

**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 toptype) 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 toptype). In the Middle East (Pakistan, Afghanistan, Iran, Turkey, Jordan, Saudi Arabia), the A-Iran-05 (ASIA toptype) has dominated for the last three years. However, since August 2007, a new sub-lineage of this strain (named A-Iran-05^{ARD-07}) 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 toptype) 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^{ARD-07}) 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

Batch	Country	Serotype	Number of samples	Status
WRLFMD/2008/00012*	Iran	A	3	Completed
WRLFMD/2008/00013*	Pakistan	O	6	Completed
WRLFMD/2008/00013*	Pakistan	A	1	Completed
WRLFMD/2008/00014*	Nigeria	O	1	Completed
WRLFMD/2008/00014*	Nigeria	SAT 2	9	Completed
WRLFMD/2008/00015*	Somalia	O	3	Completed
WRLFMD/2008/00016*	Kenya	O	11	Completed
WRLFMD/2008/00016*	Kenya	A	2	Completed
WRLFMD/2008/00016*	Kenya	SAT 1	2	Completed
WRLFMD/2008/00016*	Kenya	SAT 2	15	Completed
WRLFMD/2008/00018*	Namibia	SAT 2	1	Completed
WRLFMD/2008/00020*	Turkey	O	9	Completed
WRLFMD/2008/00020*	Turkey	A	23	Completed
WRLFMD/2008/00021	Saudi Arabia	O	1	Completed
WRLFMD/2008/00022	Thailand	O	10	Completed
WRLFMD/2008/00022	Thailand	A	2	Completed
WRLFMD/2008/00023	Laos	O	5	Completed
WRLFMD/2008/00025	Botswana	SAT 2	4	Completed
WRLFMD/2008/00026	Namibia	SAT 2	3	Completed
WRLFMD/2008/00027	Zambia	SAT 1	5	Completed
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 toptotype, 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 toptotype, 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 toptotype, 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 toptotype, PanAsia-2 lineage (Annex 2, Figure 4), while all the type A viruses belonged to a sub-lineage of Iran-05 (called Iran-05^{ARD-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 toptotype, 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 toptotype 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 toptotype, 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 toptotype, 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 toptotype 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 toptotype 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 toptotype 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 toptotype 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 previously 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).

Annex 1.

Table A: Summary of clinical sample diagnostics made by the WRL between July and September 2008

Country	WRL for FMD Sample Identification	Animal	Date of Collection	Results		
				VI/ELISA	RT-PCR	Final report
BOTSWANA	BOT 12/2008	Cattle	NK	SAT 2	Positive	SAT 2
	BOT 13/2008	Cattle	NK	SAT 2	Positive	SAT 2
	BOT 14/2008	Cattle	NK	SAT 2	Positive	SAT 2
	BOT 15/2008	Cattle	NK	SAT 2	Positive	SAT 2
ETHIOPIA	ETH 1/2008	Cattle	16.07.08	NVD	Negative	NVD
	ETH 2/2008	Cattle	16.07.08	NVD	Positive	FMDV GD
	ETH 3/2008	Cattle	16.07.08	NVD	Negative	NVD
	ETH 4/2008	Cattle	16.07.08	NVD	Negative	NVD
	ETH 5/2008	Cattle	16.07.08	NVD	Negative	NVD
	ETH 6/2008	Cattle	16.07.08	NVD	Negative	NVD
LAOS	LAO 1/2007	Buffalo	24.08.07	O	Positive	O
	LAO 2/2007	Buffalo	19.10.07	O	Positive	O
	LAO 3/2007	Cattle	19.10.07	O	Positive	O
	LAO 4/2007	Cattle	13.12.07	O	Positive	O
	LAO 1/2008	Cattle	03.01.08	O	Positive	O
NAMIBIA	NMB 2/2008	Cattle	NK	SAT 2	Positive	SAT 2
	NMB 3/2008	Cattle	NK	SAT 2	Positive	SAT 2
	NMB 4/2008	Cattle	NK	SAT 2	Positive	SAT 2
THAILAND	TAI 2/2007	Cattle	16.03.07	NVD	Negative	NVD
	TAI 3/2007	Cattle	02.06.07	A	Positive	A
	TAI 4/2007	Cattle	10.09.07	O	Positive	O
	TAI 5/2007	Buffalo	12.09.07	O	Positive	O
	TAI 6/2007	Cattle	24.09.07	O	Positive	O
	TAI 7/2007	Cattle	15.10.07	O	Positive	O
	TAI 8/2007	Cattle	19.10.07	O	Positive	O
	TAI 9/2007	Cattle	07.11.07	O	Positive	O
	TAI 10/2007	Cattle	14.11.07	O	Positive	O
	TAI 11/2007	Cattle	19.11.07	A	Positive	A
	TAI 12/2007	Cattle	20.11.07	O	Positive	O
	TAI 13/2007	Cattle	21.11.07	O	Positive	O
	TAI 14/2007	Cattle	19.12.07	O	Positive	O
	TAI 15/2007	Cattle	27.12.07	NVD	Positive	FMDV GD
	TAI 1/2008	Cattle	25.01.08	NVD	Positive	FMDV GD
ZAMBIA	ZAM 9/2008	Cattle	NK	SAT 1	Positive	SAT 1
	ZAM 10/2008	Cattle	NK	SAT 1	Positive	SAT 1
	ZAM 11/2008	Cattle	NK	SAT 1	Positive	SAT 1
	ZAM 12/2008	Cattle	NK	SAT 1	Negative	SAT 1
	ZAM 13/2008	Cattle	NK	SAT 1	Positive	SAT 1

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)

Country	No. of samples	Virus isolation in cell culture/ELISA							RT-PCR for FMD (or SVD) virus (where appropriate)			
		FMD virus serotypes			SVD virus	NVD	Positive	Negative				
		O	A	C					SAT 1	SAT 2	SAT 3	Asia
BOTSWANA	4	-	-	-	-	4	-	-	-	4	-	
ETHIOPIA	6	-	-	-	-	-	-	-	-	6	1	5
LAOS	5	5	-	-	-	-	-	-	-	-	5	-
NAMIBIA	3	-	-	-	-	3	-	-	-	-	3	-
THAILAND	15	10	2	-	-	-	-	-	-	3	14	1
ZAMBIA	5	-	-	-	5	-	-	-	-	-	4	1
TOTAL	38	15	2	-	5	7	-	-	-	9	31	7

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 C: Antigenic characterisation of FMD field isolates by matching with vaccine strains by VNT – r₁ value data from 1st July to 30th September 2008

	r₁ Values by neutralisation test against vaccine strains below
WRL Ref Number	O Manisa
O TUR 4/2008	0.35
O TUR 10/2008	0.50
O TUR 26/2008	0.41
O TUR 30/2008	0.45

	r₁ Values by neutralisation test against vaccine strains below		
WRL Ref Number	A22 Irq	A Tur 4/06	A 5925
A TUR 1/2008	0.18		
A TUR 6/2008		>0.83	
A TUR 7/2008	0.11	>0.97	0.28
A TUR 8/2008		>1.0	
A TUR 11/2008	0.17	0.91	0.34
A TUR 24/2007	0.67		
A TUR 28/2008	0.19		
A TUR 32/2008	<0.16		
A TUR 33/2008	0.19		

Field Isolate	r1 Values by 2dmVNT	
	Sat1 Rho 12/78	
Sat1 Bot 20/2006	0.37	
Sat1 Bot 22/2006	0.43	
Sat1 Zam 9/2008	0.17	
Sat1 Zam 13/2008	0.22	

Field Isolate	r1 Values by 2dmVNT		r1 Values by LPBE			
	Sat2 Zim 7/83	Sat2 Eritrea	Sat2 K52/84	Sat2 K65/82	Sat2 Nig 6/81	Sat2 Zim 11/89
Sat2 Nig 2/07	0.24	0.49				
Sat2 Nig 7/07	0.13					
Sat2 Nmb 1/08	0.04	0.16				
Sat2 Nmb 2/08	0.07	0.10	0.22	0.43	0.14	0.61
Sat2 Nmb 4/08	0.09	0.14	0.38	0.22	0.11	0.75
Sat2 Bot 6/08						
Sat2 Bot 11/08	0.15	0.16		0.26	0.17	
Sat2 Bot 12/08	0.29	0.33		0.29	0.17	
Sat2 Ken 2/08	0.09	0.18				
Sat2 Ken 7/07	0.20	0.18				
Sat2 Ken 9/07	0.08					
Sat2 Ken 16/07	0.04					
Sat2 Eth 2/07		0.15				

