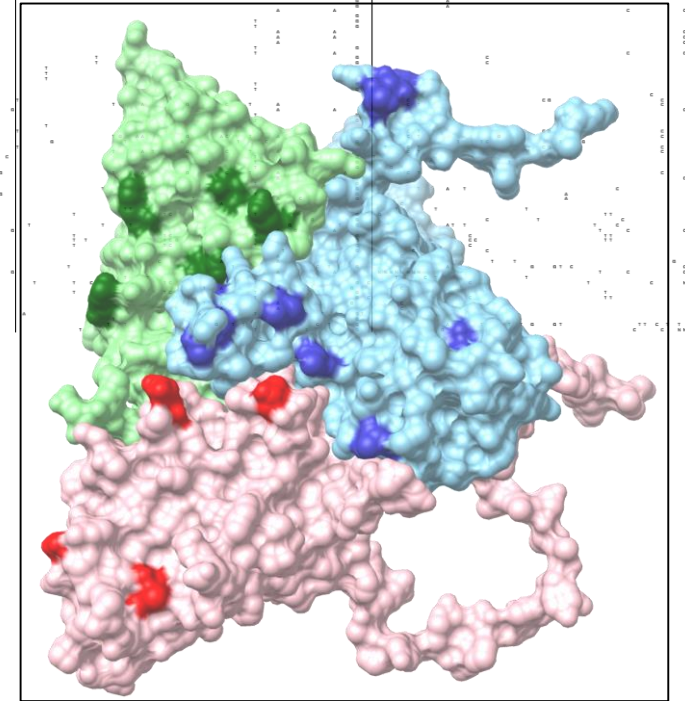
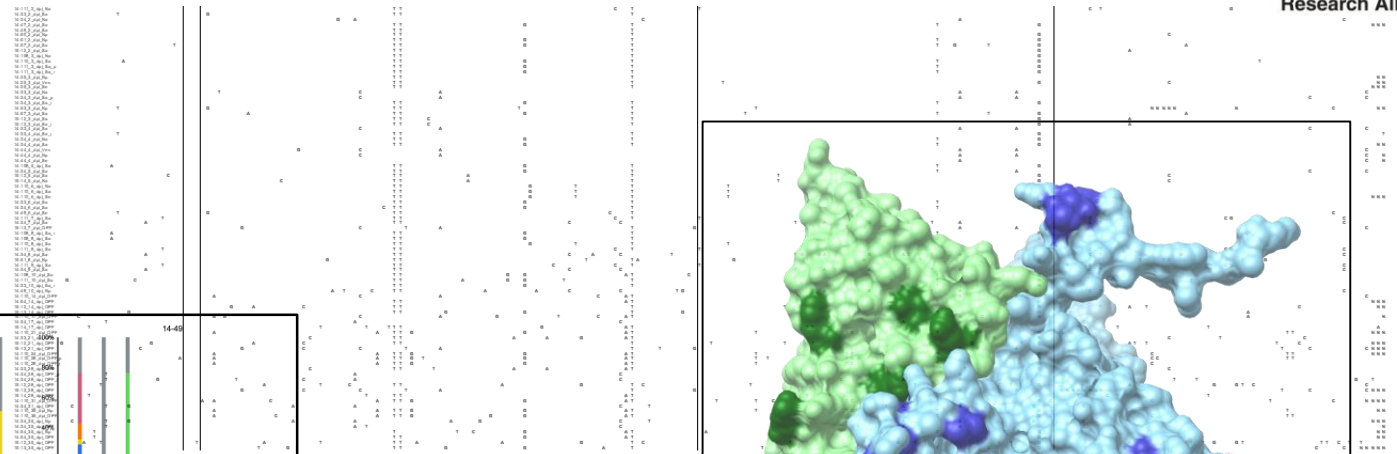
