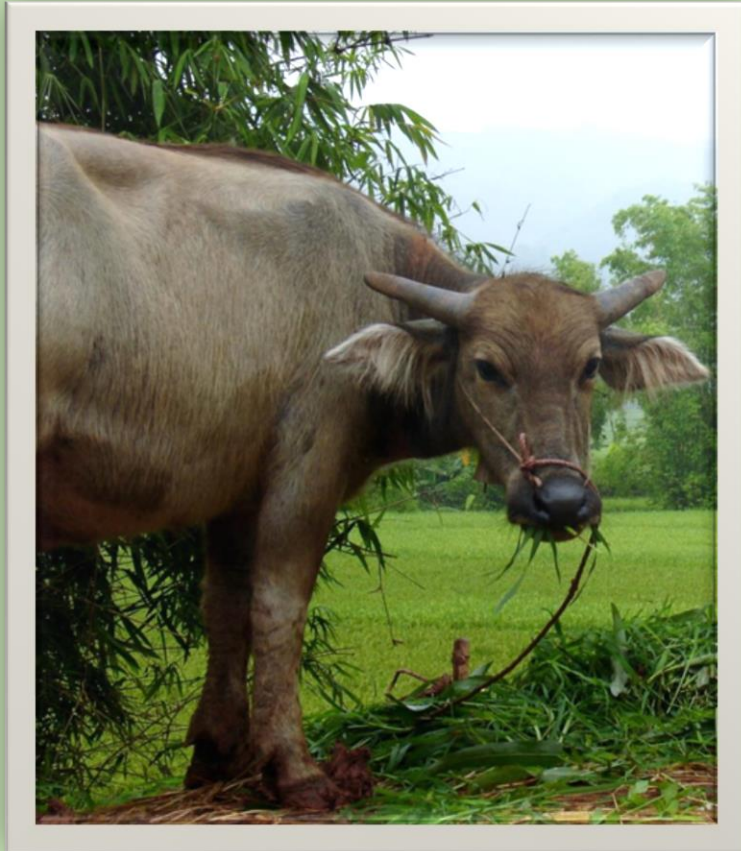
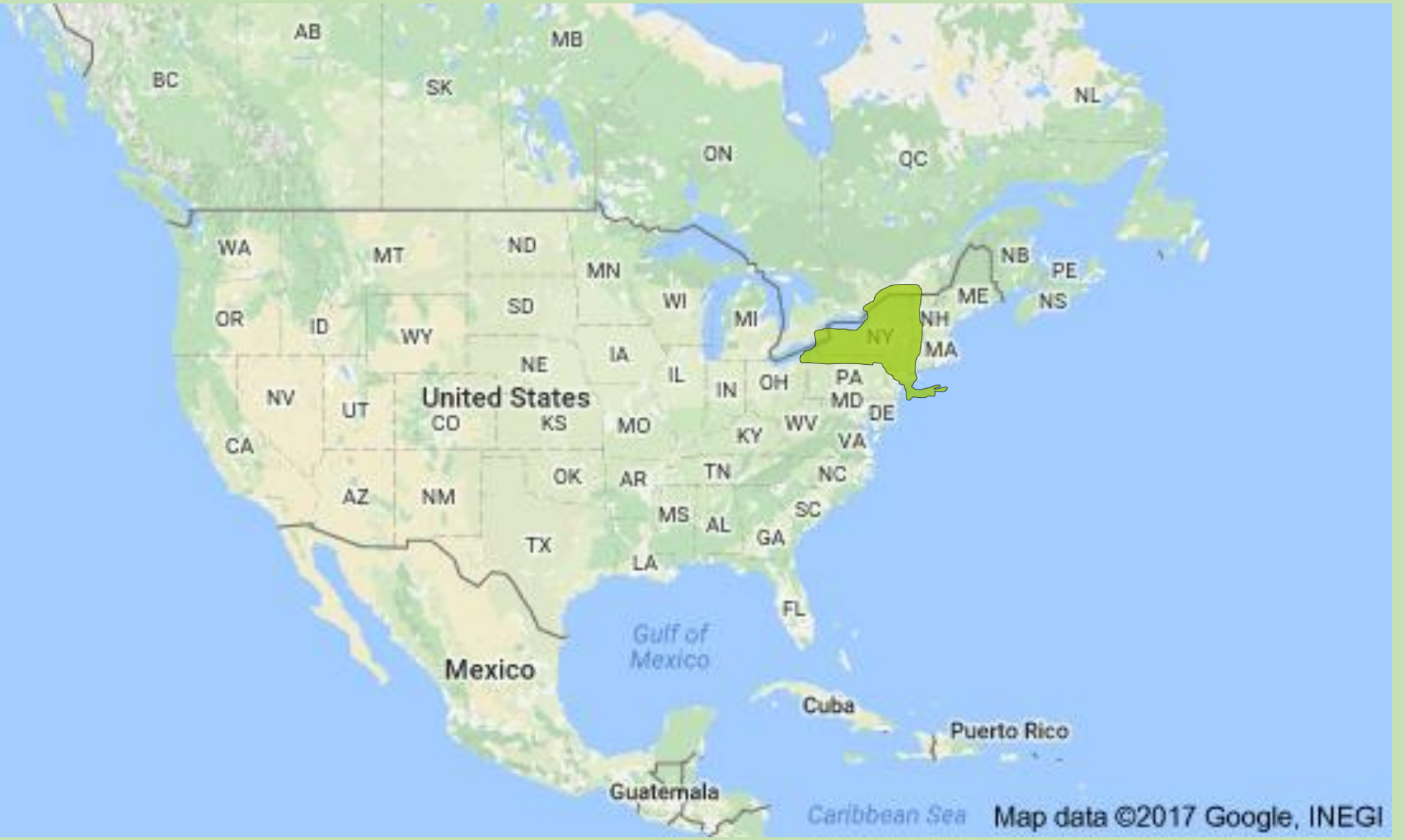


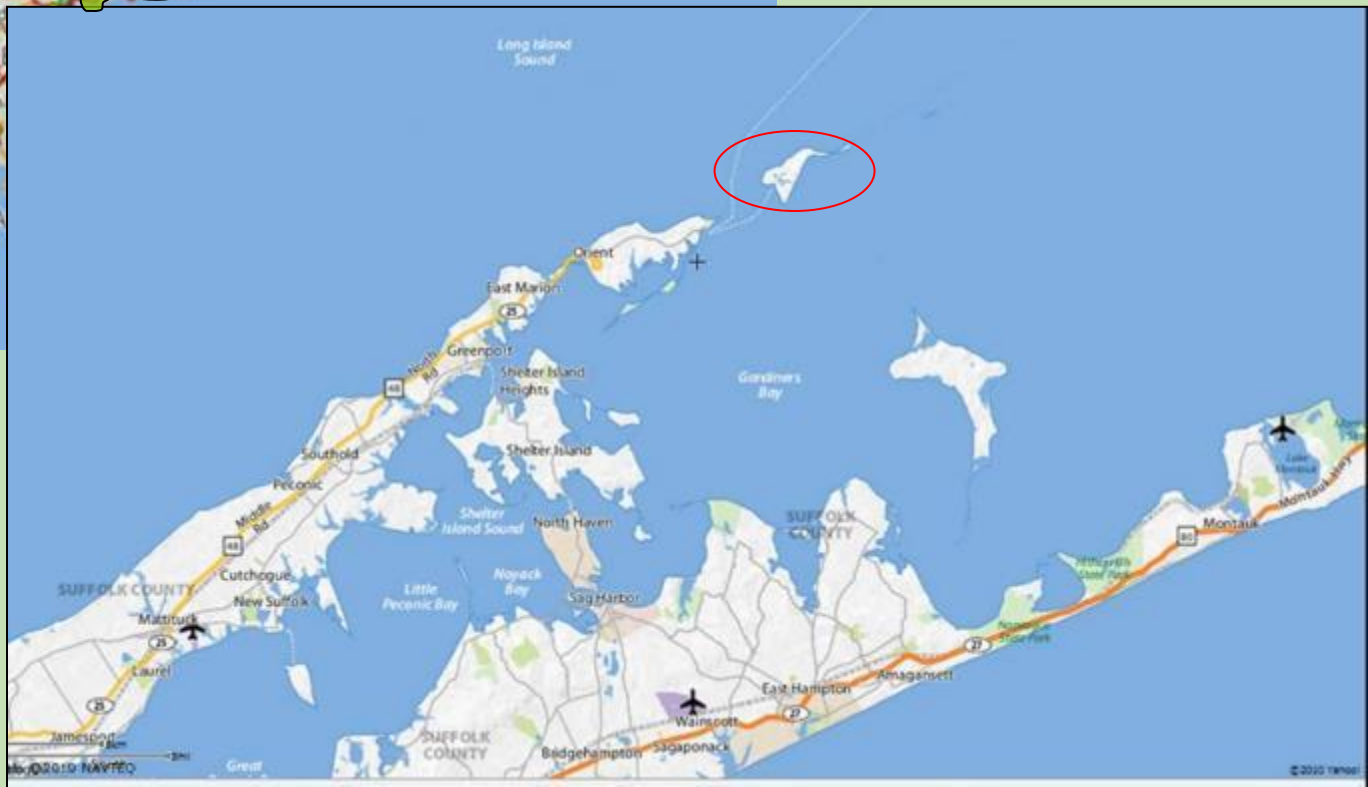
FMDV Research Within the Clinical Studies Group at Foreign Animal Disease Research Unit at Plum Island



