

An Epidemiological Investigation of Foot-and-Mouth Disease Virus Carriers in Vietnam

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Overarching Study Goals

1. Elucidate the conventional and molecular epidemiology of circulating FMDV strains in Vietnam

Focus 1: Source of emergent strains

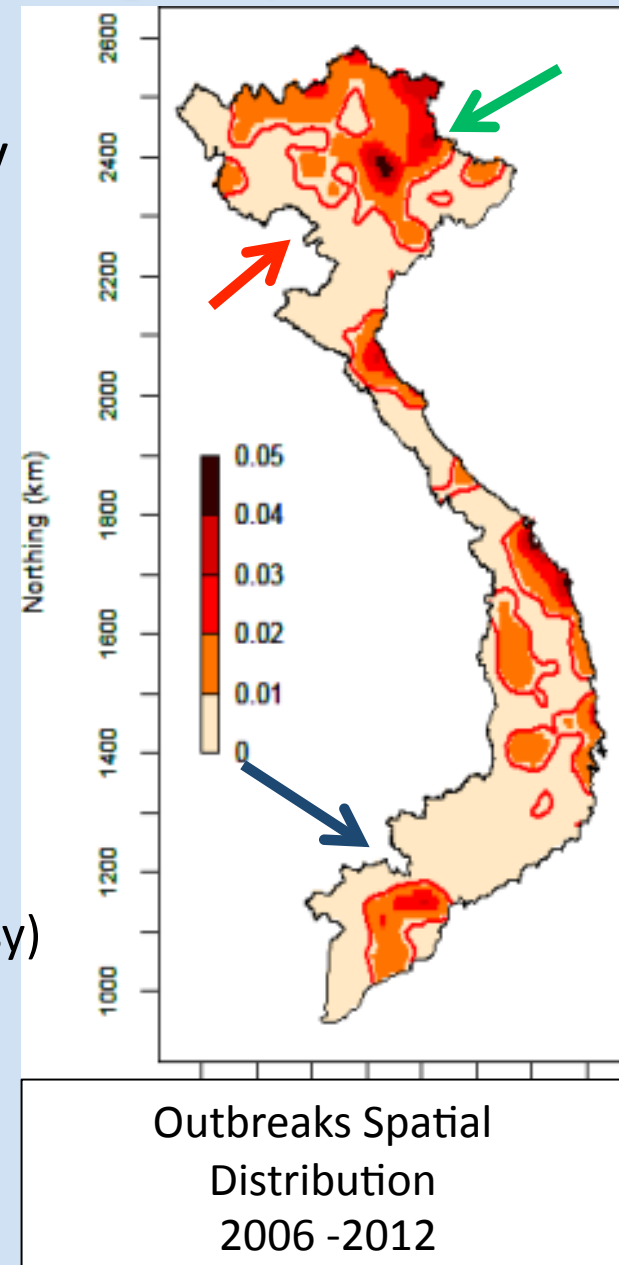
Focus 2: Risk factors associated with infection

Study Approach

1. Passive surveillance (reported FMD outbreaks)
 - Obtain outbreak viruses (sequence)
2. Active (targeted) surveillance of asymptomatic cattle and buffalo
 - Identify carriers & obtain subclinical viruses (sequence)
3. Collect/assimilate descriptive data
 - Outbreaks
 - Carrier suspects

Study Site Selection (targeting suspect carriers)

- Three Provinces identified with history of FMD outbreaks in cattle, buffalo
 - Lang Son (north)
 - SonLa (north)
 - LongAn (south)
- Established study sites:
 - Targeted surveillance study
 - NSP serology → Probang sampling
 - Buffalo (longitudinal & necropsy)
 - Cattle (longitudinal, transmission, necropsy)