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

Annex 2: Phylogenetic analysis of characterised FMDV isolates

Software: MEGA 4.0
 No. of Taxa : 172
 Data File : n:\evd\meg\db\fmv\la\tUR2008b.meg
 Data Title : Turkey 2008
 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 : 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

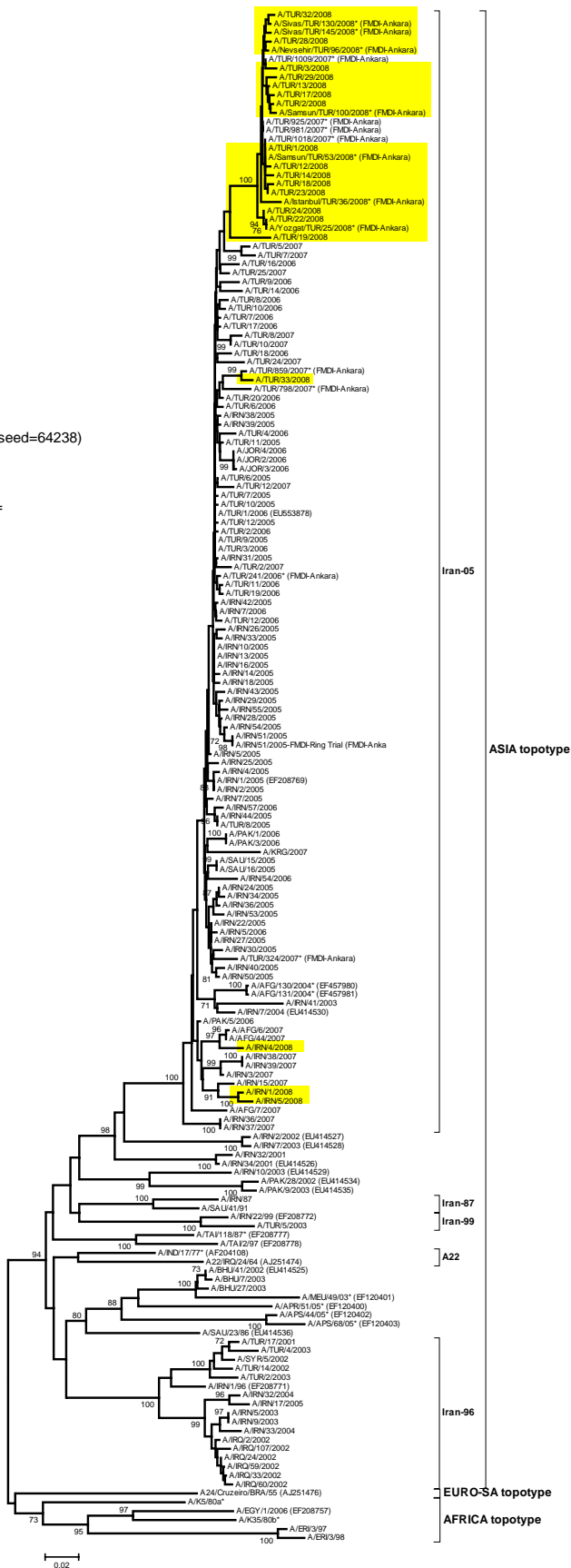


Fig. 1. FMDV type A in Iran in 2008.

Software: MEGA 4.0
 No. of Taxa : 268
 Data File : n:\evd\meg\db\fmv\o\PAK2008b.meg
 Data Title : O Pakistan 2007-2008
 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 : 639
 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, 5 August 2008

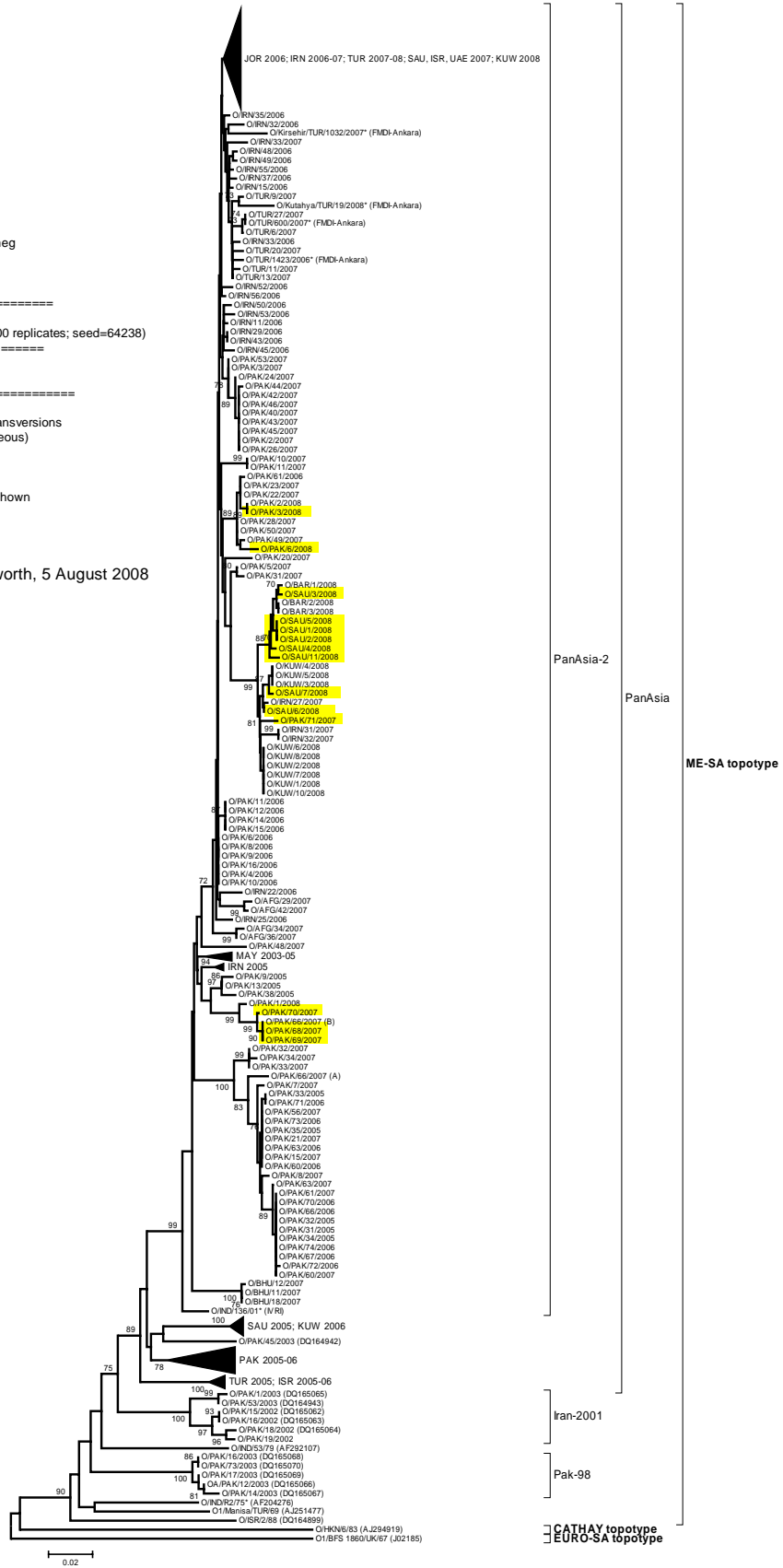


Fig. 2. FMDV type O in Pakistan (2007) and Saudi Arabia (2008).

Software: MEGA 4.0
 No. of Taxa : 162
 Data File : n:\evd\meg\db\fm\md\va\PAK2007a.meg
 Data Title : A Pakistan 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 : 645
 No Of Bootstrap Reps = 1000
 Only bootstrap values of 70% and above are shown

*, not a WRLFMD Ref. No.