Dr. Jonathan Arzt
Plum Island Animal Disease Center
Agricultural Research Service, USDA



Map data ©2017 Google, INEGI



Plum Island Animal Disease Center



Foreign Animal Disease Research Unit (**FADRU**) ARS/USDA
(Distinct from **FADDL** APHIS/USDA (OIE Network Lab))

FMDV Research Within the **Clinical Studies Group** at Foreign Animal Disease Research Unit at Plum Island



FMD Field Studies



FMD Laboratory Experiments

FMDV molecular epidemiology field studies

- Vietnam
- India
- Pakistan
- Kenya
- Cameroon
- Nigeria



ARS/USDA Projects in Vietnam

2 Collaborative projects with DAH/MARD/Vietnam

- Hanoi: NCVD/DAH
- HCMC: RAHO6

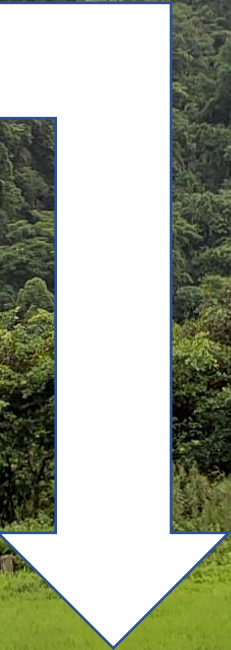
1. 2009-2013 BEP/DOS Project

Molecular epidemiology, surveillance and predictive tools for FMD control in Vietnam

2. 2014-2019 BTRP/DTRA/DOD Project

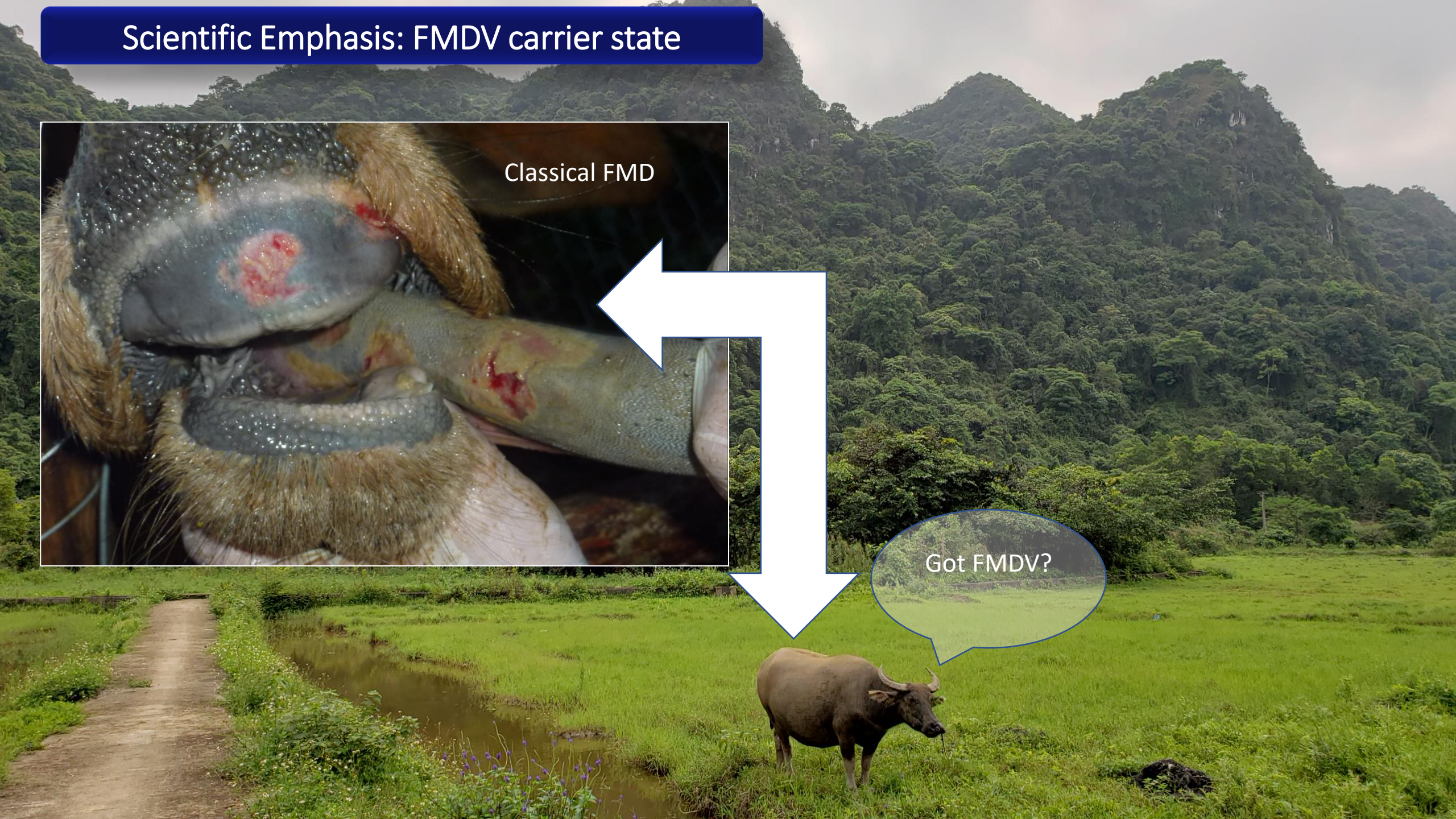
Foot-and-mouth disease surveillance in Vietnam

Scientific Emphasis: FMDV carrier state



Got FMDV?

A blue speech bubble containing the text "Got FMDV?" is positioned near the water buffalo in the field, suggesting a question about the animal's carrier status.



1. Active (targeted) surveillance

- On-farm sampling of asymptomatic Cattle and Buffalo
 - Collect blood (serology) and oropharyngeal fluid (Probang)
 - Longitudinal design (serial sampling farm and individual animal level)
- Slaughterhouse sampling (serial cross-sectional)

2. Passive surveillance (DAH *enhanced* outbreak investigations)

- Outbreaks (all species); Vesicle Epithelium samples

3. Laboratory methods

- Serology (NSP-ELISA)
- Virus detection (rRT-PCR & VI)
- Viral sequencing (Sanger → Illumina NGS → Sanger)



Results: Field Sampling Achieved



Longitudinal (2011-15)

1446
Total Samples



Longitudinal 2015-2019
13 rounds

3505
Total Samples



Slaughterhouse
(2017-2019)
16 rounds

1200
Total Samples



Outbreaks
(2009-2019)

254
Total Samples

Regions (Longitudinal Sampling)

Northern Vietnam (Hanoi, Lang Son, **Son La**, **Bac Kan**, Phu Tho, Ha Tinh)

Southern Vietnam (HCMC, **Long An**, Binh Phuoc, Dak Lak, Dong Thap, **Ninh Thuan**, Tien Giang)

Regions (Slaughterhouse Sampling)

Northern Vietnam (Hanoi (TBD))

Southern Vietnam (**Long An**, Tay Ninh)

Results: Descriptive Epidemiology and Risk Factors for FMDV carrier state

	Infected (%) ^a		Total infected ^c	Never infected (%) ^b	Total sampled
	Carrier	Non-carrier			
Type					
Buffalo	14 (8.0) ^{2,4*}	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
5-14	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)	189 (92.2)	205 (21.9)	730 (78.1)	935
Total	35 (10.8)	288 (89.2)	323 (22.3)	1123 (77.7)	1446

N/A, no information available.