Targeted Longitudinal Sampling

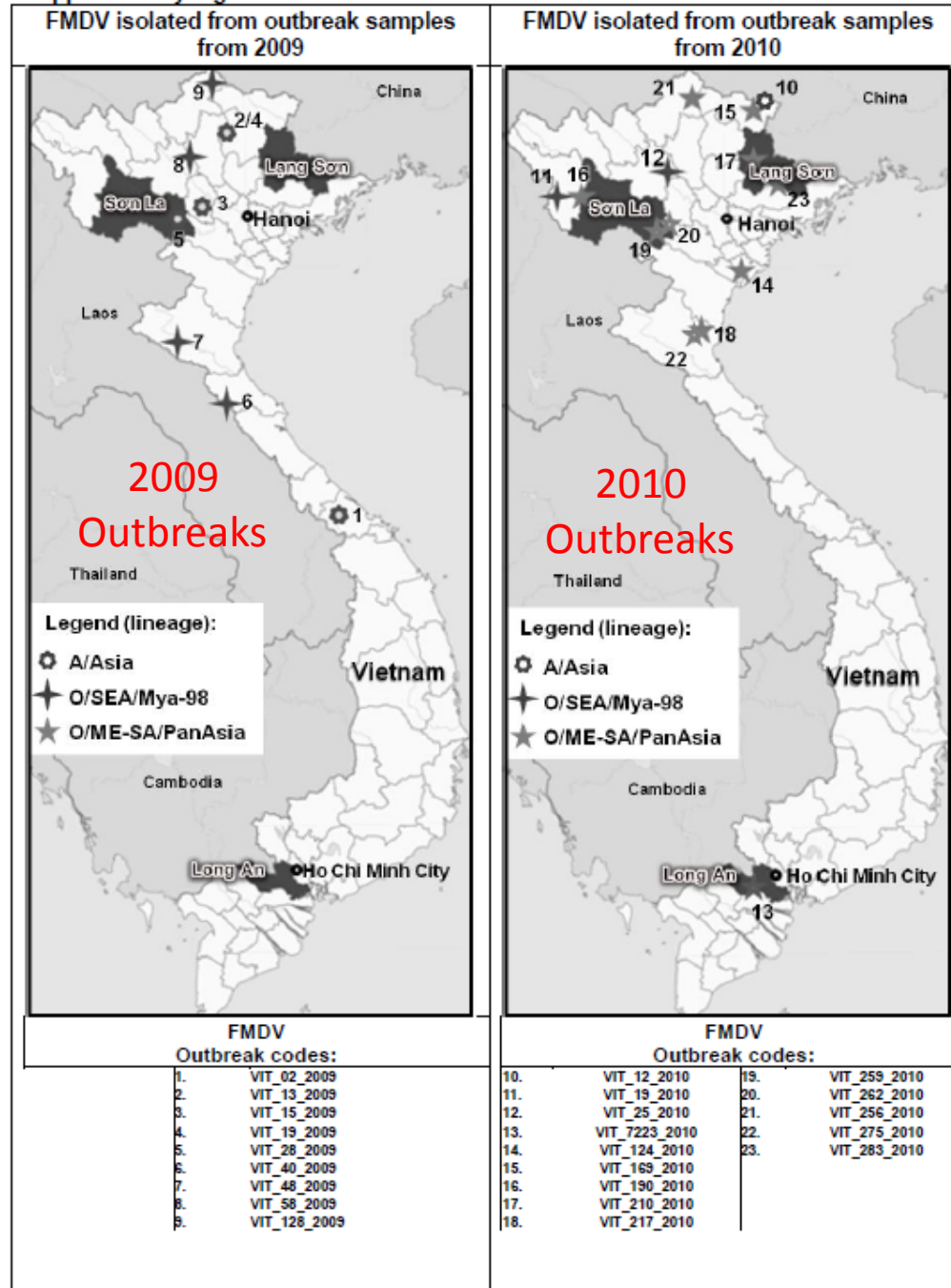


Timeline of Samples included: 2009-2013

- Outbreak Samples
 - 2009-2013
- Serum and Probang Samples (asymptomatic)
 - 2012-2013