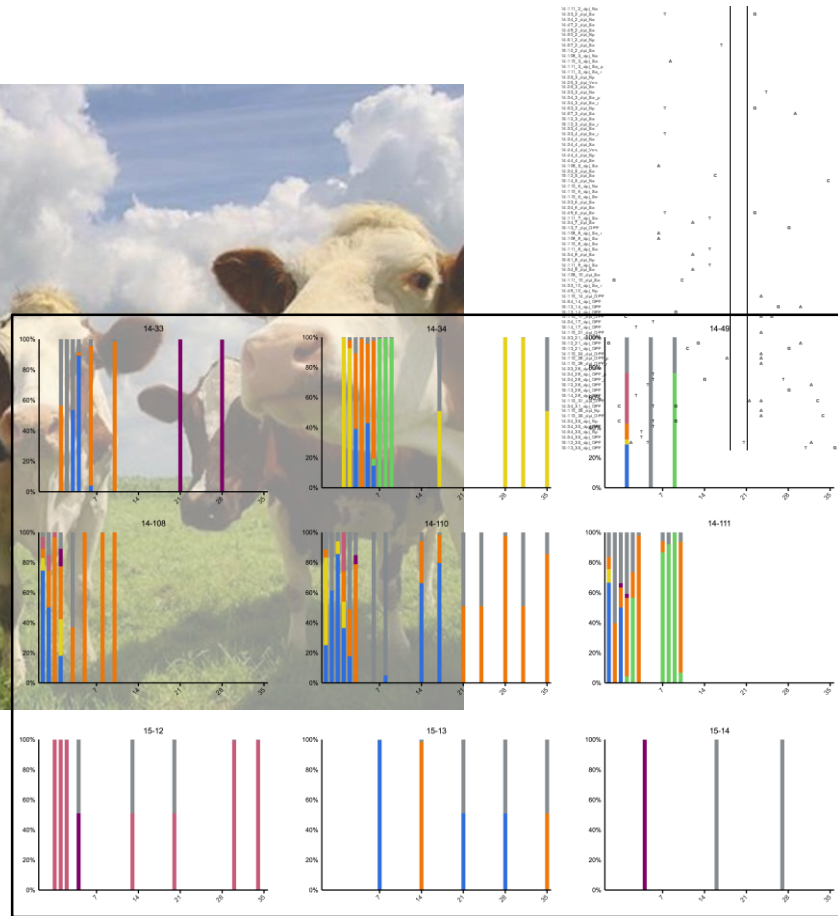
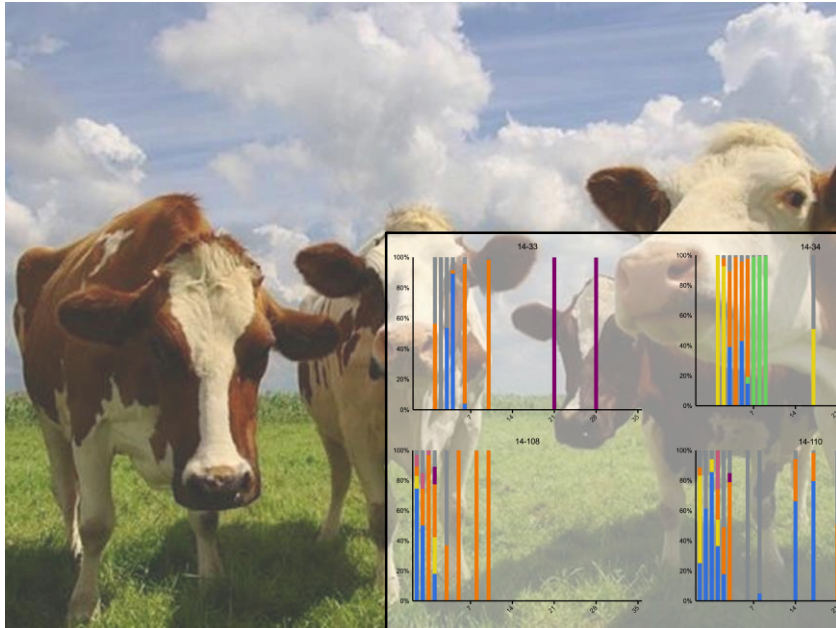