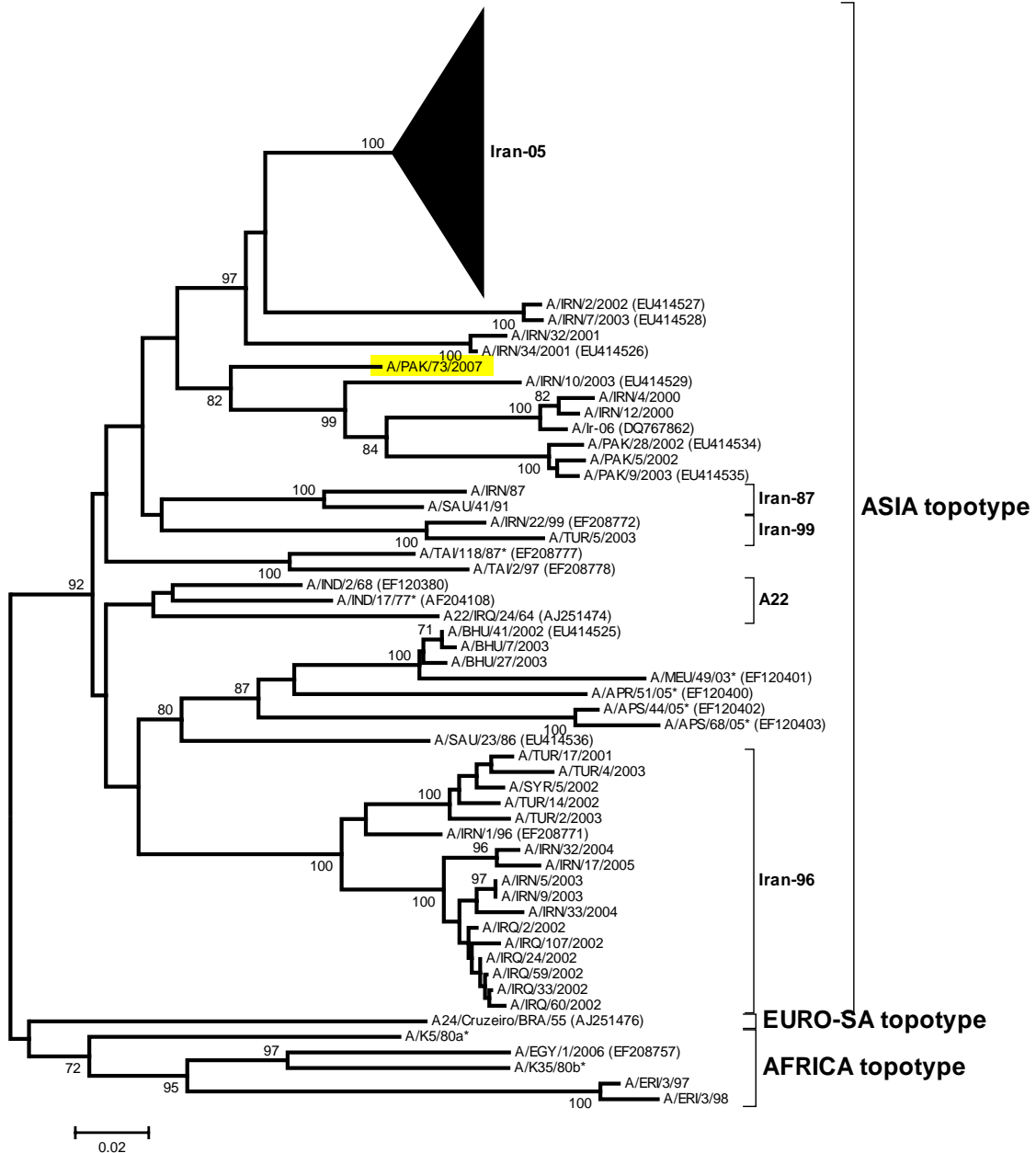


Fig. 3. FMDV type A in Pakistan (2007).

Software: MEGA 4.0
 No. of Taxa : 219
 Data File : n:\evd\meg\db\fmvd\o\tUR2008b.meg
 Data Title : Turkey 2008
 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 : 639
 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

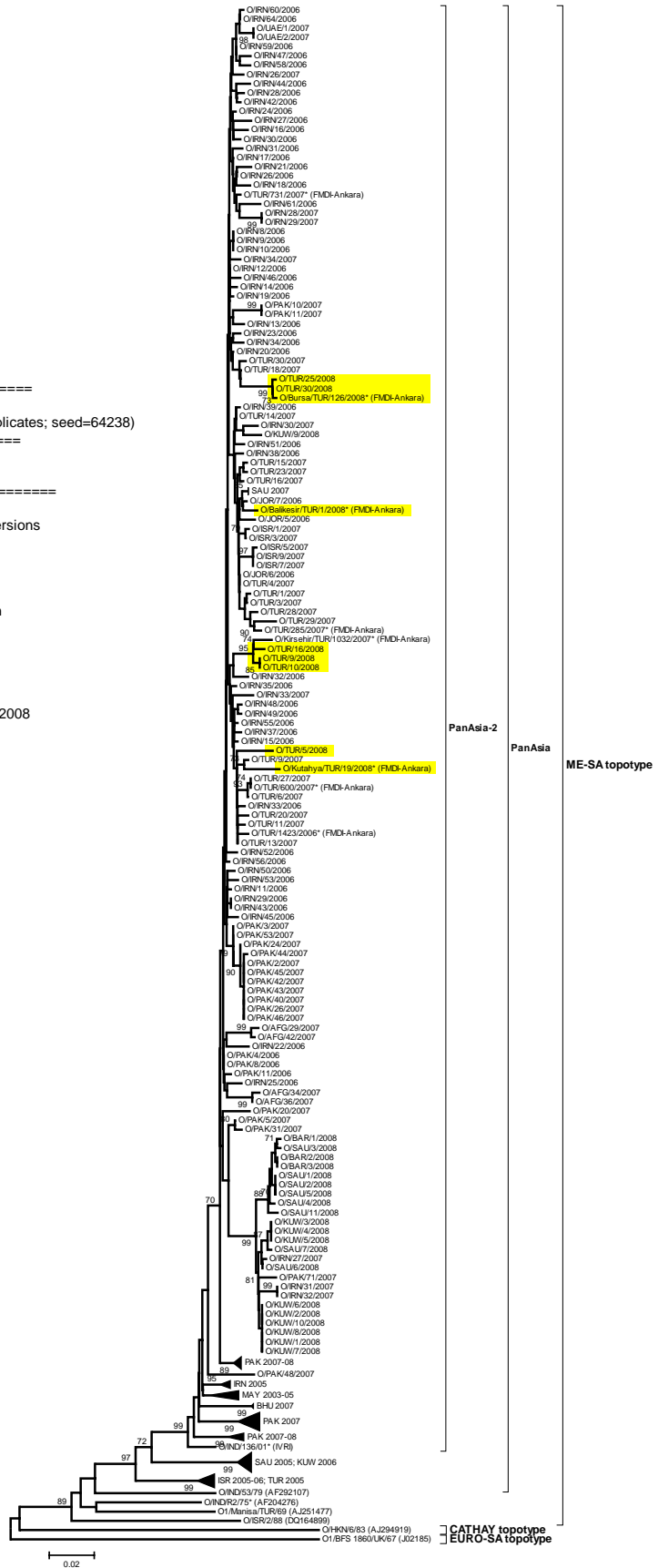


Fig. 4. FMDV type O in Turkey in 2008.