Geo-temporal distribution of samples collected

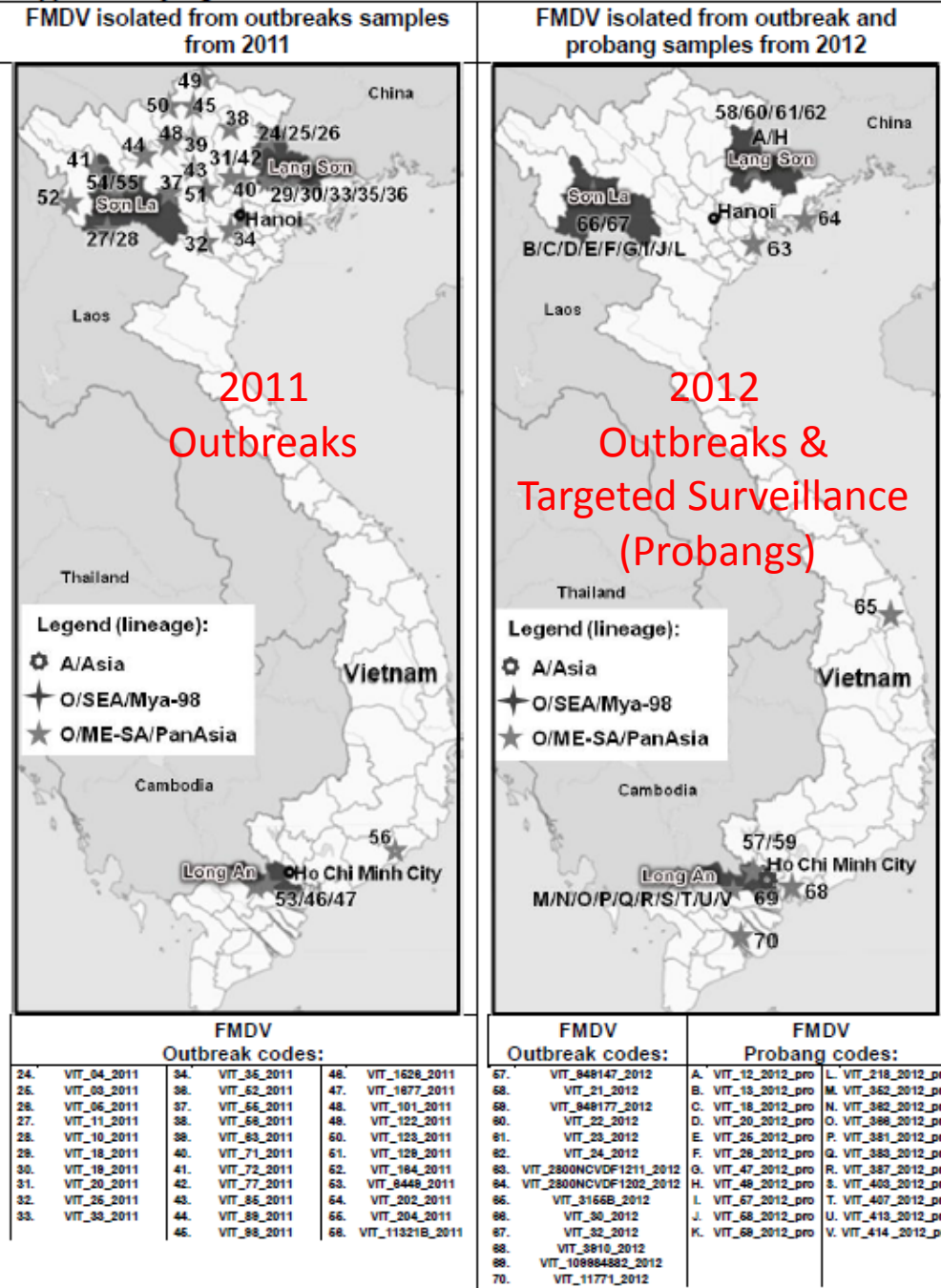
Supplementary Figure 1



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Geo-temporal distribution of samples collected

Supplementary Figure 2



Supplementary Figure 3



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Table 1: FMDV exposure status classification per province, district and commune

Province	District	Commune	Infected ^a			Never Infected ^b	Total Tested
			Carrier	Non-carrier	Total infected		
Long An	Duc Hoa	An Ninh Đông	8 (16.0%) [¶]	42 (84.0%)	50 (41.0%) [¶]	72 (59.0%)	122
		Hiệp Hòa	0 (0.0%)	31 (100.0%)	31 (15.8%)	165 (84.2%)	196
		Tân Mỹ	3 (13.0%)	20 (87.0%)	23 (12.8%)	157 (87.2%)	180
	Total	11 (10.6%)	93 (89.4%)	104 (20.9%)	394 (79.1%)	498	
Lạng Sơn	Chi Lăng	Quan Sơn	1 (25.0%)	3 (75.0%)	4 (30.4%)	7 (63.6%)	11
		Quang Lang	0 (0.0%)	6 (100.0%)	6 (54.5%) [§]	5 (45.5%)	11
		Thượng cường	0 (0.0%)	14 (100.0%)	14 (73.7%) [§]	5 (26.3%)	19
		Total	1 (4.2%)	23 (95.8%)	24 (58.5%)	17 (41.5%)	41
	Hữu Lũng	Đồng Tân	0 (0.0%)	22 (100.0%)	22 (16.5%)	111 (83.5%)	133