Significant differences ($P < 0.05$) in odds of being 'Carrier' or 'Total infected' between categories (within type and within age) are marked with different superscript numbers, with 1 = higher odds, 2 = intermediate odds, 3 = lower odds.

^a'Infected' = 'Total Infected' = (3ABC positive), with subsets: 'Carrier' = (3ABC positive, probang positive), 'Non-carrier' = (3ABC positive, probang negative).

^b'Never infected' = (3ABC negative).

^cPercentages in 'Total infected' and in 'Never infected' are relative to 'Total sampled'.

^dEach data cell indicates number of animals (percentage of animals per total in corresponding row).

^ePercentages in 'Carrier' and 'Non-carrier' are relative to 'Total infected'.

Table 3. Odds ratio for each variable associated with 'infected' farm status

Predicting variables	n ^a	OR of 'infected' ^b (95% CI)	P-value
Farm capacity			
1-5 animals ^c	30	0.05 (0.0; 0.72)	0.028*
6-12 animals	27	95.03 (2.4; 1262.91)	0.012*
>13 animals	21	42.38 (2.02; 887.8)	0.016*
Purpose			
Purpose = Milk?	56	0.05 (0.0; 0.81)	0.035*
Purpose = Breed?	52	3.76 (0.75; 18.75)	0.106
Date of last infection			
No infection	45	1.74 (0.28; 10.93)	0.556
Infected in 2010	19	152.74 (7.45; 3132.75)	0.001*
Infected in 2011	6	0.95 (0.05; 17.67)	0.973

OR, odds ratio; CI, confidence interval; N/A, no information available.

*Significant effect ($P < 0.05$).

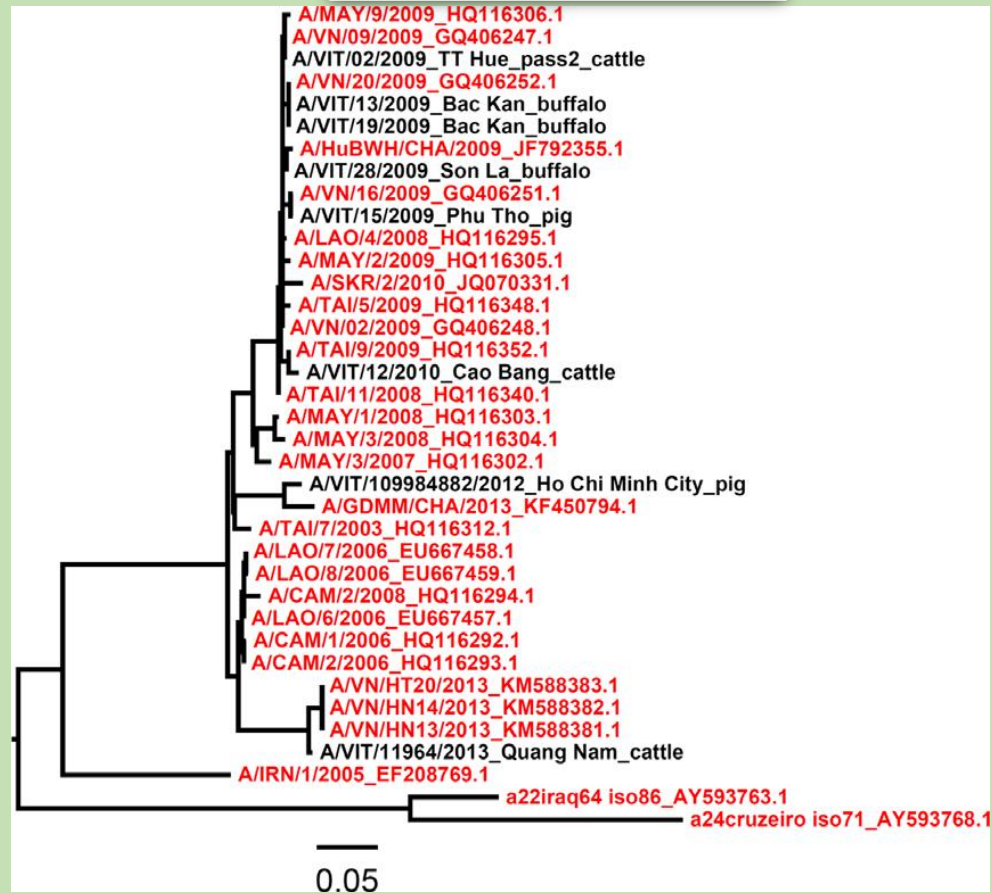
^aTotal number of farms = 78.

^b'Infected' = seropositive on 3ABC ELISA.

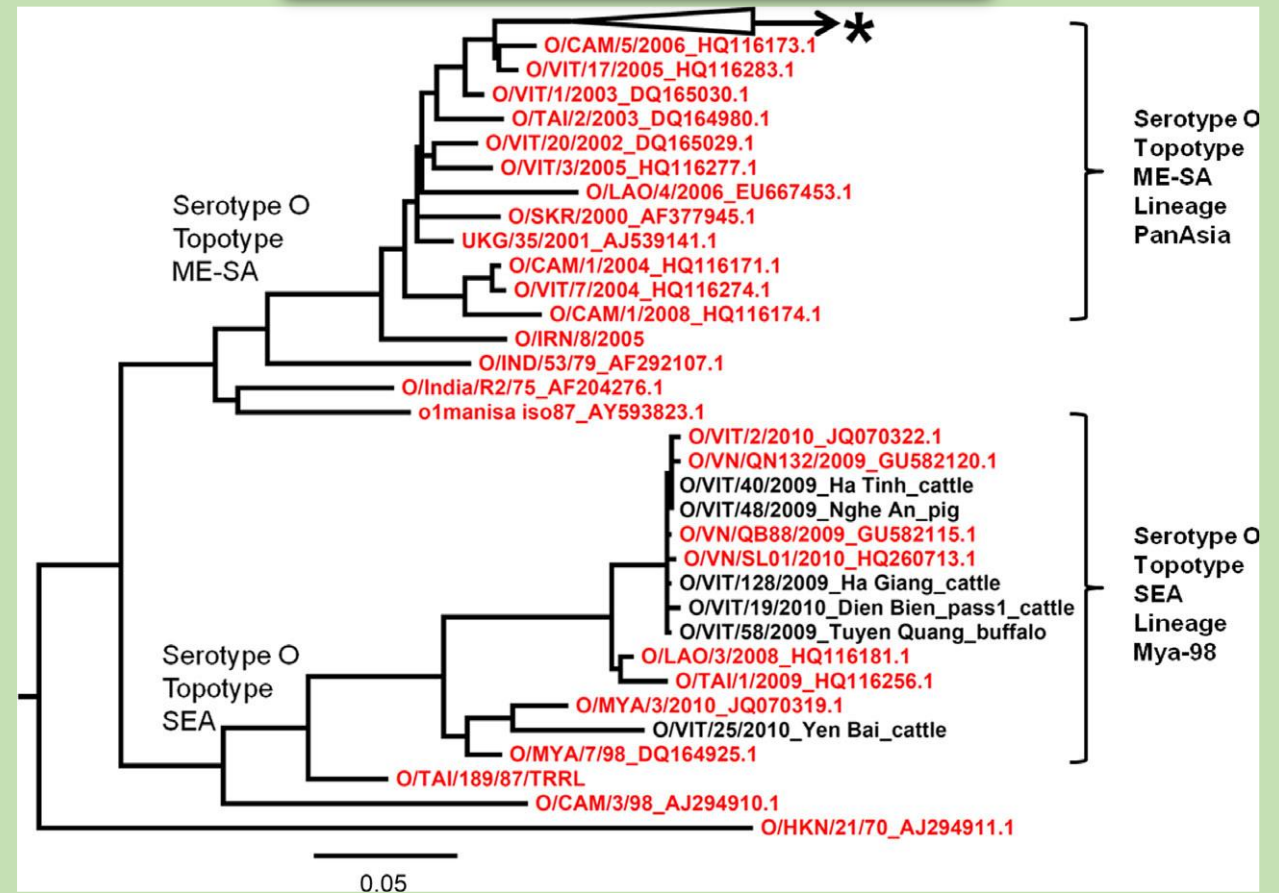
2.4% overall prevalence of subclinical FMDV infection