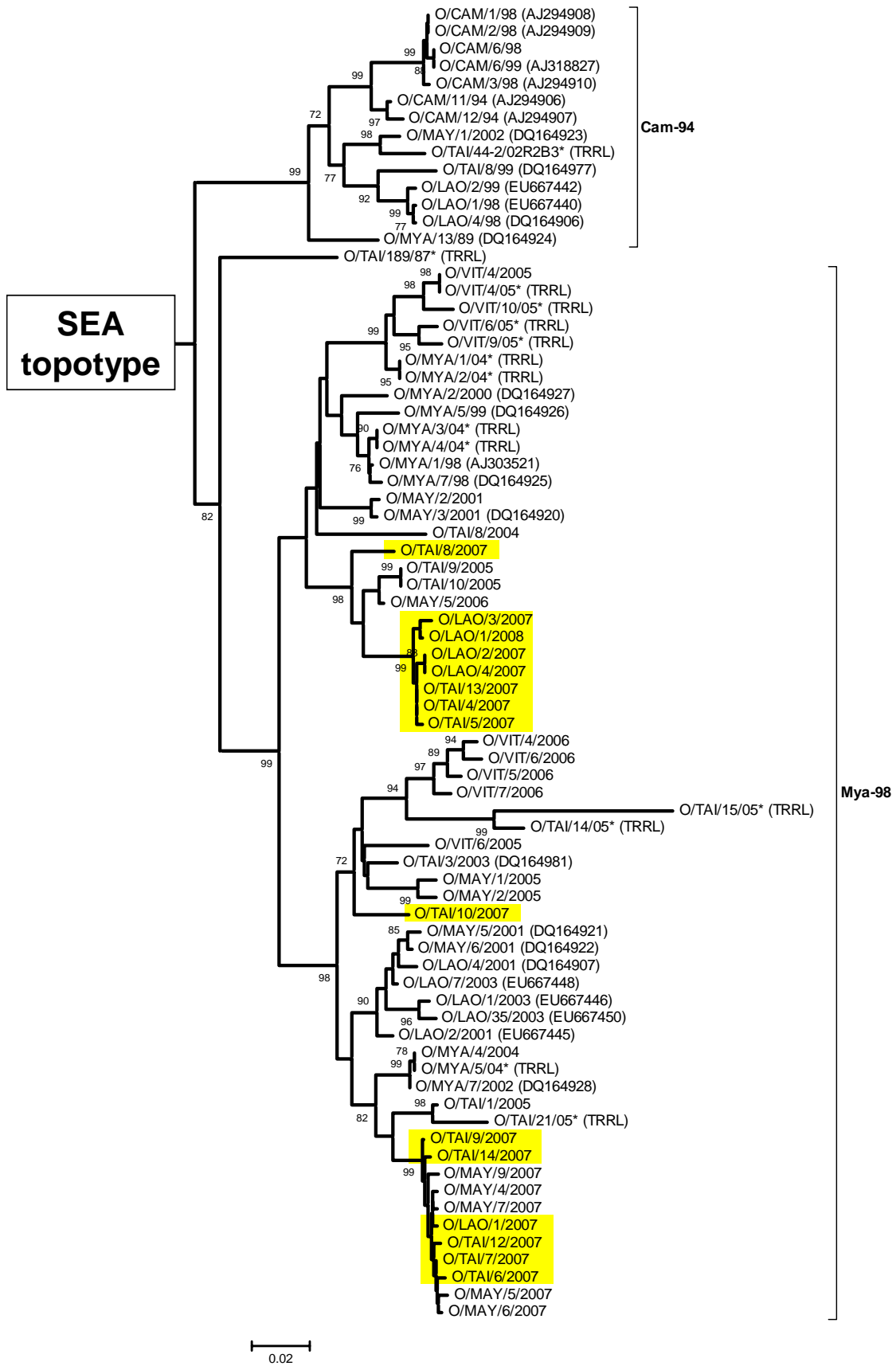


Fig. 5. FMDV type O in Thailand (2007) and Laos (2007-2008)

Software: MEGA 4.0
 No. of Taxa : 122
 Data File : n:\evd\meg\db\fmvd\A\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

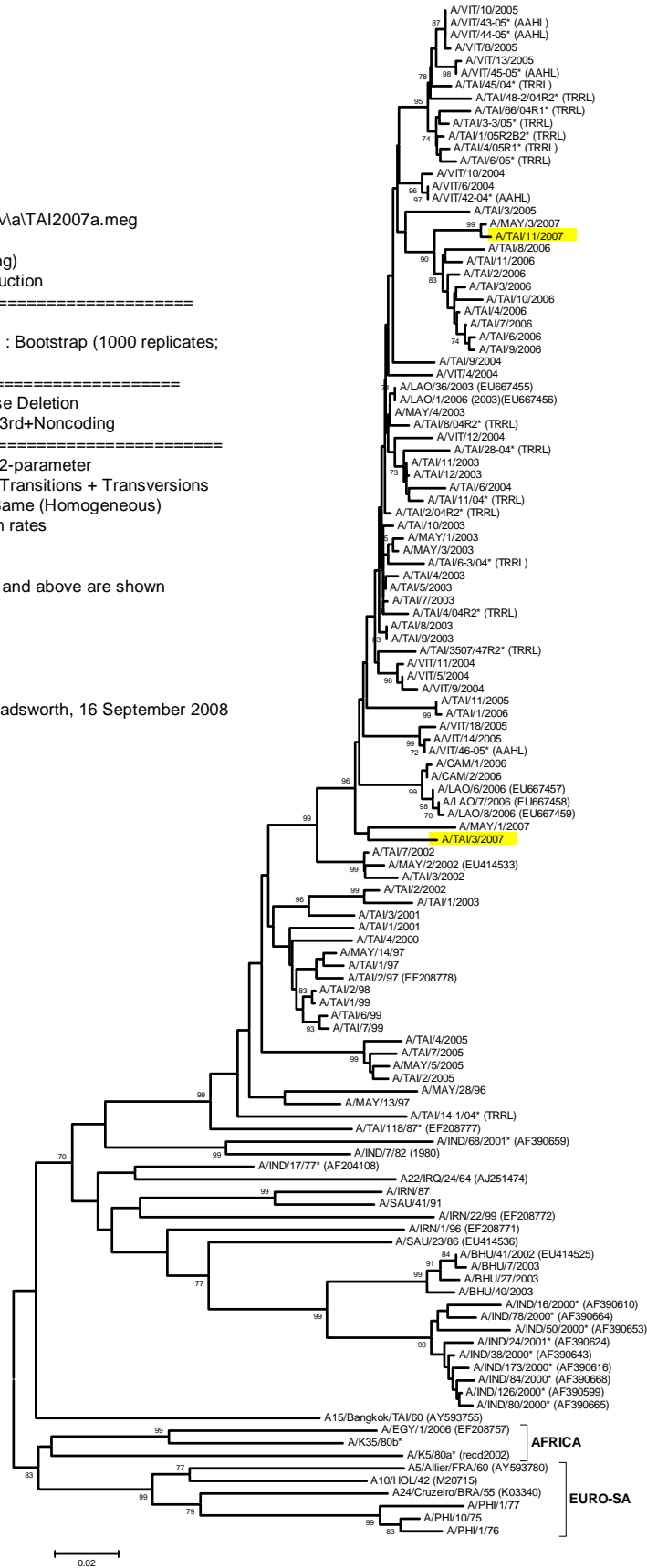


Fig. 6. FMDV type A in Thailand in 2007.

Software: MEGA 4.0
 No. of Taxa : 128
 Data File : n:\evd\meg\db\fmv\asia1\ARRIAHb.meg
 Data Title : Asia 1
 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 : 636
 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, 24/10/2008

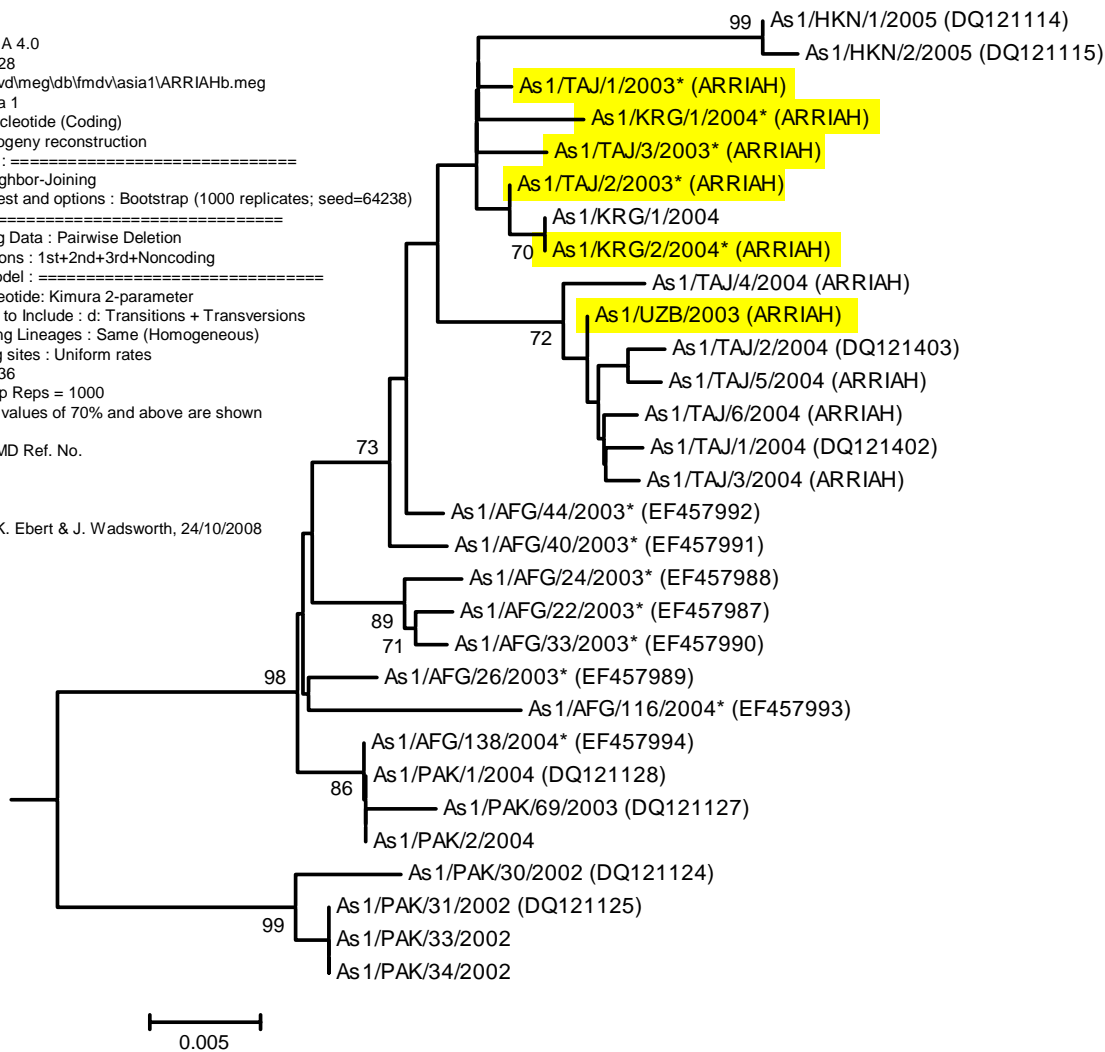


Fig. 7. FMDV type Asia 1 in Central Asia in 2003-2004.