		Yên Vượng	0 (0.0%)	10 (100.0%)	10 (47.6%) [§]	11 (52.4%)	21
Total	0 (0.0%)	32 (100.0%)	32 (20.8%)	122 (79.2%)	154		
Total		1 (1.8%)	55 (98.2%)	56 (28.7%)	139 (71.3%)	195	
Cò Nòi			3 (15.8%)	16 (84.2%)	19 (15.2%)	106 (84.8%)	125
	Total		3 (10.8%)	288 (89.2%)	323 (22.3%)	1123 (77.7%)	1446
Sơn La	TP Sơn La	Chiềng Ngạn	0 (0.0%)	7 (70.0%)	10 (12.5%)	70 (87.5%)	80
		Chiềng Sinh	1 (10.0%)	9 (90.0%)	10 (24.4%) [§]	31 (75.6%)	41
		Total	4 (20.0%)	16 (80.0%)	20 (16.5%)	101 (83.5%)	121
	Yên Châu	Chiềng Đông	0 (0.0%)	0 (0.0%)	0 (0.0%)	15 (100.0%)	15
		Chiềng Pả	3 (8.1%)	34 (91.9%)	37 (37.8%) [§]	61 (62.2%)	98
		Chiềng Sàng	6 (14.0%)	37 (86.0%)	43 (55.8%) [§]	34 (44.2%)	77
		Phiềng khoáng	0 (0.0%)	0 (0.0%)	0 (0.0%)	21 (100.0%)	21
		Sấp Vạt	0 (0.0%)	4 (100.0%)	4 (13.8%)	24 (82.8%)	29
		Viêng Lầu	5 (14.7%)	29 (85.3%)	34 (28.1%) [§]	85 (70.2%)	121
	Total	14 (11.9%)	104 (88.1%)	118 (33.0%)	240 (67.0%)	358	
Total	23 (14.1%)[¶]	140 (85.9%)	163 (21.6%)	590 (78.4%)	753		
Total		35 (10.8%)	288 (89.2%)	323 (22.3%)	1123 (77.7%)	1446	