Results: 2009-2013 Viruses, VP1s

Serotype A/SEA-97



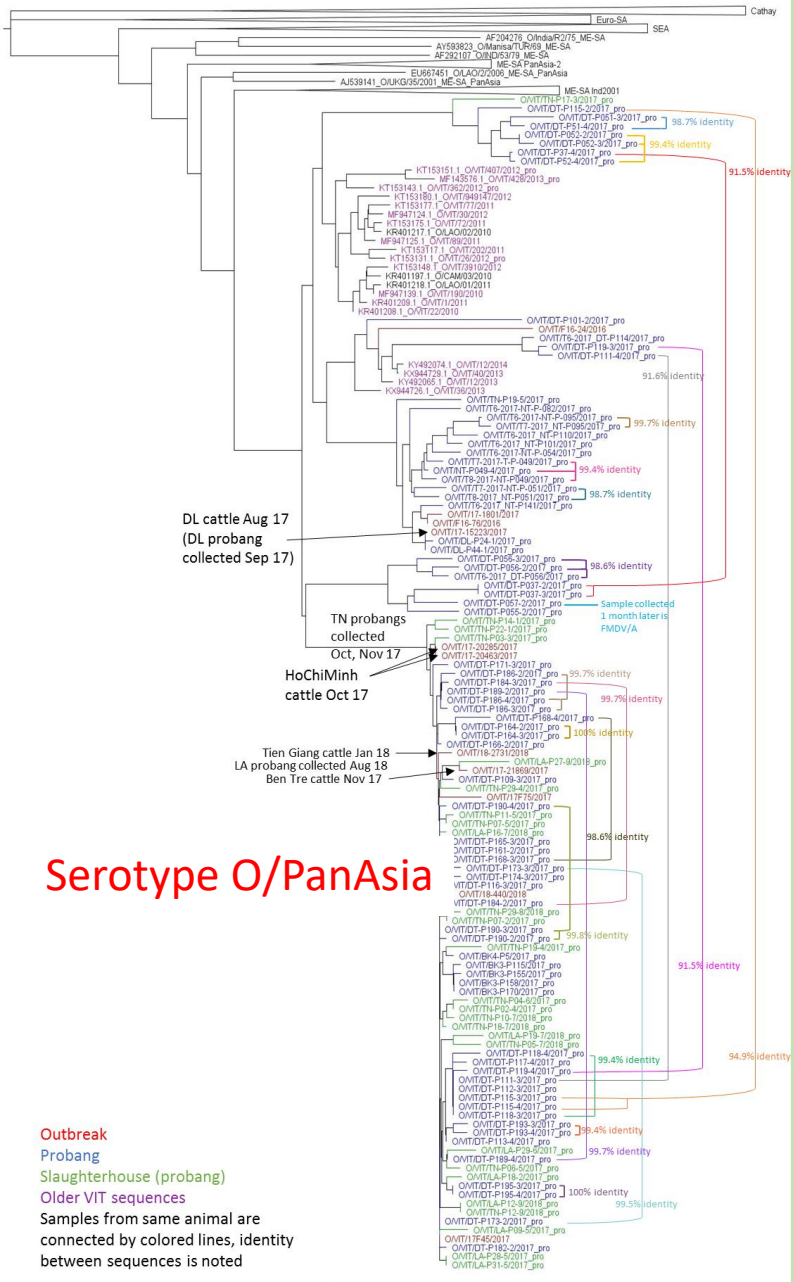
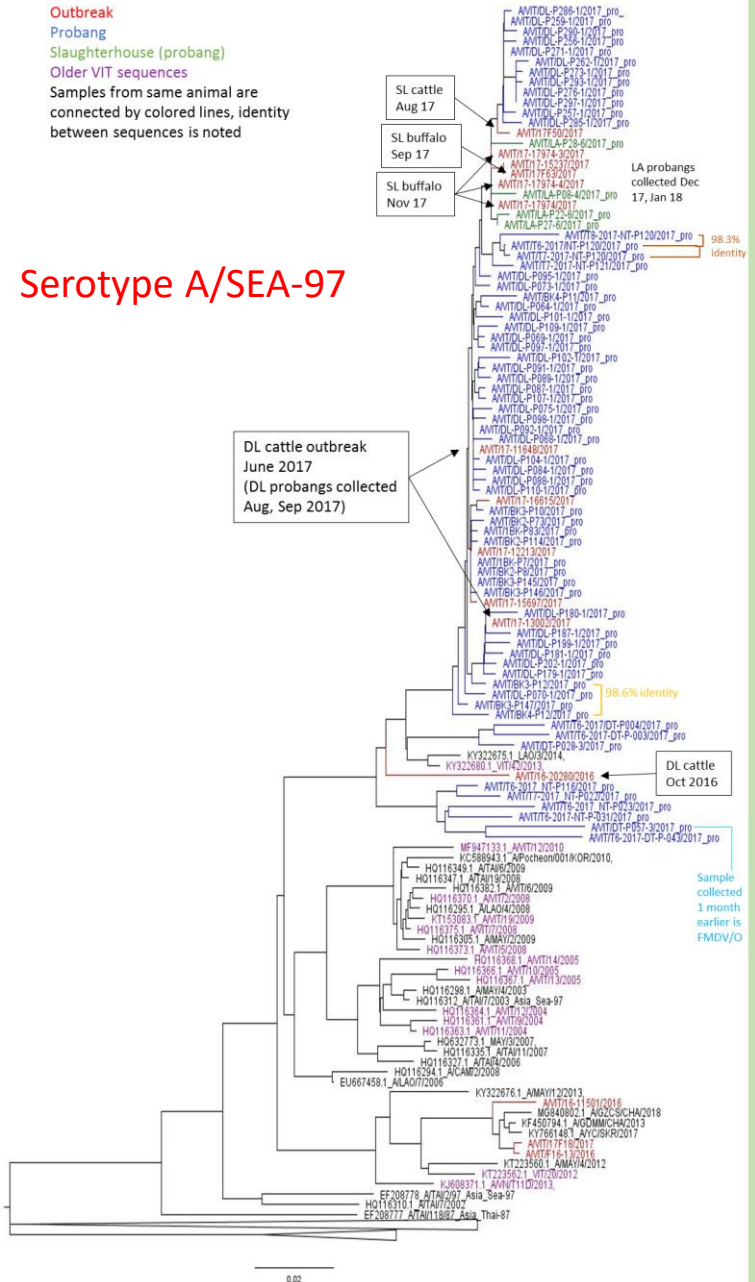
Serotype O/PanAsia & MYA98