No. of Taxa : 150
 Data File : N:\evd\MEG\vb\FMDV\O\KEN2008a.meg
 Data Title : O/Kenya/2007-08
 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

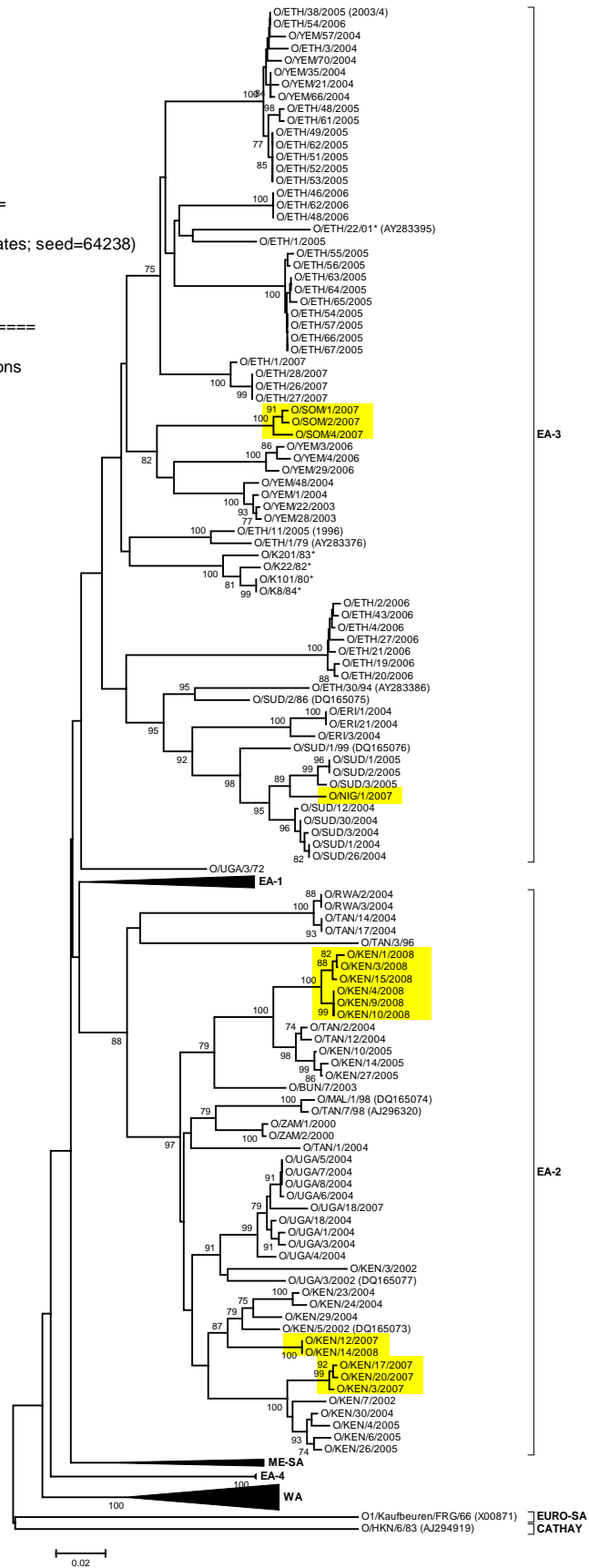


Fig. 8. FMDV type O in Kenya (2007-2008), Somalia (2007) and Nigeria (2007).

Software: MEGA 4.0
 No. of Taxa : 119
 Data File : n:\evd\megldb\fmdiv\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)
 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.

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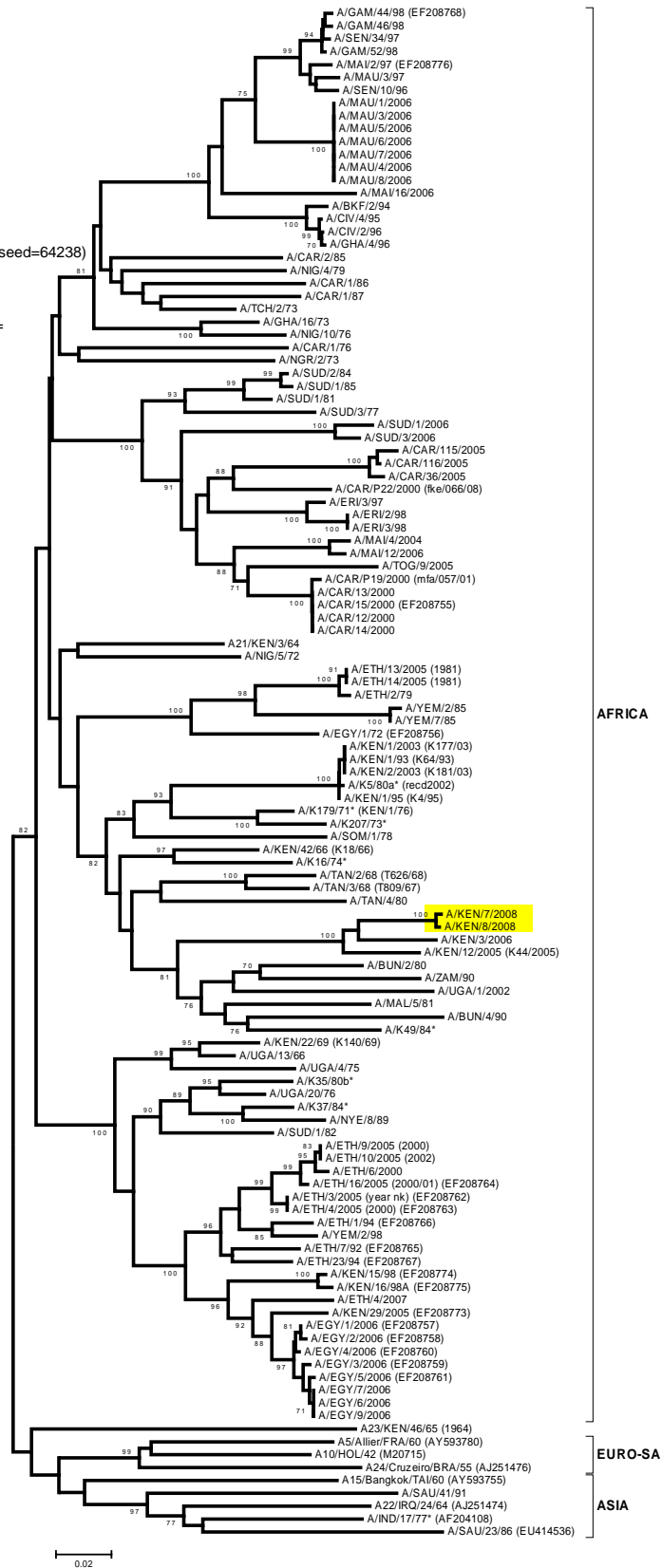


Fig. 9. FMDV type A in Kenya in 2008.