Overall:

22.3% of all cattle and buffalo NSP+ (total infected)

2.4% of all cattle and buffalo probang+ (qRT-PCR)

Descriptive epidemiological data translated to modeling risk factors for Infection/Carriers

18 Table 2: Comparison of FMDV exposure status between animal types and age ranges

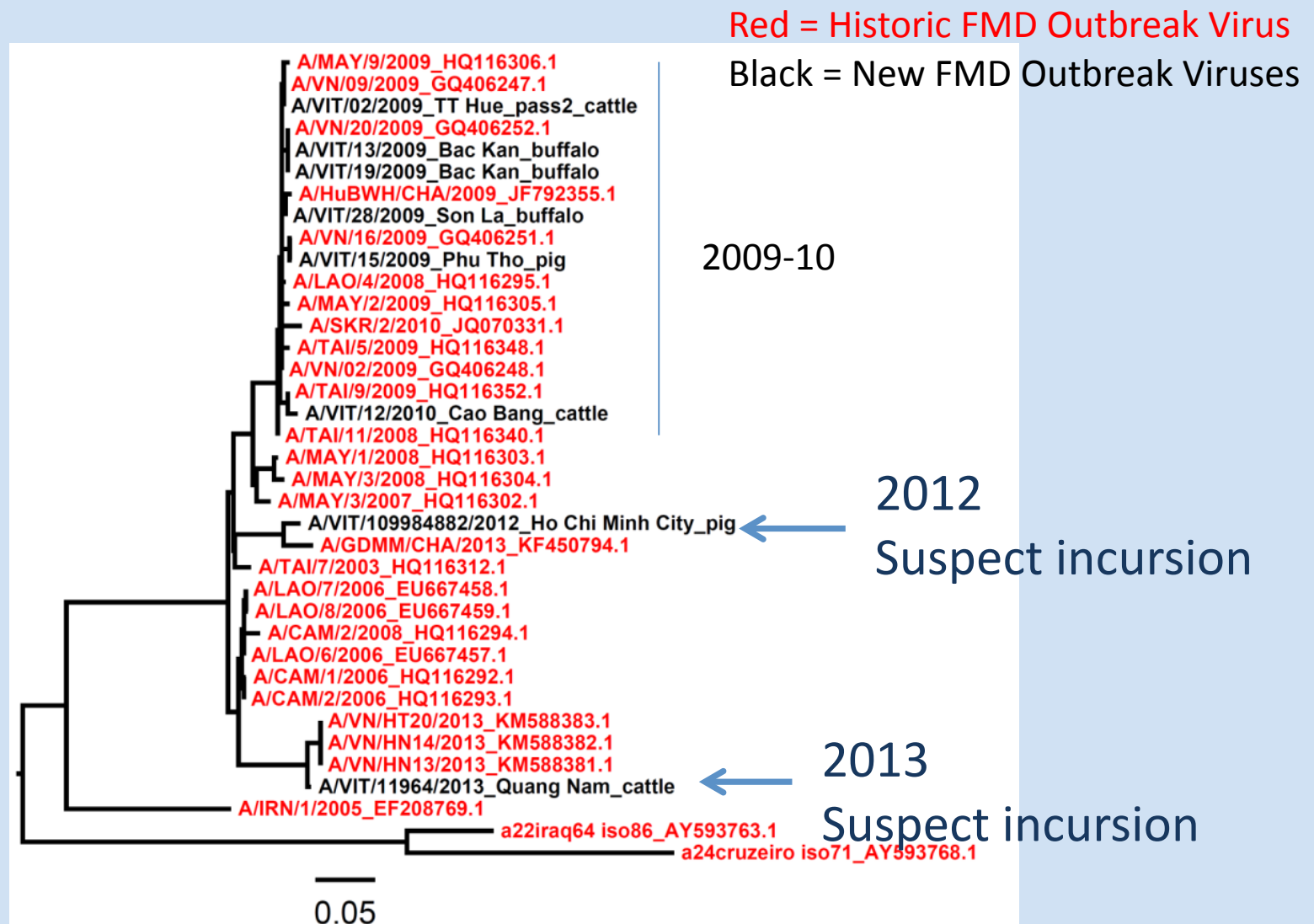
		Infected ^a			Never infected ^b	Total sampled
		Carrier	Non-carrier	Total infected ^e		
Type	Buffalo	14 (8.0 %) ^{2,c,d}	162 (92.0 %)	176 (29.8 %) ¹	414 (70.2%)	590
	Dairy cattle	3 (3.6 %) ³	80 (96.4 %)	83 (18.4 %) ^{2,3}	367 (81.6%)	450
	Beef cattle	18 (28.1 %) ¹	46 (71.9 %)	64 (15.8 %) ²	342 (84.2%)	406
Age range	≤1	0 (0.0 %) ³	2 (100.0 %)	2 (2.3 %) ³	86 (97.7%)	88
	5-Jan	15 (15.3 %) ^{1,2}	83 (84.7 %)	98 (26.5 %) ^{1,2}	272 (73.5%)	370
	>5	4 (22.2 %) ¹	14 (77.8 %)	18 (34.0 %) ¹	35 (66.0%)	53
	N/A	16 (7.8 %) ¹	189 (92.2 %)	205 (21.9 %) ¹	730 (78.1%)	935
Total		35 (10.8 %) ¹	288 (89.2 %) ²	323 (22.3 %) ¹	1123 (77.7%) ²	1446