Deep sequencing of FMDV reveals associations between vaccination, population bottlenecks, and adaptation



Ian Fish, PhD
ORISE, ARS/USDA
GFRA 2019



Acknowledgements & Source Studies

Arzt-lab

*Jonathan Arzt

*Carolina Stenfeldt

Michael Eschbaumer

Steve Pauszek

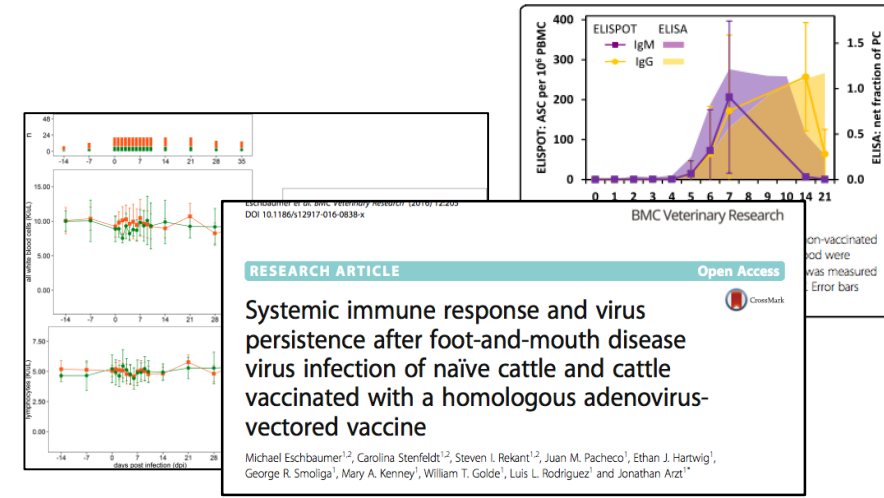
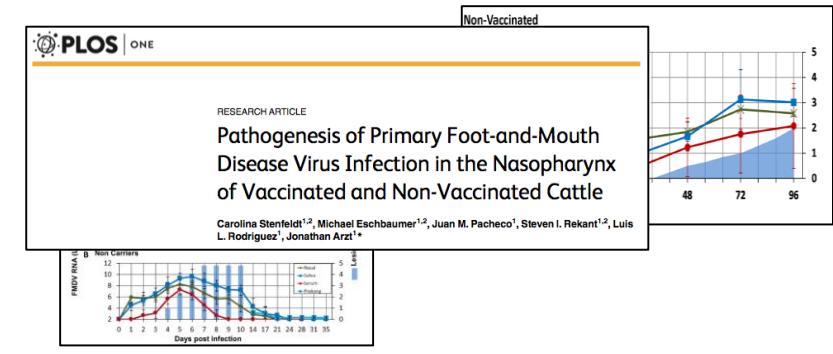
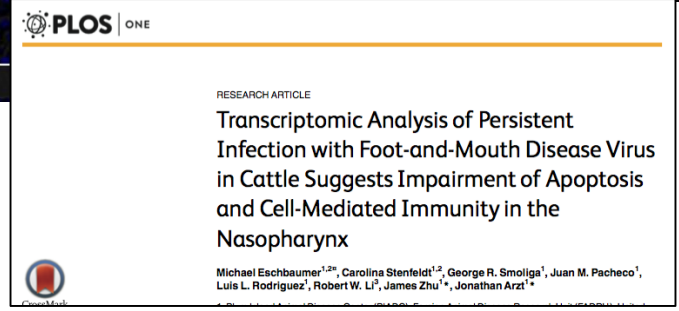
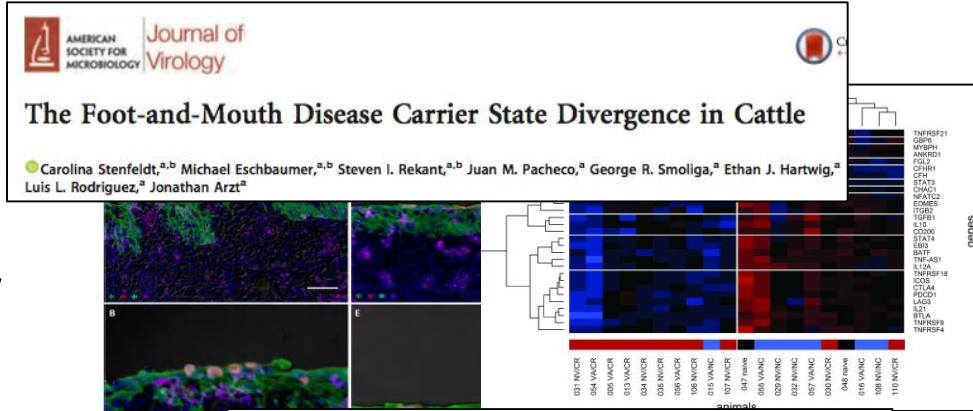
George Smoliga