Software: MEGA 4.0
 No. of Taxa: 112
 Data File: n:\evd\meg\db\fmvd\sat1KEN2006c.meg
 Data Title: SAT 1 Kenya 2006
 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: 663
 No Of Bootstrap Reps = 1000
 Only bootstrap values of 70% and above are shown

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

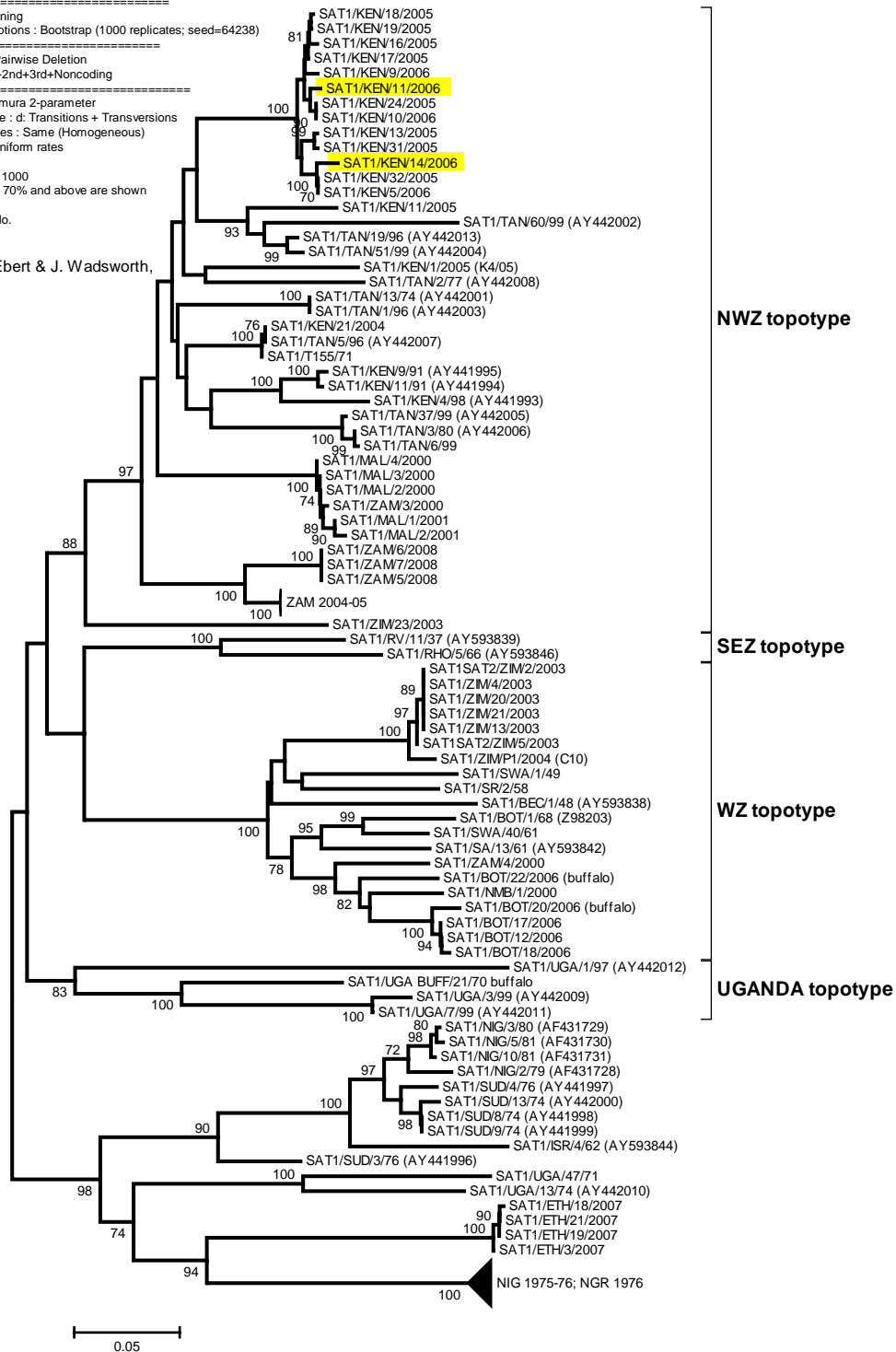


Fig. 10. FMDV type SAT 1 in Kenya in 2006.

Software: MEGA 4.0
 No. of Taxa : 262
 Data File : n:\evd\meg\db\fmv\sat2\KEN2008a.meg
 Data Title : SAT 2
 Data Type : Nucleotide (Coding)
 Analysis : Phylogeny reconstruction
 Tree Inference : =====
 ->Method : Neighbor-Joining
 ->Phylogeny Test and options : Bootstrap (1000 replicates; seed=31764)
 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 : 651
 No Of Bootstrap Reps = 1000
 Only bootstrap values of 70% and above are shown

*, not a WRLFMD Ref. No.

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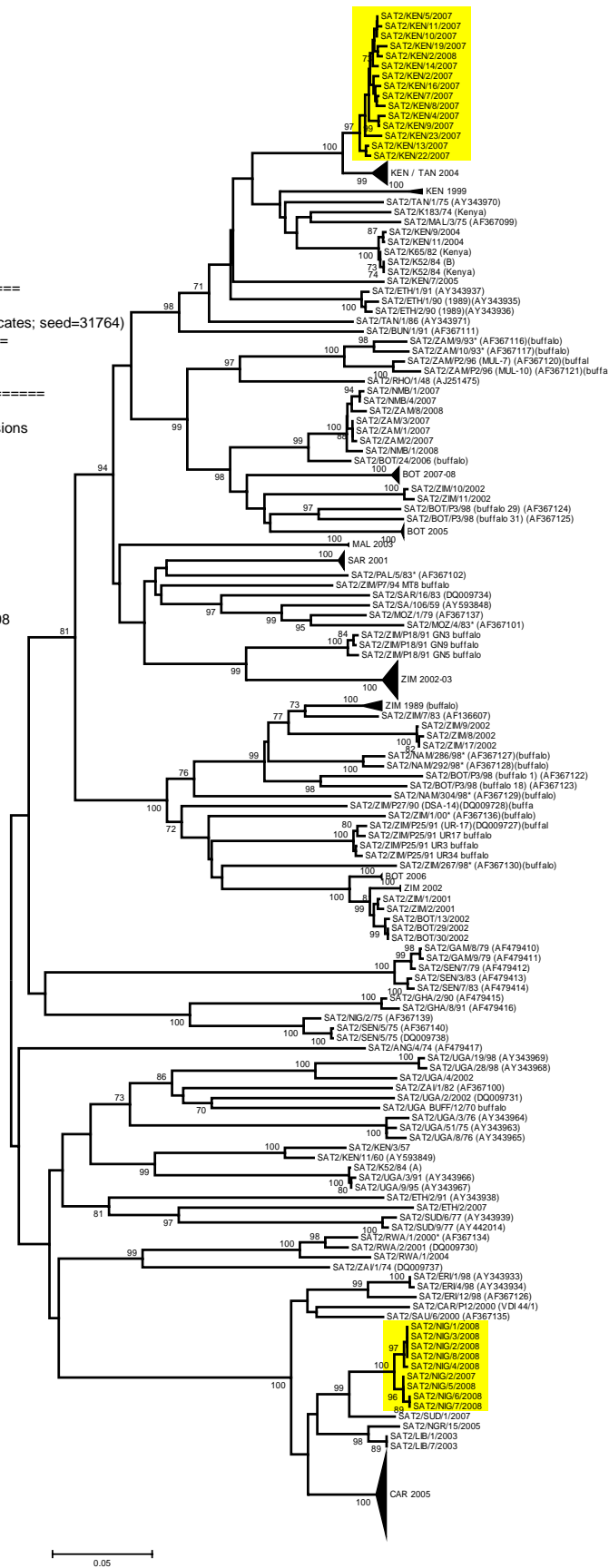


Fig. 11. FMDV type SAT 2 in Kenya and Nigeria in 2007-2008.

Software: MEGA 4.0
 No. of Taxa : 136
 Data File : n:\evd\meg\db\fm\sv\sat2\NMB2008b.meg
 Data Title : SAT2 2008
 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 : 648
 No Of Bootstrap Reps = 1000
 Only bootstrap values of 70% and above are shown

*, not a WRLFMD Ref. No.