Results: 2016-2017 Viruses, VP1s

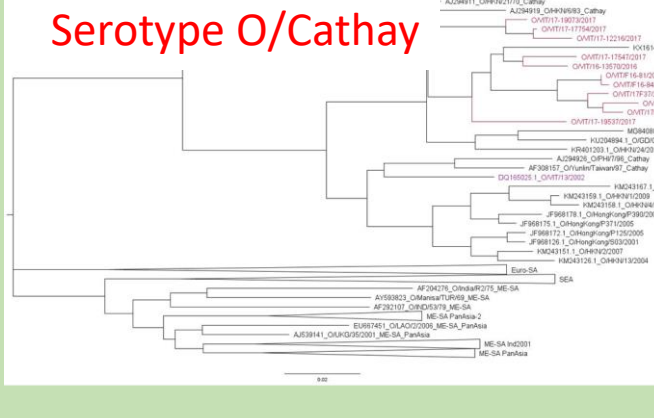
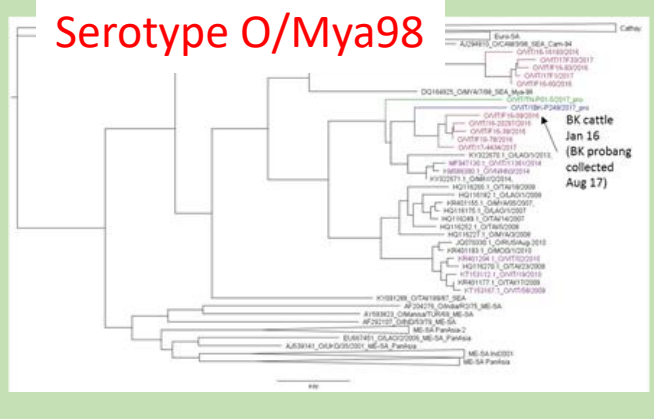
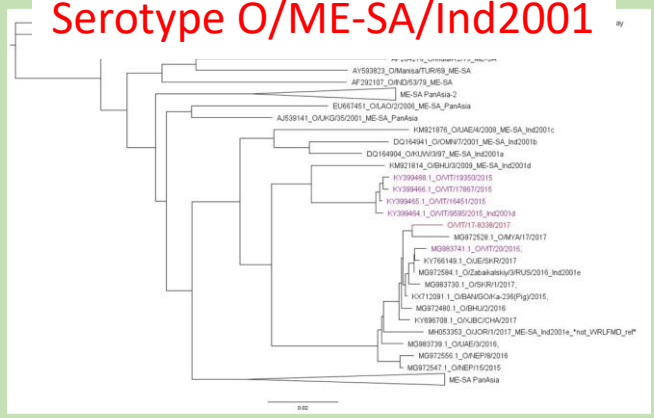
- Outbreak**
- Probang**
- Slaughterhouse (probang)**
- Older VIT sequences**
- Samples from same animal are connected by colored lines, identity between sequences is noted

Serotype A/SEA-97



Serotype O/PanAsia

- Outbreak**
- Probang**
- Slaughterhouse (probang)**
- Older VIT sequences**
- Samples from same animal are connected by colored lines, identity between sequences is noted



Serotype O/Cathay

Results: full genome discovery / analyses

Genome Sequence of Foot-and-Mouth Disease Virus Serotype O Lineage Ind-2001d Collected in Vietnam in 2015

Jonathan Arzt,^a Barbara Brito,^{a,b} Steven J. Pauszek,^a Ethan J. Hartwig,^a George R. Smoliga,^a Le T. Vu,^a Pham P. Vu,^a Carolina Stanfeldt,^{a,b} Luis L. Rodriguez,^a Ngo T. Long,^a Do H. Dung^a

^aDivision of Animal Disease Research Unit, Plum Island Animal Disease Center, APIS, USDA, Central Islip, New York

First Genome Sequence of Foot-and-Mouth Disease Virus Serotype O Sublineage Ind2001e from Southern Vietnam

Rachel M. Pallinski,^{a,d} Miranda R. Bertram,^{a,b} Le T. Vu,^a Steven J. Pauszek,^a Ethan J. Hartwig,^a George R. Smoliga,^a Carolina Stanfeldt,^{a,e} Ian H. Fish,^{a,b} Bui H. Hoang,^a Nguyen T. Phuong,^a Vo V. Hung,^a Pham P. Vu,^a Nguyen K. Dung,^a Pham V. Dong,^f Nguyen N. Tien,^f Nguyen D. Tho,^f Do H. Dung,^g Jonathan Arzt^a

First Detection and Genome Sequence of Senecavirus A in Vietnam

Jonathan Arzt,^a Miranda R. Bertram,^{a,b} Le T. Vu,^a Steven J. Pauszek,^a Ethan J. Hartwig,^a George R. Smoliga,^a Rachel Pallinski,^{a,d} Carolina Stanfeldt,^{a,e} Ian H. Fish,^{a,b} Bui H. Hoang,^a Nguyen T. Phuong,^a Vo V. Hung,^a Pham P. Vu,^a Nguyen K. Dung,^a Pham V. Dong,^f Nguyen N. Tien,^f Do H. Dung^g

Genome Sequences of Seven Foot-and-Mouth Disease Virus Isolates Collected from Serial Samples from One Persistently Infected Carrier Cow in Vietnam

Steven J. Pauszek,^a Miranda R. Bertram,^{a,b} Le T. Vu,^a Ethan J. Hartwig,^a George R. Smoliga,^a Barbara Brito,^{a,b} Carolina Stanfeldt,^{a,e} Kimberley VanderWaal,^c Ian H. Fish,^{a,b} Vo V. Hung,^d Nguyen T. Phuong,^d Bui H. Hoang,^d Luis L. Rodriguez,^a Do H. Dung,^a Jonathan Arzt^a

