr>
<th>Field Isolate:</th>
<th>BFS 1860</th>
<th>O Ind R2/75</th>
<th>O Kaufbeuren</th>
<th>O Manisa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VNT</td>
<td>VNT</td>
<td>VNT</td>
<td>VNT</td>
</tr>
<tr>
<td>O Bhu 2/2008</td>
<td>0.75</td>
<td>1.0</td>
<td>1.0</td>
<td>0.38</td>
</tr>
<tr>
<td>O Bhu 3/2008</td>
<td>0.84</td>
<td>1.0</td>
<td>0.81</td>
<td>0.39</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.

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.
Annex 2: Phylogenetic analysis of characterised FMDV isolates

Report on FMDV A from Bahrain in 2008
Batch: WRLFMD/2008/00033

Software: MEGA 4.0
No. of Taxa : 174
Data File : n:\evd\meg\db\fmdv\a\BAR2008a.meg
Data Title : Bahrain 2008
Data Type : Nucleotide (Coding)
Analysis : Phylogeny reconstruction
Tree Inference : Neo-Neighbor-Joining
-Phylogeny Test and options : Bootstrap (1000 replicates; seed=31332)
Include Sites : Pairwise Deletion
-Codon Positions : 1st+2nd+3rd+Noncoding
Substitution Model : Kimura 2-parameter
-Substitutions to Include : d: Transitions + Transversions
-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, 10 December 2008

Figure 1. FMDV type A in Bahrain.

Report on FMDV A from Bahrain in 2008
Batch: WRLFMD/2008/00033

Software: MEGA 4.0
No. of Taxa : 174
Data File : n:\evd\meg\db\fmdv\a\BAR2008a.meg
Data Title : Bahrain 2008
Data Type : Nucleotide (Coding)
Analysis : Phylogeny reconstruction
Tree Inference : Neo-Neighbor-Joining
-Phylogeny Test and options : Bootstrap (1000 replicates; seed=31332)
Include Sites : Pairwise Deletion
-Codon Positions : 1st+2nd+3rd+Noncoding
Substitution Model : Kimura 2-parameter
-Substitutions to Include : d: Transitions + Transversions
-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.

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Figure 1. FMDV type A in Bahrain.
Figure 2. FMDV type O in Bhutan.
Figure 3. FMDV type SAT 2 in Botswana.
Report on FMDV O from Ethiopia in 2008

Software: MEGA 4.0
No. of Taxa: 164
Data File: \n:\evd\meg\db\fmdv\o\ETH2008a.meg
Data Title: Ethiopia 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

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 and J. Wadsworth, 6 January 2009

Figure 4. FMDV type O in Ethiopia.
Figure 5. FMDV type A in Ethiopia.
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 – December 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)