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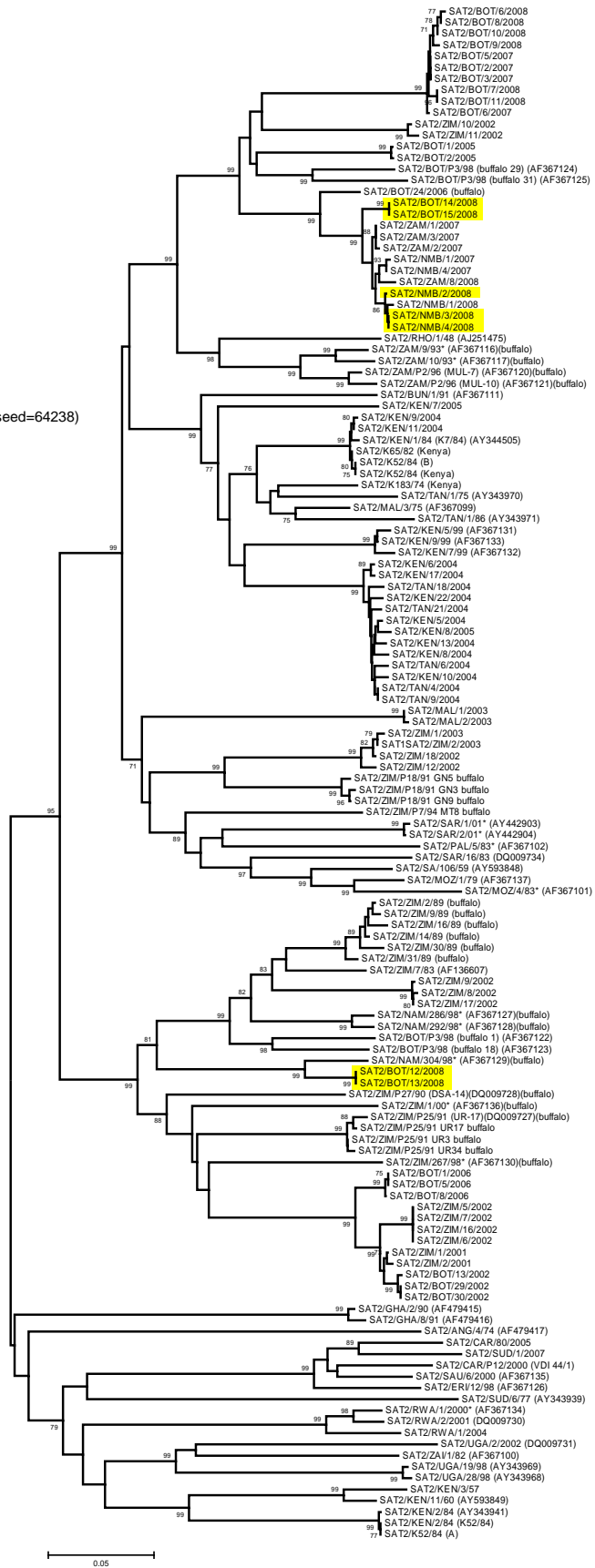


Fig. 12. FMDV type SAT 2 in Botswana and Namibia in 2008.

Software: MEGA 4.0
 No. of Taxa : 128
 Data File : n:\evd\meg\db\fm\dsat1\ZAM2008b.meg
 Data Title : Zambia 2008
 Data Type : Nucleotide (Coding)
 Analysis : Phylogeny reconstruction
 Tree Inference : =====
 ->Method : Neighbor-Joining
 ->Phylogeny Test and options : Bootstrap (1000 replicates; seed=56677)
 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 : 663
 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

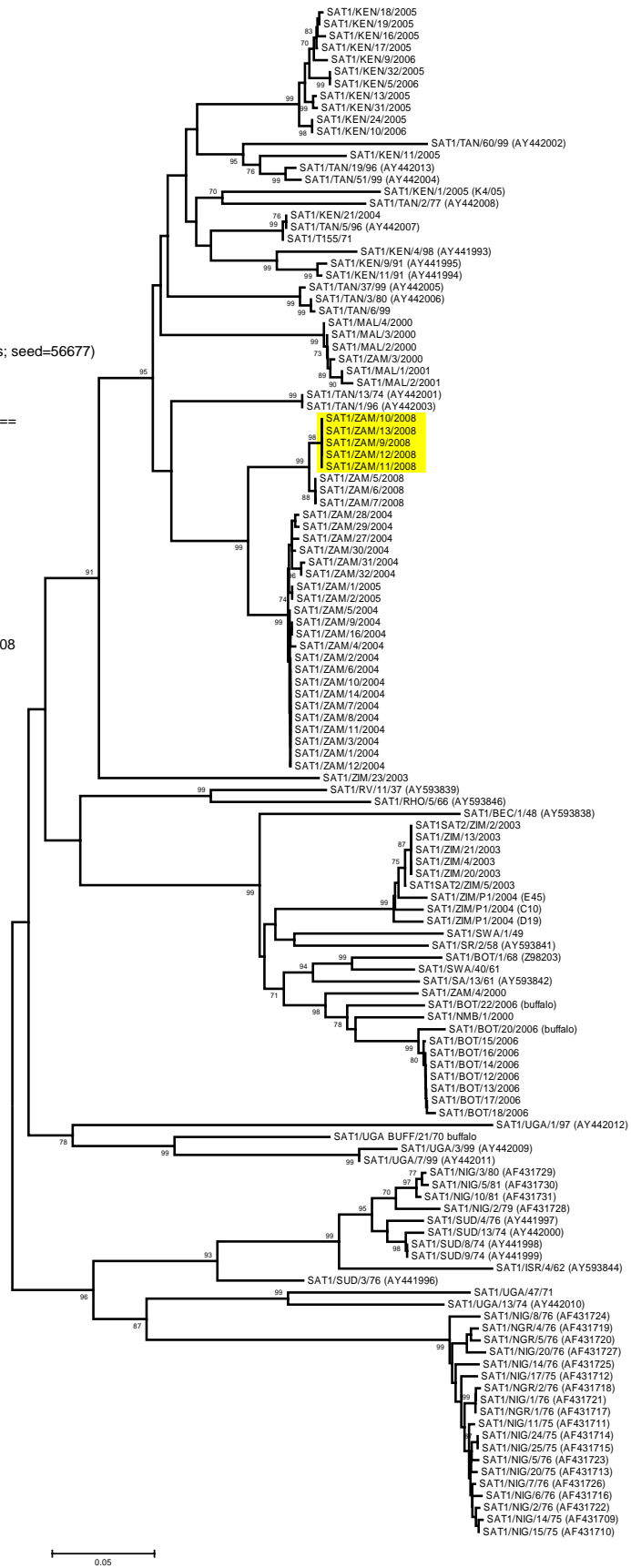


Fig. 13. FMDV type SAT 1 in Zambia in 2008.