Genome announcement (submitted) O-Cathay FLS
2019

Results: Phylodynamics 2009-2013 Viruses, VP1s

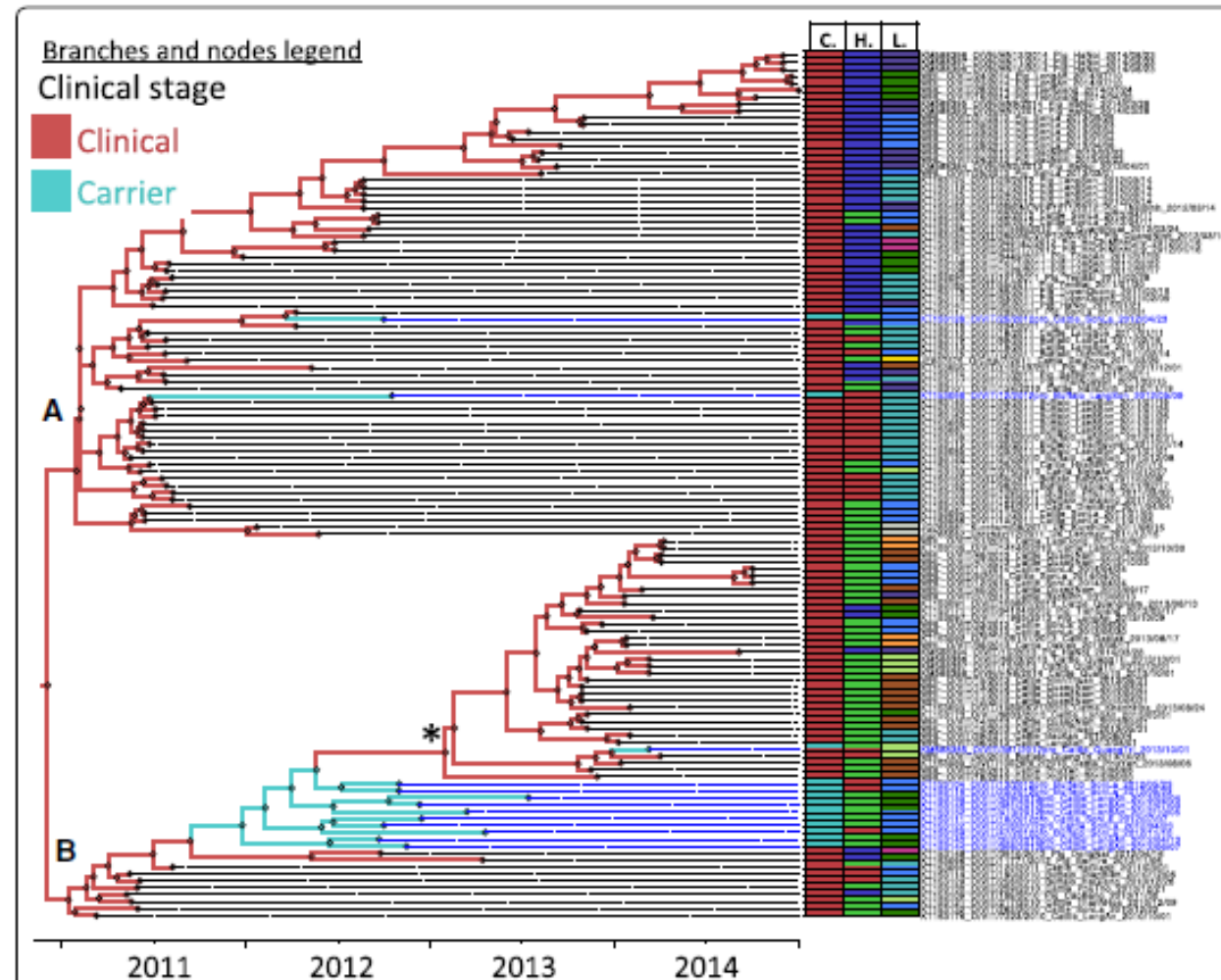


Figure 2 Maximum clade credibility FMDV O/ME-SA/PanAsia viruses collected in Vietnam (and additional 3 sequences from China and Kazakhstan) between 2010 and 2014. Nodes and branches of the trees are colored according to the clinical stage reconstructed in the phylogeny. The ancestral reconstruction of the viruses analyzed suggests 1 instance where outbreak viruses may have originated from carriers (*). Clades **A** and **B** represent the two main O/ME-SA/PanAsia sublineages that have diverged recently in Vietnam. Characteristics of the sampled viruses (clinical stage: C, host species: H, and location: L) are indicated in colored columns aligned to the right of the tree (color coding legend for the columns is indicated in Figure 1).

RESEARCH ARTICLE Open Access

Phylodynamics of foot-and-mouth disease virus O/PanAsia in Vietnam 2010–2014

Barbara Brito^{1,2}, Steven J. Paauze¹, Michael Eschbaumer^{1,2,3}, Carolina Stenfeldt^{1,2}, Helena C. de Carvalho Ferreira^{1,2}, Le T. Wu¹, Nguyen T. Phuong¹, Bui H. Hoang¹, Nguyen D. Tho¹, Pham V. Dong¹, Phan Q. Minh¹, Ngo T. Long¹, Donald P. King¹, Nick J. Knowles¹, Do H. Dung¹, Luis L. Rodriguez¹ and Jonathan Arzt¹

Results

Phylogenetics (Viral Movement Pathways)

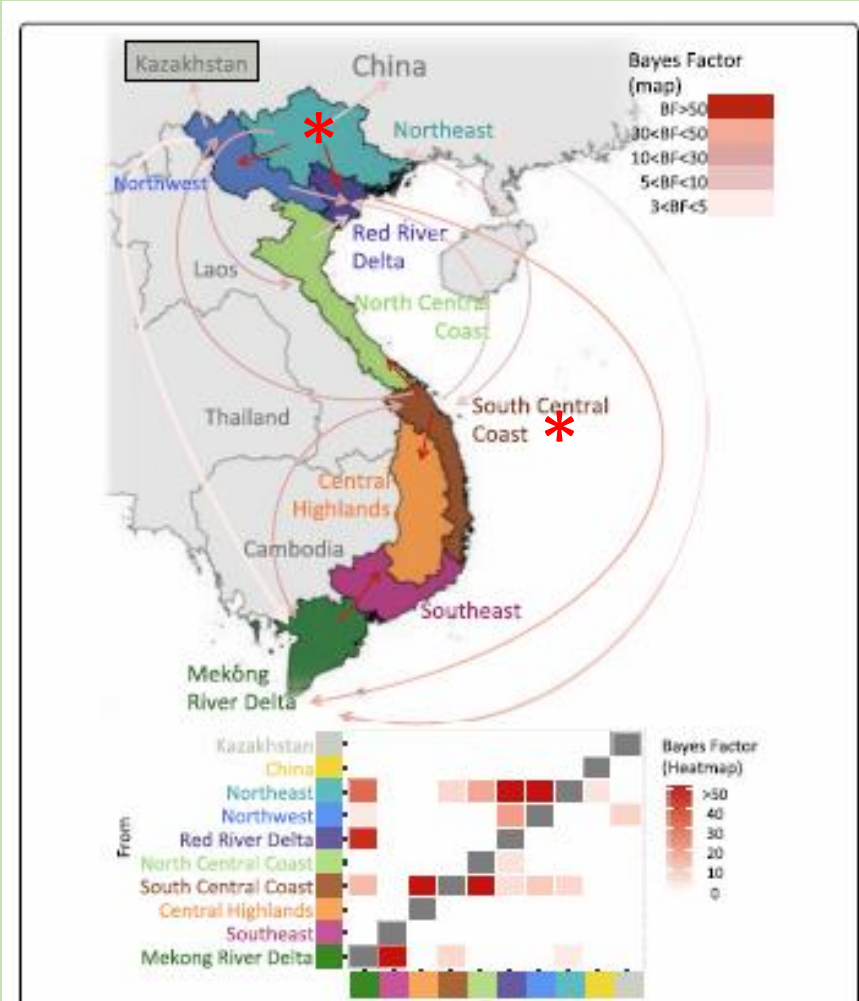


Figure 4 Results from the Bayesian stochastic reconstruction of the transmission pathways of FMDV in Vietnam, and related sequences in Vietnam regions, and related sequences in other regions. The color scale indicates the Bayes Factor (BF) for transmission to occur from South Central Coast and the other regions. Incoming transmission from other regions is indicated by red arrows.

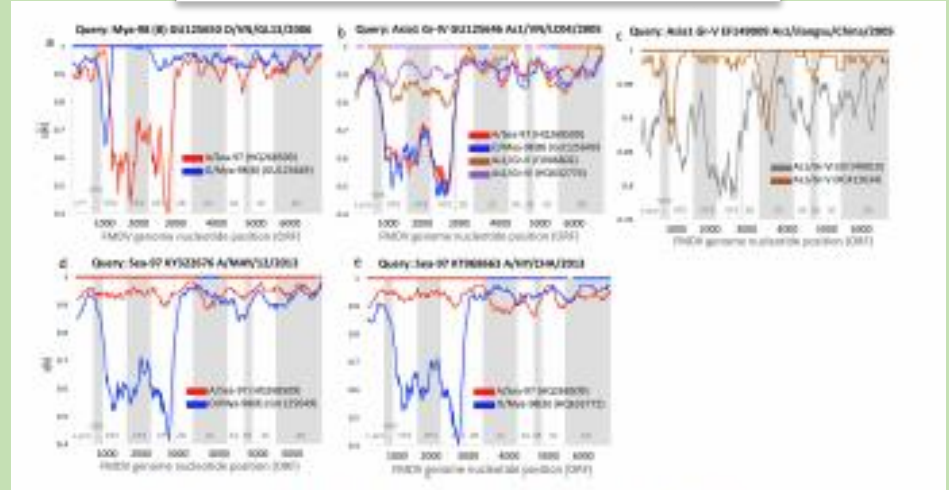
RESEARCH ARTICLE [Open Access](#)

Phylogenetics of foot-and-mouth disease virus O/PanAsia in Vietnam 2010–2014

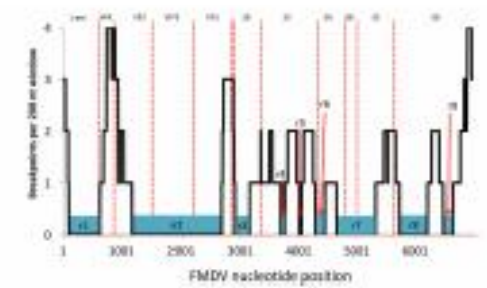
Barbara Brito^{1,2}, Steven J. Paauw^{1,2}, Michael Eschbauer^{1,2,3}, Carolina Steinfeldt^{1,2}, Helena C. de Carvalho Ferreira^{1,2}, Le T. Vu¹, Nguyen T. Phuong¹, Bui H. Hoang¹, Nguyen D. Tho¹, Pham V. Dong¹, Phan Q. Minh¹, Ngo T. Long¹, Donald P. King¹, Nick J. Knowles¹, Do H. Dung¹, Luis L. Rodriguez¹ and Jonathan Arzt¹

SAPanAsia is a significant transmission route for the virus with...