Ethan Hartwig

Miranda Bertram

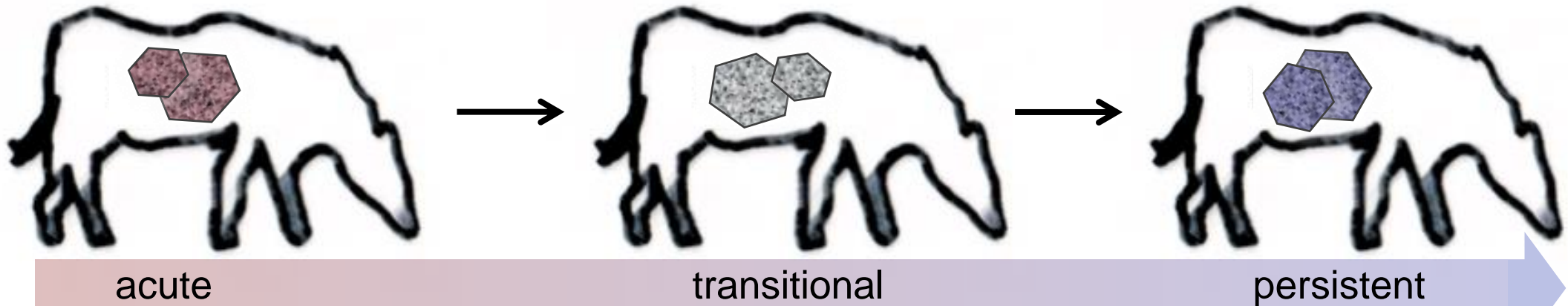
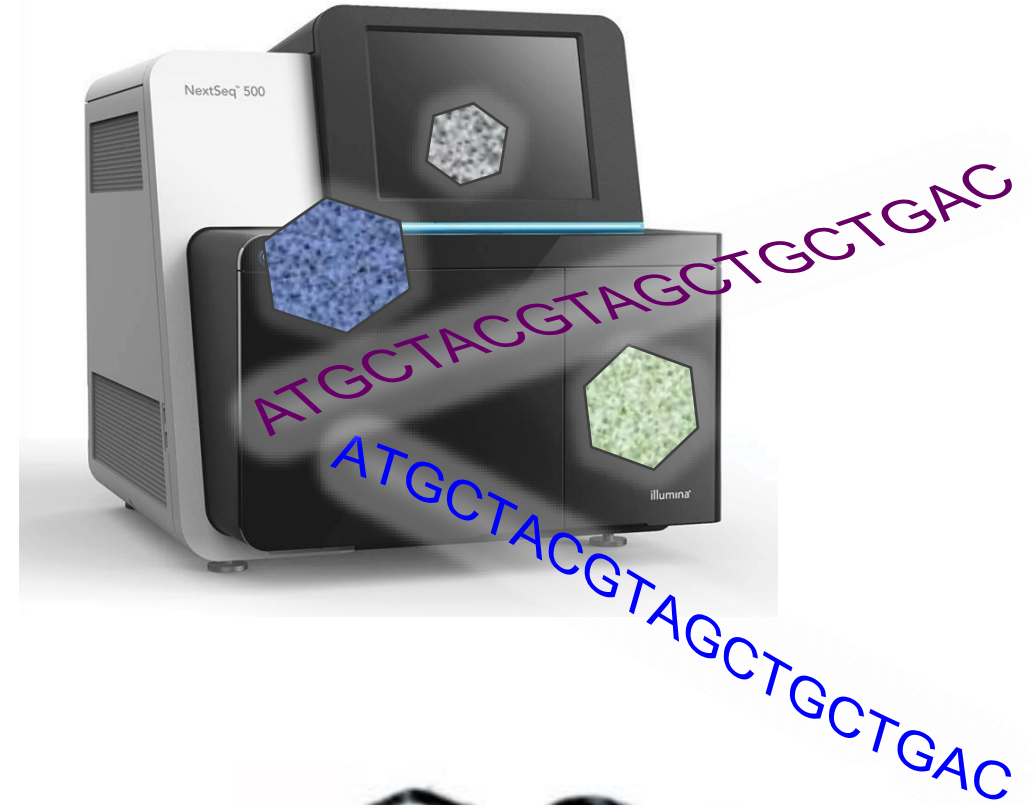
PIADC ARS Leader