Annex 3. Recent FMD Publications cited by PubMed

1. Nuanualsuwan S, Thongtha P, Kamolsiripichaiporn S, Subharat S. UV inactivation and model of UV inactivation of foot-and-mouth disease viruses in suspension. *Int J Food Microbiol.* 2008 Sep 30;127(1-2):84-90. Epub 2008 Jun 18. PMID: 18625534 [PubMed - in process]
2. Joyappa DH, Sasi S, Ashok KC, Reddy GR, Suryanarayana VV. The plasmid constructs producing shRNA corresponding to the conserved 3D polymerase of Foot and Mouth Disease virus protects guinea pigs against challenge virus. *Vet Res Commun.* 2008 Sep 23. [Epub ahead of print] PMID: 18810649 [PubMed - as supplied by publisher]
3. Wang X, Zhang X, Kang Y, Jin H, Du X, Zhao G, Yu Y, Li J, Su B, Huang C, Wang B. Interleukin-15 enhance DNA vaccine elicited mucosal and systemic immunity against foot and mouth disease virus. *Vaccine.* 2008 Sep 19;26(40):5135-44. Epub 2008 May 6. PMID: 18462848 [PubMed - in process]
4. Su B, Wang J, Wang X, Jin H, Zhao G, Ding Z, Kang Y, Wang B. The effects of IL-6 and TNF-alpha as molecular adjuvants on immune responses to FMDV and maturation of dendritic cells by DNA vaccination. *Vaccine.* 2008 Sep 19;26(40):5111-22. Epub 2008 Apr 21. PMID: 18462845 [PubMed - in process]
5. Gloster J, Ryan E, Wright C, Doel C, Parida S, Cox S, Barnett P, Schley D, Gubbins S, Paton D. Foot-and-mouth disease: How much airborne virus do animals exhale? *Vet J.* 2008 Sep 16. [Epub ahead of print] PMID: 18801678 [PubMed - as supplied by publisher]
6. Alexandersen S, Wernery U, Nagy P, Frederiksen T, Normann P. Dromedaries (*Camelus dromedarius*) are of Low Susceptibility to Inoculation with Foot-and-Mouth Disease Virus Serotype O. *J Comp Pathol.* 2008 Sep 10. [Epub ahead of print] PMID: 18789453 [PubMed - as supplied by publisher]
7. Martínez-Azorín F, Remacha M, Martínez-Salas E, Ballesta JP. Internal translation initiation on the foot-and-mouth disease virus IRES is affected by ribosomal stalk conformation. *FEBS Lett.* 2008 Sep 3;582(20):3029-32. Epub 2008 Aug 7. PMID: 18675807 [PubMed - in process]
8. O'Donnell V, Larocco M, Baxt B. Heparan sulfate-binding foot-and-mouth disease virus enters cells via caveola-mediated endocytosis. *J Virol.* 2008 Sep;82(18):9075-85. Epub 2008 Jul 9. PMID: 18614639 [PubMed - indexed for MEDLINE]
9. Valarcher JF, Gloster J, Doel CA, Bankowski B, Gibson D. Foot-and-mouth disease virus (O/UKG/2001) is poorly transmitted between sheep by the airborne route. *Vet J.* 2008 Sep;177(3):425-8. Epub 2007 Jul 12. PMID: 17629524 [PubMed - indexed for MEDLINE]
10. Mingqiu Z, Qingli S, Jinding C, Lijun C, Yanfang X. Sequence analysis of the protein-coding regions of foot-and-mouth disease virus O/HK/2001. *Vet Microbiol.* 2008 Aug 25;130(3-4):238-46. Epub 2008 Feb 7. PMID: 18343054 [PubMed - indexed for MEDLINE]
11. Cooke JN, Westover KM. Serotype-specific differences in antigenic regions of foot-and-mouth disease virus (FMDV): A comprehensive statistical analysis. *Infect Genet Evol.* 2008 Aug 23. [Epub ahead of print] PMID: 18790086 [PubMed - as supplied by publisher]
12. Martínez-López B, Perez AM, De la Torre A, Rodriguez JM. Quantitative risk assessment of foot-and-mouth disease introduction into Spain via importation of live animals. *Prev Vet Med.* 2008 Aug 15;86(1-2):43-56. Epub 2008 Apr 21. PMID: 18430478 [PubMed - indexed for MEDLINE]
13. Du Y, Dai J, Li Y, Li C, Qi J, Duan S, Jiang P. Immune responses of recombinant adenovirus co-expressing VP1 of foot-and-mouth disease virus and porcine interferon alpha in mice and guinea pigs. *Vet Immunol Immunopathol.* 2008 Aug 15;124(3-4):274-83. Epub 2008 Apr 22. PMID: 18511133 [PubMed - indexed for MEDLINE]

14. Larska M, Wernery U, Kinne J, Schuster R, Alexandersen G, Alexandersen S. Differences in the susceptibility of dromedary and Bactrian camels to foot-and-mouth disease virus. *Epidemiol Infect.* 2008 Aug 8;1-6. [Epub ahead of print] PMID: 18687160 [PubMed - as supplied by publisher]
15. Busch GK, Tate EW, Gaffney PR, Rosivatz E, Woscholski R, Leatherbarrow RJ. Specific N-terminal protein labelling: use of FMDV 3C pro protease and native chemical ligation. *Chem Commun (Camb).* 2008 Aug 7;(29):3369-71. Epub 2008 Jun 25. PMID: 18633492 [PubMed - indexed for MEDLINE]
16. Durand S, Murphy C, Zhang Z, Alexandersen S. Epithelial distribution and replication of foot-and-mouth disease virus RNA in infected pigs. *J Comp Pathol.* 2008 Aug-Oct;139(2-3):86-96. Epub 2008 Jul 11. PMID: 18620703 [PubMed - in process]
17. Muthukrishnan M, Singanallur NB, Ralla K, Villuppanoor SA. Evaluation of FTA cards as a laboratory and field sampling device for the detection of foot-and-mouth disease virus and serotyping by RT-PCR and real-time RT-PCR. *J Virol Methods.* 2008 Aug;151(2):311-6. Epub 2008 Jun 26. PMID: 18584888 [PubMed - in process]
18. Lu Z, Cao Y, Bao H, Qi S, Guo J, Shang Y, Jiang T, Zhang Q, Ma J, Liu Z, Liu X, Yin H, Xie Q. Techniques developed in China for foot-and-mouth disease diagnosis. *Transbound Emerg Dis.* 2008 Aug;55(5-6):196-9. PMID: 18666962 [PubMed - indexed for MEDLINE]
19. Mohan MS, Gajendragad MR, Gopalakrishna S, Singh N. Comparative study of experimental Foot-and-Mouth Disease in cattle (*Bos indicus*) and buffaloes (*Bubalis bubalus*). *Vet Res Commun.* 2008 Aug;32(6):481-9. Epub 2008 May 20. PMID: 18491212 [PubMed - indexed for MEDLINE]
20. Choudary S, Ravikumar P, Ashok Kumar C, Suryanarayana VV, Reddy GR. Enhanced immune response of DNA vaccine (VP1-pCDNA) adsorbed on cationic PLG for foot and mouth disease in guinea pigs. *Virus Genes.* 2008 Aug;37(1):81-7. Epub 2008 May 31. PMID: 18516668 [PubMed - indexed for MEDLINE]
21. Rana SK, Bagchi T. Partial sequence analysis of VP1 of Indian isolates of foot-and-mouth disease virus type Asia-1. *Virus Genes.* 2008 Aug;37(1):60-8. Epub 2008 May 30. PMID: 18512139 [PubMed - indexed for MEDLINE]
22. Seki C, Robiolo B, Periolo O, Iglesias M, D'Antuono A, Maradei E, Barros V, La Torre J, Mattion N. Rapid methodology for antigenic profiling of FMDV field strains and for the control of identity, purity and viral integrity in commercial virus vaccines using monoclonal antibodies. *Vet Microbiol.* 2008 Jul 26. [Epub ahead of print] PMID: 18774662 [PubMed - as supplied by publisher]
23. Pengyan W, Yan R, Zhiru G, Chuangfu C. Inhibition of foot-and-mouth disease virus replication in vitro and in vivo by small interfering RNA. *Virol J.* 2008 Jul 25;5:86. PMID: 18652701 [PubMed - indexed for MEDLINE]
24. Mohan MS, Gajendragad MR, Kishore S, Gopalakrishna S, Singh N. Kinetics of immune response to foot-and-mouth disease virus (type Asia 1) in experimental cattle. *Vet Res Commun.* 2008 Jul 22. [Epub ahead of print] PMID: 18648998 [PubMed - as supplied by publisher]
25. Ojosnegros S, Agudo R, Sierra M, Briones C, Sierra S, González-López C, Domingo E, Cristina J. Topology of evolving, mutagenized viral populations: quasispecies expansion, compression, and operation of negative selection. *BMC Evol Biol.* 2008 Jul 17;8:207. PMID: 18637173 [PubMed - indexed for MEDLINE]
26. Perez AM, König G, Späth E, Thurmond MC. Variation in the VP1 gene of foot-and-mouth disease virus serotype A associated with epidemiological characteristics of outbreaks in the 2001 epizootic in Argentina. *J Vet Diagn Invest.* 2008 Jul;20(4):433-9. PMID: 18599847 [PubMed - indexed for MEDLINE]
27. Morioka K, Fukai K, Ohashi S, Sakamoto K, Tsuda T, Yoshida K. Comparison of the characters of the plaque-purified viruses from foot-and-mouth disease virus O/JPN/2000.

J Vet Med Sci. 2008 Jul;70(7):653-8. PMID: 18685235 [PubMed - indexed for MEDLINE]

28. Harwood LJ, Gerber H, Sobrino F, Summerfield A, McCullough KC. Dendritic cell internalization of foot-and-mouth disease virus: influence of heparan sulfate binding on virus uptake and induction of the immune response. *J Virol*. 2008 Jul;82(13):6379-94. Epub 2008 Apr 30. PMID: 18448534 [PubMed - indexed for MEDLINE]
29. Cubillos C, de la Torre BG, Jakab A, Clementi G, Borrás E, Bárcena J, Andreu D, Sobrino F, Blanco E. Enhanced mucosal immunoglobulin A response and solid protection against foot-and-mouth disease virus challenge induced by a novel dendrimeric peptide. *J Virol*. 2008 Jul;82(14):7223-30. Epub 2008 Apr 30. PMID: 18448530 [PubMed - indexed for MEDLINE]
30. Muller JD, McEachern JA, Bossart KN, Hansson E, Yu M, Clavijo A, Hammond JM, Wang LF. Serotype-independent detection of foot-and-mouth disease virus. *J Virol Methods*. 2008 Jul;151(1):146-53. Epub 2008 Apr 25. PMID: 18440078 [PubMed - indexed for MEDLINE]

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)