Recombination/Emergence (full-genome sequence)



Intra-lineage and between-lineage recomb.
Unique inter-lineage relationships
(A/Sea-97 & O/Mya-98)



SCIENTIFIC REPORTS

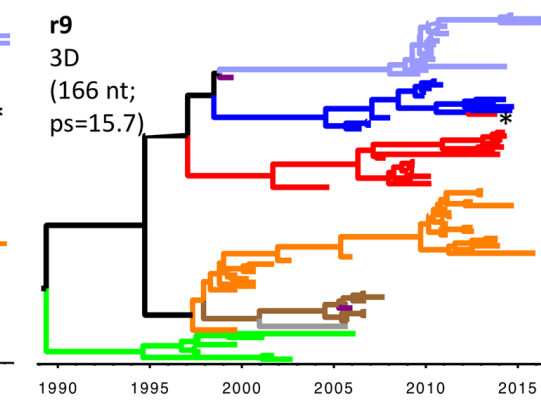
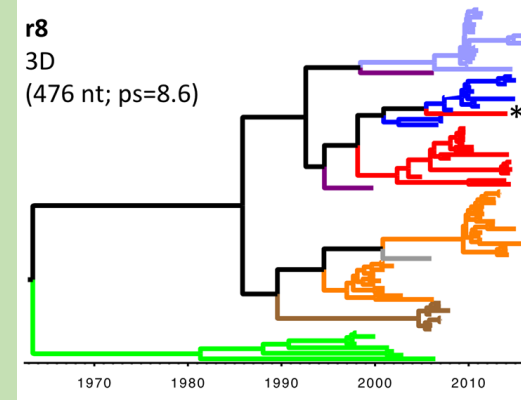
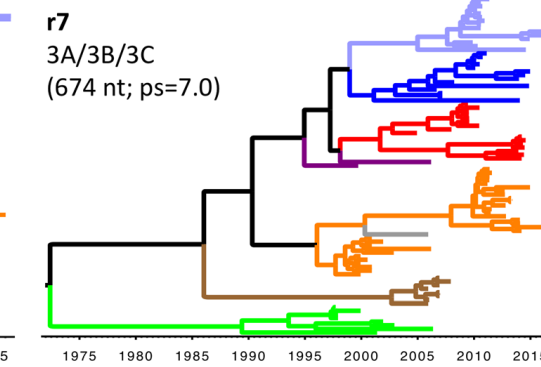
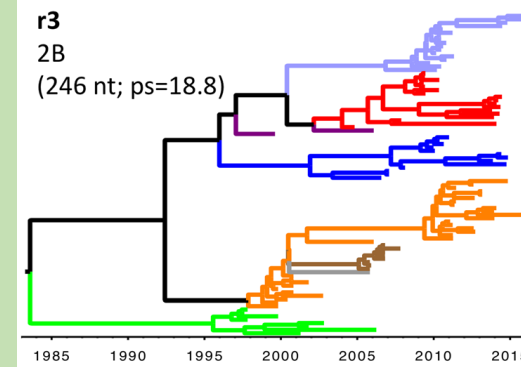
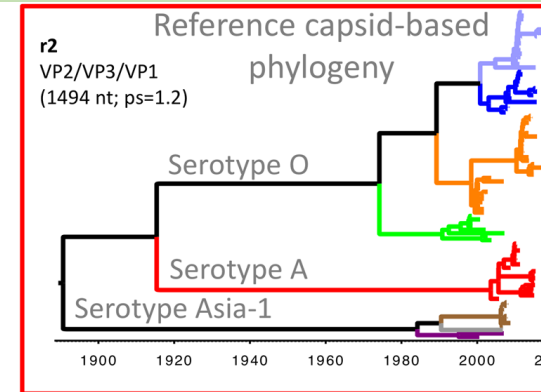
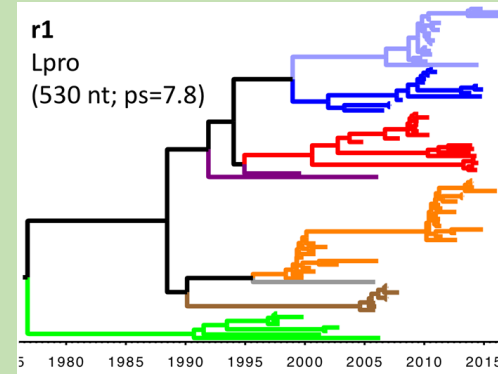
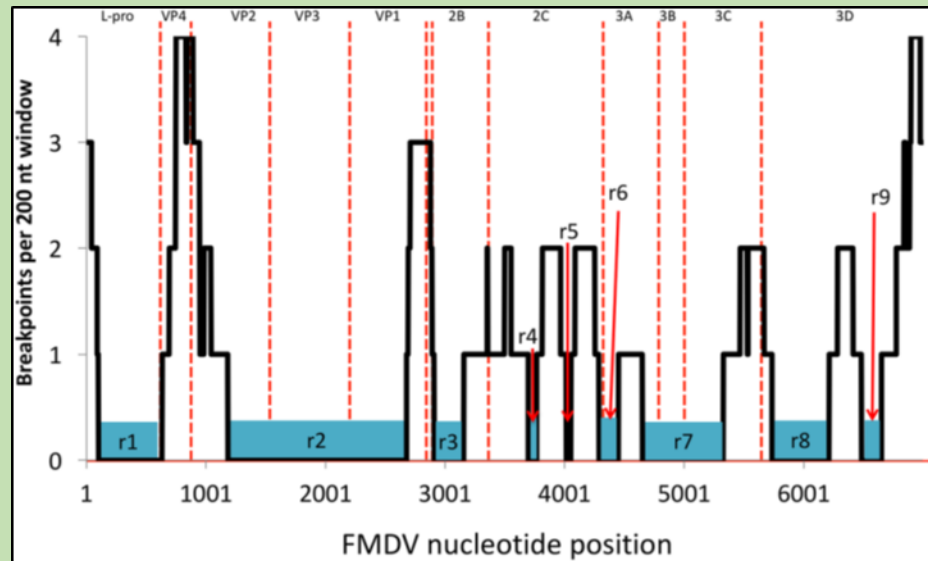
OPEN A traditional evolutionary history of foot-and-mouth disease viruses in Southeast Asia challenged by analyses of non-structural protein coding sequences

23 November 2017
6134 Article
First published: 24 April 2018

Results: Recombination

Event Number	Recombinant detected (lineage; virus name; GenBank accession number)	Description of recombination: protein coding region (nucleotide position in alignment of the breakpoints detected), lineage from which parental recombinant was inferred	Methods that detected recombination ($P < 0.05$)	Similarity plots (Fig. 1)
1	O/Mya-98(B); O/VN/GL13/2006; GU125650 (host: cattle)	Recombination at VP4 (731–1081) with lineage A/Sea-97	RDP, GC, BS, CS, CHM, SIS	Fig. 1(a)
2	As1/Gr-IV; As1/VN/LC04/2005; GU125646 (host: buffalo)	Recombination at 3D (6303–6741) with lineage O/Mya-98	RDP, GC, BS, CS, CHM	Fig. 1(b)
3	As1/Gr-IV; As1/VN/LC04/2005; GU125646 (host: buffalo)	Recombination at 2C-3A (3903–4540) with lineage A/Sea-97	RDP, GC, CS, CHM	Fig. 1(b)
4	As1-Gr-V; As1/Jiangsu/China/2005; EF149009 (host: cattle)	Recombination at 2C (3443–3587) with As1/Gr-VI As1/HNK/CHA/05 (#EF149010)	RDP, GC	Fig. 1(c)
5	A/Sea-97; A/MAY/12/2013; KY322676 (host: cattle)	Recombination at 2C (3497–4151) with lineage O/Mya-98	RDP, GC, CS, CHM	Fig. 1(d)
6	A/Sea-97; A/HY/CHA/2013; KT968663 (host: yak)	Recombination at in 2C and 3C-3D with lineage O/Mya-98(B)	Bayesian time divergence estimation	Fig. 1(e)