- Buffalo “protected” from FMD persistence relative to beef cattle by unknown mechanism
- Dairy Cattle lowest prevalence of carrier

Other Analyses Modeled

- Type of farm
- Animal age
- type of housing; Mingle on pasture?
- pigs present in the farm?
- poultry present in the farm?
- other animals present in the farm?
- source of animal
- type of manure handling
- purpose milk, meat, breeding

Phylogenetic Tree; FMDV serotype A



Captions for figures

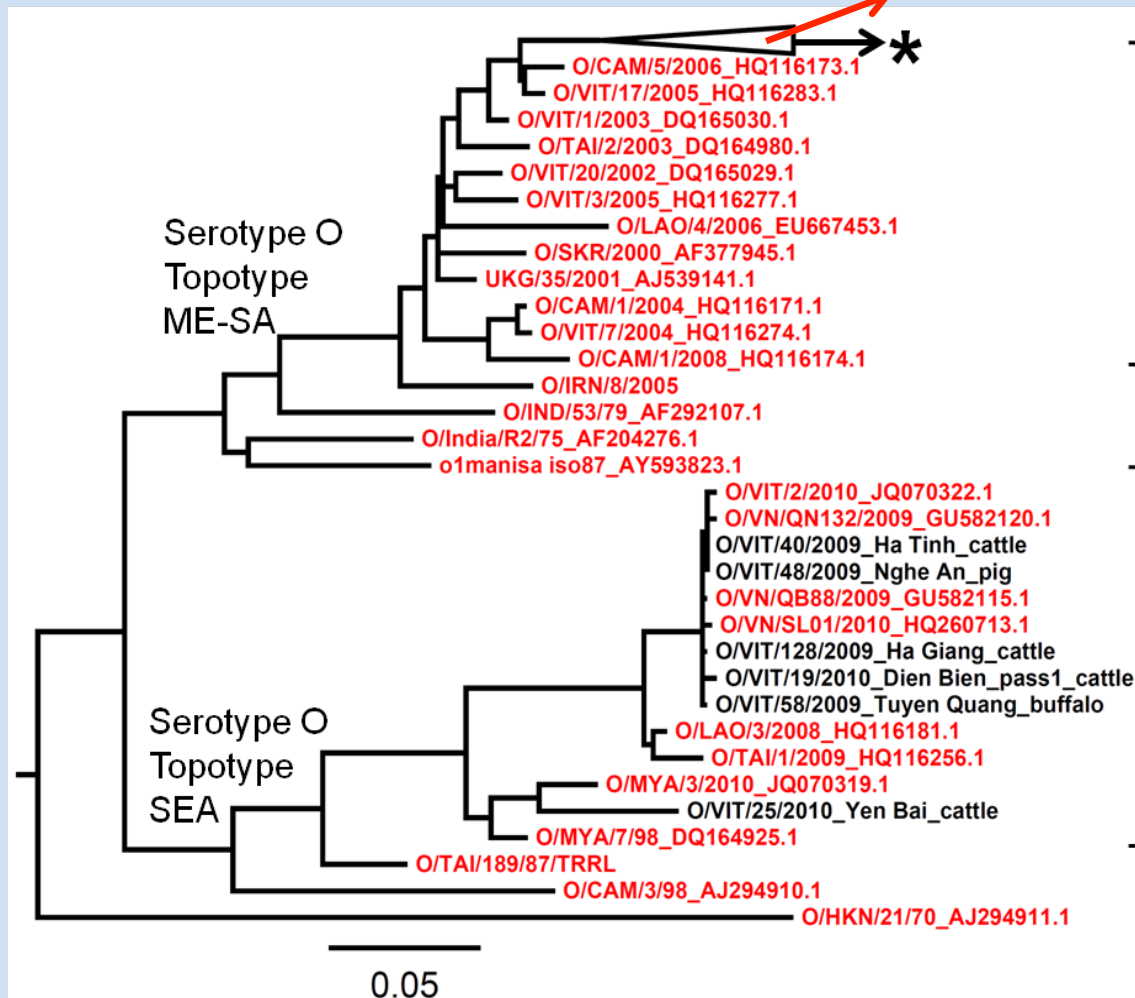
Figure 1: Mid-point rooted maximum likelihood phylogenetic tree of ARS sequenced FMDV Serotype A isolates (starting with VIT) and other phylogenetically related published VIT sequences (in red, starting with "A", with Accession numbers). Note: Information regarding animal species from which virus was originally isolated is appended to the end of the