Luis Rodriguez



Background: The FMDV Intra-host Population

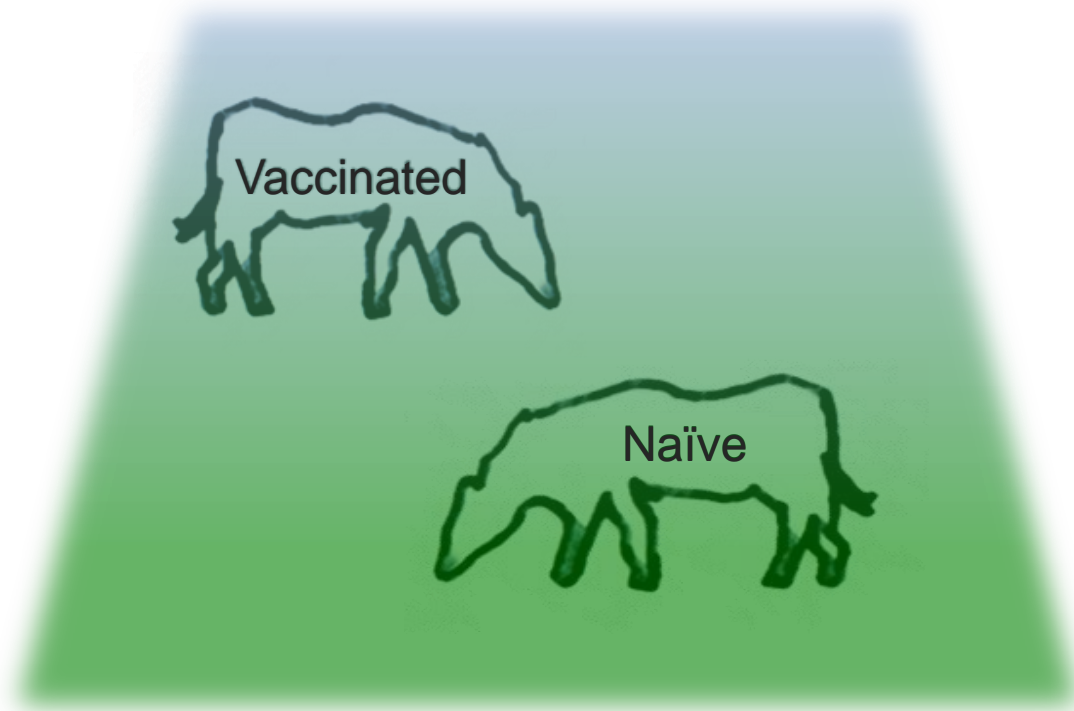
- ☆ FMDV exists as a diverse mutant swarm
- ☆ Illumina deep sequencing provides a powerful tool to investigate the FMDV population changing over time