Results: Recombination



Results: FMDV persistent infection in Vietnam

Carrier State Extinction

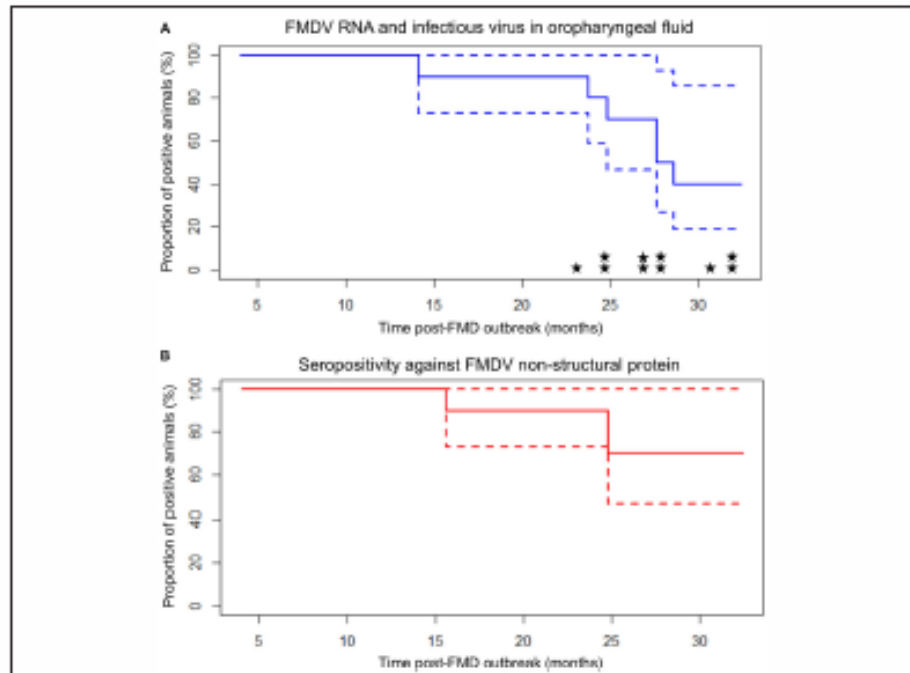
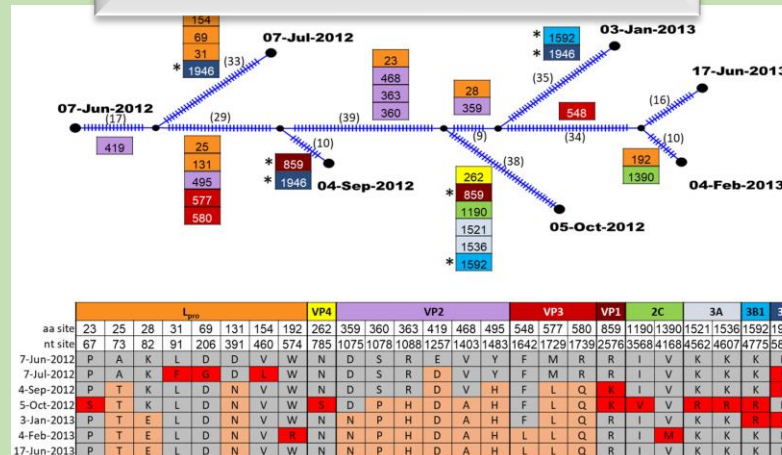


FIGURE 2 | Carrier state extinction curves for 10 cattle persistently infected with FMDV in Long An, Vietnam. Elapsed time (x-axis) is from the midpoint of the reported year of FMD outbreak in each animal's resident herd (9 or 21 months prior to the start of sample collection). Dashed lines represent 95% confidence intervals. **(A)** Probing (CPS) samples. Extinction curve is based upon detection of FMDV RNA in oropharyngeal fluid. Stars represent detection of infectious virus (one star = one positive sample). **(B)** Serum samples screened by anti-FMDV non-structural protein competitive ELISA.

FMDV Transmission from Carriers?: not



Within-host evolution



frontiers
in Veterinary Science

ORIGINAL RESEARCH
published: 27 July 2015
doi: 10.3389/fvets.2015.00174

Lack of Transmission of Foot-and-Mouth Disease Virus From Persistently Infected Cattle to Naïve Cattle Under Field Conditions in Vietnam

OPEN ACCESS

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Luis L. Rodriguez^{1,2}, Ngo T. Long⁴, Do H. Dung⁴ and Jonathan Arzt^{1,2*}

Threat Reduction (non-scientific)



Publications and Presentations

- **Publications**

1. Ferreira et al, **An Integrative Analysis of Foot-and-Mouth Disease Virus Carriers in Vietnam Achieved Through Targeted Surveillance and Molecular Epidemiology**, TBED, 2015
2. Pauszek et al, **Site-specific substitution (Q172R) in the VP1 protein of FMDV isolates collected from asymptomatic carrier ruminants in Vietnam**, Virology Reports, 2016
3. Vu LT, et al. (2017) **First detection of foot-and-mouth disease virus O/Ind-2001d in Vietnam**. PLoS ONE 12(6): e0177361. Brito, et al., A traditional evolutionary history of foot-and-mouth disease viruses in Southeast Asia challenged by analyses of non-structural protein coding sequences. Scientific Reports. 2018 8(1):1-13. doi: 10.1038/s41598-018-24870-6
4. Brito et al. (2017) **Phylogenetics of foot-and-mouth disease virus O/PanAsia in Vietnam 2010–2014**. Vet Res. 48:24 DOI 10.1186/s13567-017-0424-7
5. M Bertram, et al., **Lack of transmission of foot-and-mouth disease virus from persistently infected cattle to naïve cattle under field conditions in Vietnam**. Frontiers in Veterinary Science: Veterinary Infectious Diseases. 2018 5(174). doi:10.3389/fvets.2018.00174
6. Brito et al. (2018) **A traditional evolutionary history of foot-and-mouth disease viruses in Southeast Asia challenged by analyses of non-structural protein coding sequences**. Sci Rep. 2018 Apr 24;8(1):6472. doi: 10.1038/s41598-018-24870-6.
7. J Arzt, et al., (2019) **First detection and genome sequence of Senecavirus A in Vietnam**. MRA-ASM, Jan 17;8(3).
8. Palinski, et al., (2019) **First genome sequence of Foot-and-mouth disease virus serotype O sublineage Ind2001e from Southern Vietnam**. MRA. Mar 7;8(10).
9. J Arzt, et al., **Genome Sequence of Foot-and-Mouth Disease Virus Serotype O Lineage Ind-2001d Collected in Vietnam in 2015** . Genome Announc 5:e00223-17.

- **Presentation**

- GFRA 2015, Dung
- GFRA 2015, Arzt
- GFRA 2015, Stenfeldt
- EUFMD, 2016, Arzt
- EUFMD 2016, Stenfeldt
- DTRA-SPR 2017, Dung
- **Poster**
 - GFRA 2017, Bertram et al
 - GFRA 2017, Vu et al
 - GFRA 2017, Brito
 - VEME 2017, Brito

Next Steps (currently no funding or commitment)

- Continued analyses of existing samples (2009-2019)
- Validation of slaughterhouse sampling surveillance (in progress)
- Meta-analyses merging Vietnam+India+Pakistan data sets on extinction (in progress)
- Continuity into similar FMDV biosurveillance in Vietnam (pending)
- Regional expansion to include Lao PDR for transboundary tracing and further enhance regional capacity (pending)

Acknowledgements

DAH,MARD,Vietnam

Le T. Vu,^c
Bui H. Hoang,^c
Nguyen T. Phuong,^c
Vo V. Hung,^c
Pham P. Vu,^c
Nguyen K. Dung,^c
Pham V. Dong,^f
Nguyen N. Tien,^f
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Ian H. Fish
Anna Ludi

WRLFMD

Don King
Nick Knowles
Anna Ludi

- Do Huu Dung DAH, MARD
- Marty Stokes, BTRP, DTRA, DOD
- Luis Rodriguez ARS, USDA
- Cyril Gay, ARS, USDA



Thank You!

A water buffalo is standing in a lush green field. Above its head is a large blue thought bubble containing the text "Got Questions?". The background features a dense forest of green trees and several large, rounded hills under a cloudy sky. A dirt path is visible on the left side of the field.

Got Questions?