Phylogenetic Tree; FMDV serotype 0

Red = Historic FMD

ME-SA Break-out
Next Slide

Black = New FMD
Outbreak Viruses

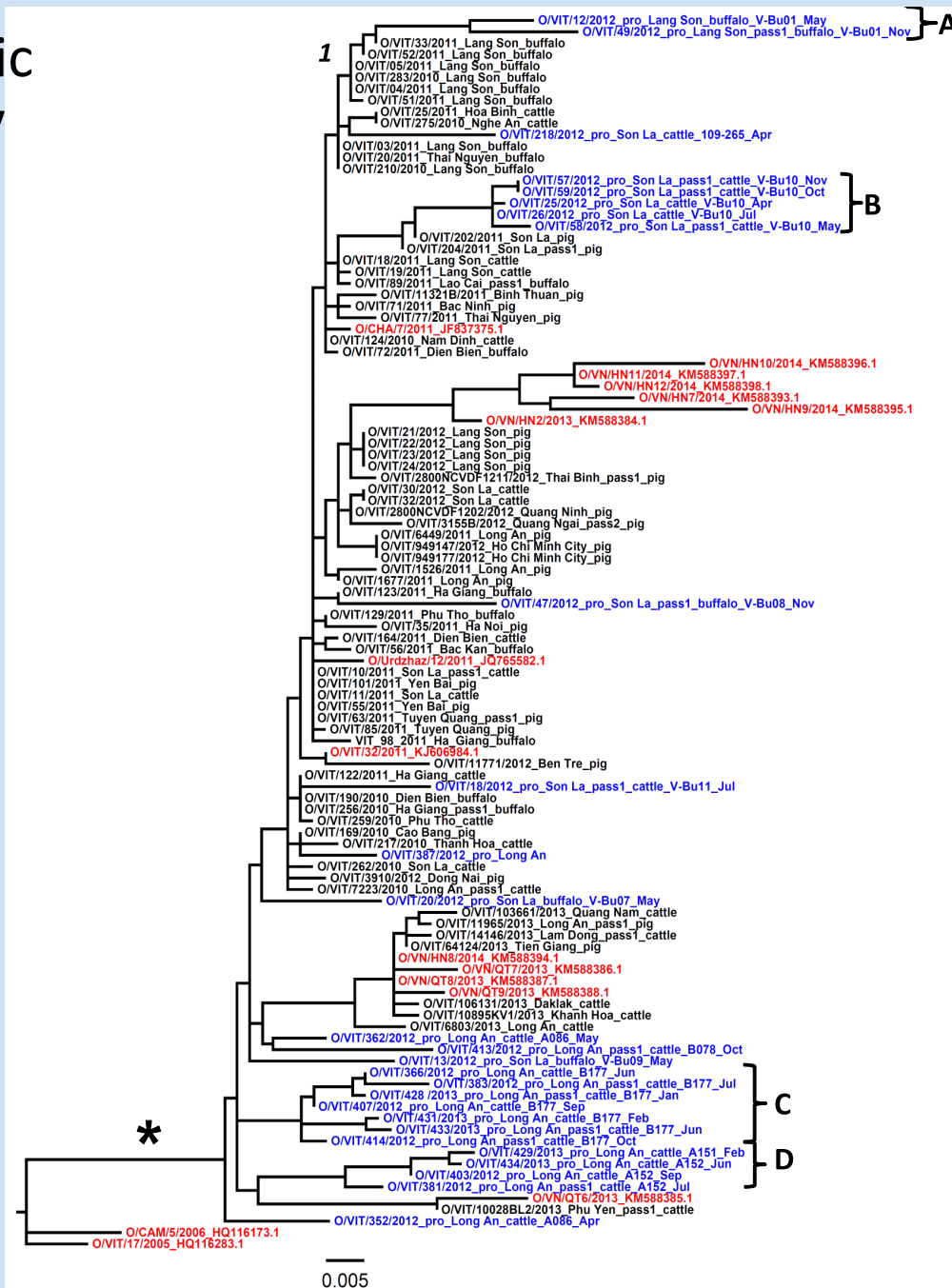


Serotype O
Topotype
ME-SA
Lineage
PanAsia

Some Overlap
in Time and
Space (2010)

Serotype O
Topotype
SEA
Lineage
Mya-98

Phylogenetic Tree; FMDV serotype 0, ME-SA



Red = Historic FMD Outbreak Virus
 Black = New FMD Outbreak Viruses
 Blue = New FMD Probang Viruses

79 Novel Outbreak Viruses

27 Novel Carrier Viruses (within host serials)

Temporo-spatial analyses ongoing to establish relationships of carriers to outbreaks



Necropsy Study



Yen Chau



Son La



Long An



Necropsy study: 13 carriers

	Virus strain											Virus strain	
	ET#	VBU-01	VBU-02	VBU-03	VBU-04	VBU-05	VBU-06	VBU-07	VBU-08	VBU-09	VBU-10	VBU-11	ET#
	Route of inoculation											Route of inoculation	
	Necropsy performed at DPI											Necropsy performed at DPI	
Nasopharynx / Larynx	Dorsal Soft Palate (rostral)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	38.56	NEG	38.43	NEG	Dorsal Soft Palate (rostral)
	Dorsal Soft Palate (caudal)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	39.36	NEG	36.55	NEG	Dorsal Soft Palate (caudal)
	Dorsal Nasopharynx (rostral)	NEG	NEG	NEG	NEG	27.27	38.19	NEG	40.88	NEG	38.52	NEG	Dorsal Nasopharynx (rostral)
	Dorsal Nasopharynx (caudal)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	39.37	NEG	Dorsal Nasopharynx (caudal)
	(Ventral) Epiglottis	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	43.80	NEG	(Ventral) Epiglottis
Larynx	30.78	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	30.69	NEG	Larynx
Tonsils and Lymph Nodes	Palatine tonsil	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Palatine tonsil
	Retropharyngeal LN	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	34.55	NEG	Retropharyngeal LN
	Submandibular LN	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Submandibular LN
	Right Popliteal LN	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Right Popliteal LN
Serial Probangs	Probang 1st						NEG	37.23			36.30		
	Probang 2nd						37.27	35.64		33.61	32.08		
	Probang 3rd	33.86					NEG	38.06		31.32	32.32	39.14	
	Probang 4th	35.38	NEG	38.55	38.19	38.48	NEG	NEG	36.95		NEG	39.76	
	Probang 5th	35.43	NEG	NEG	37.97	42.79	39.19	36.73	38.41	40.29	33.46	37.98	
													Cattle
													Cattle

Sites of FMDV persistence in Asian buffalo and Vietnamese cattle in nasopharynx and larynx (as previously published).



Longitudinal Transmission Study (Long An) (pilot study)



An FMDV-carrier (Holstein) feeds with sentinel steer at Duc Hua Dairy

- 8 Farms
- 10 Donors (Carriers)
- 16 Sentinels



- 12 months sampling
- 0 transmission events (NSP & Clinical)

Manuscript in TBED, ePub

Transboundary and Emerging Diseases

Transboundary and Emerging Diseases

ORIGINAL ARTICLE

An Integrative Analysis of Foot-and-Mouth Disease Virus Carriers in Vietnam Achieved Through Targeted Surveillance and Molecular Epidemiology

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Ferreira et. al.

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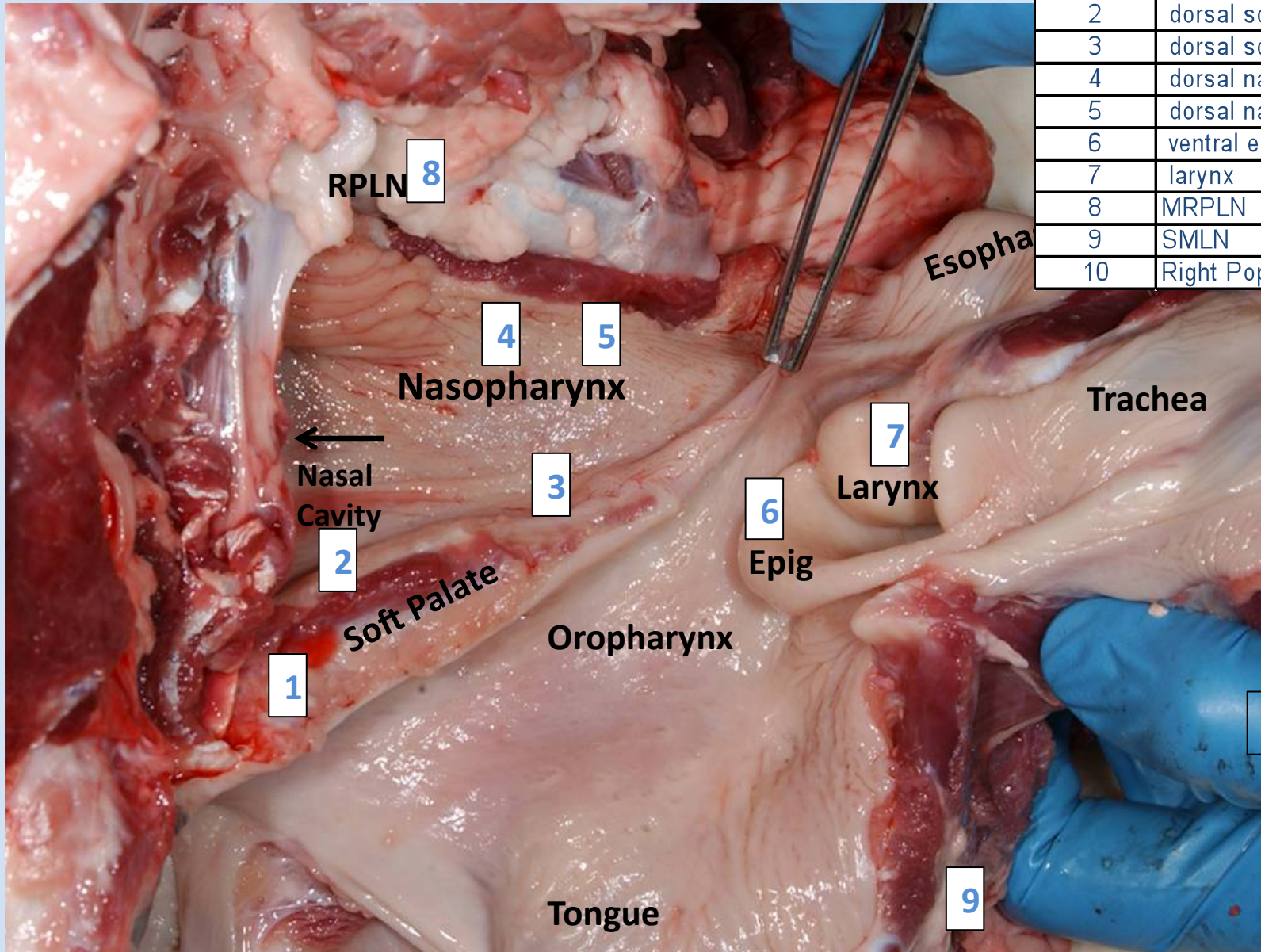


Thank you.



Background (Anatomy)

	Tissue ID
1	palatine tonsil
2	dorsal soft palate 1
3	dorsal soft palate 2
4	dorsal nasopharynx 1
5	dorsal nasopharynx 2
6	ventral epiglottis
7	larynx
8	MRPLN
9	SMLN
10	Right Popliteal LN



(for demonstration; not how the dissection is performed)