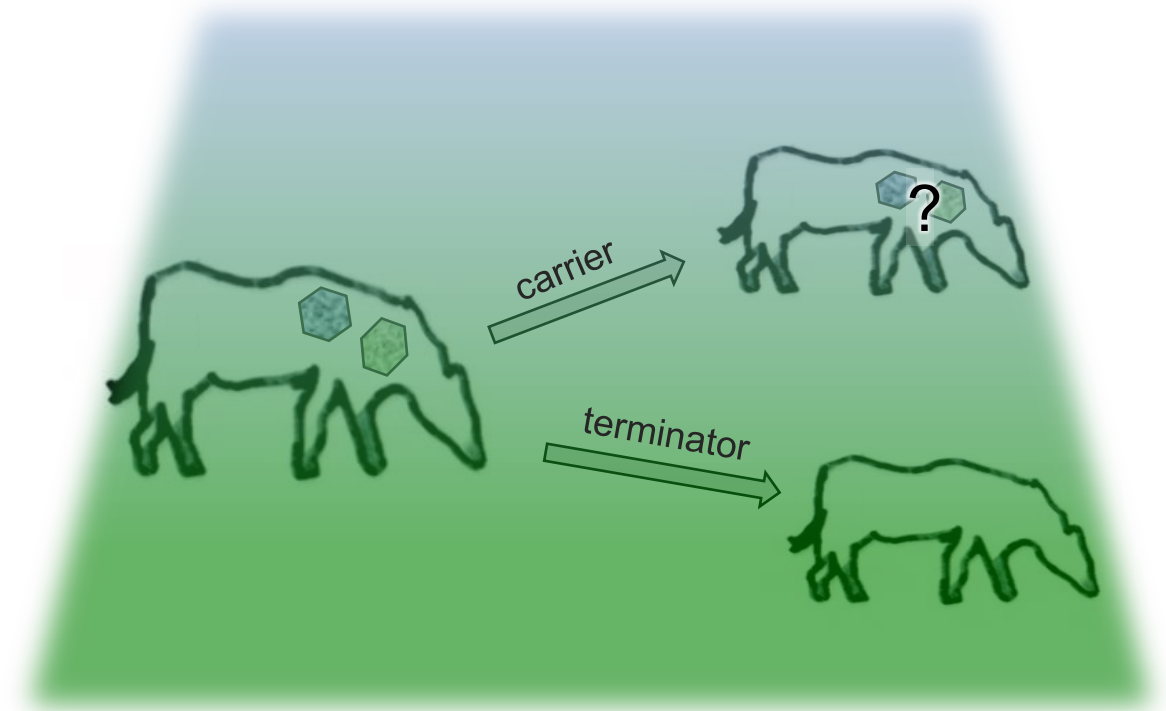
Background: Aims of Study

In describing how the FMDV population changes over time, two important questions:

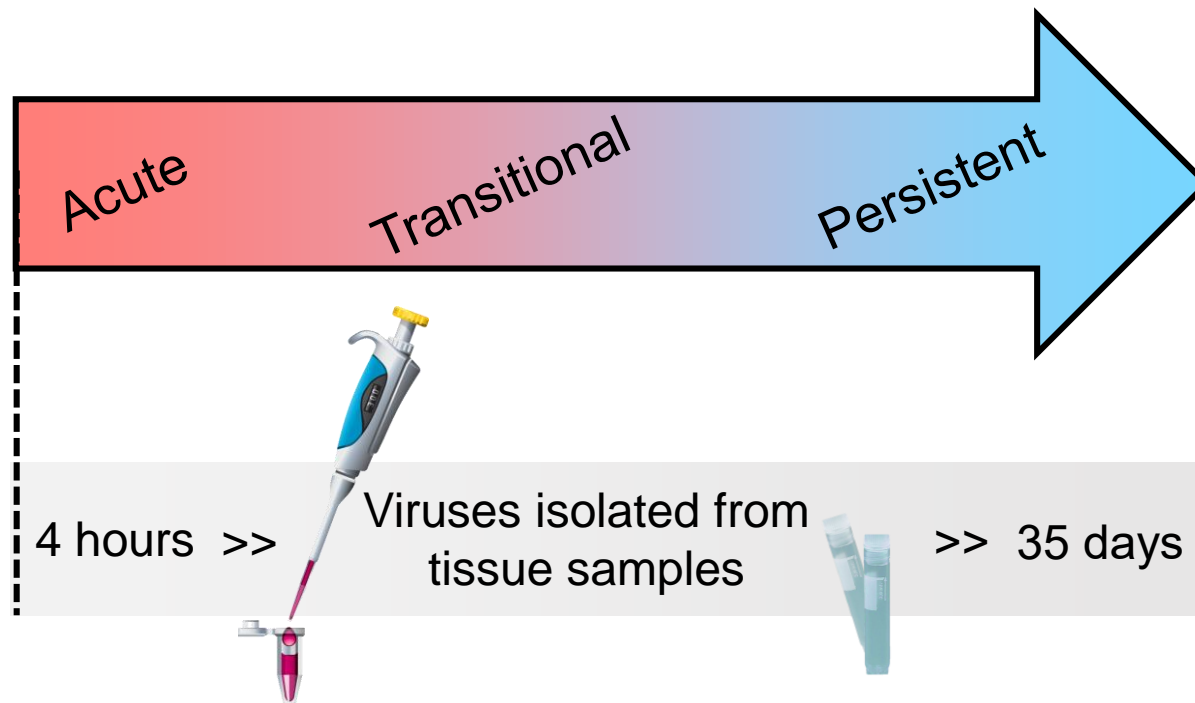
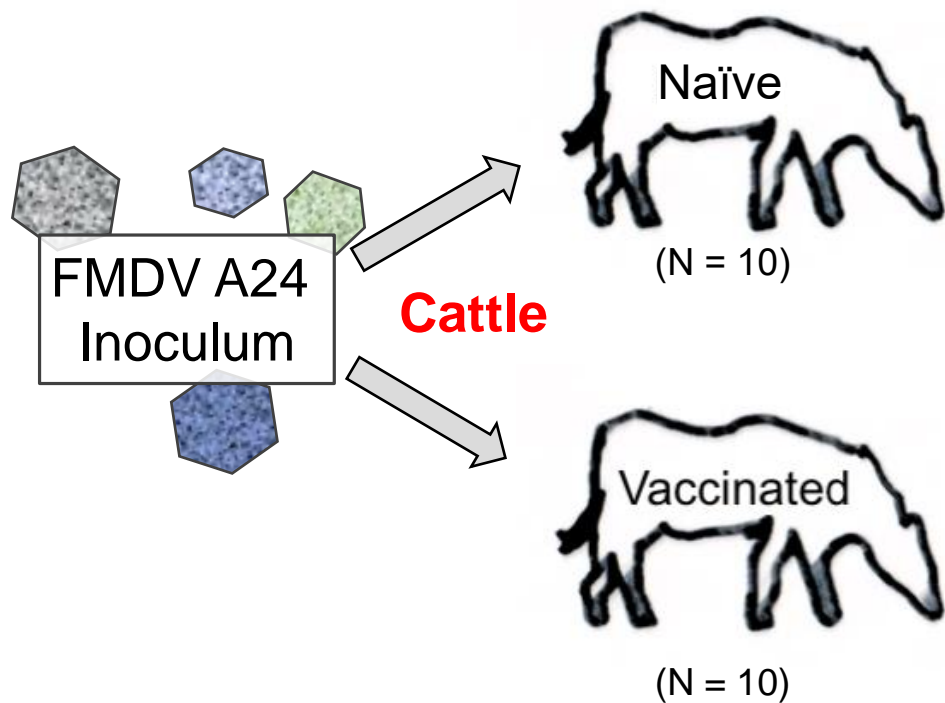
☆ What are the effects of vaccination?



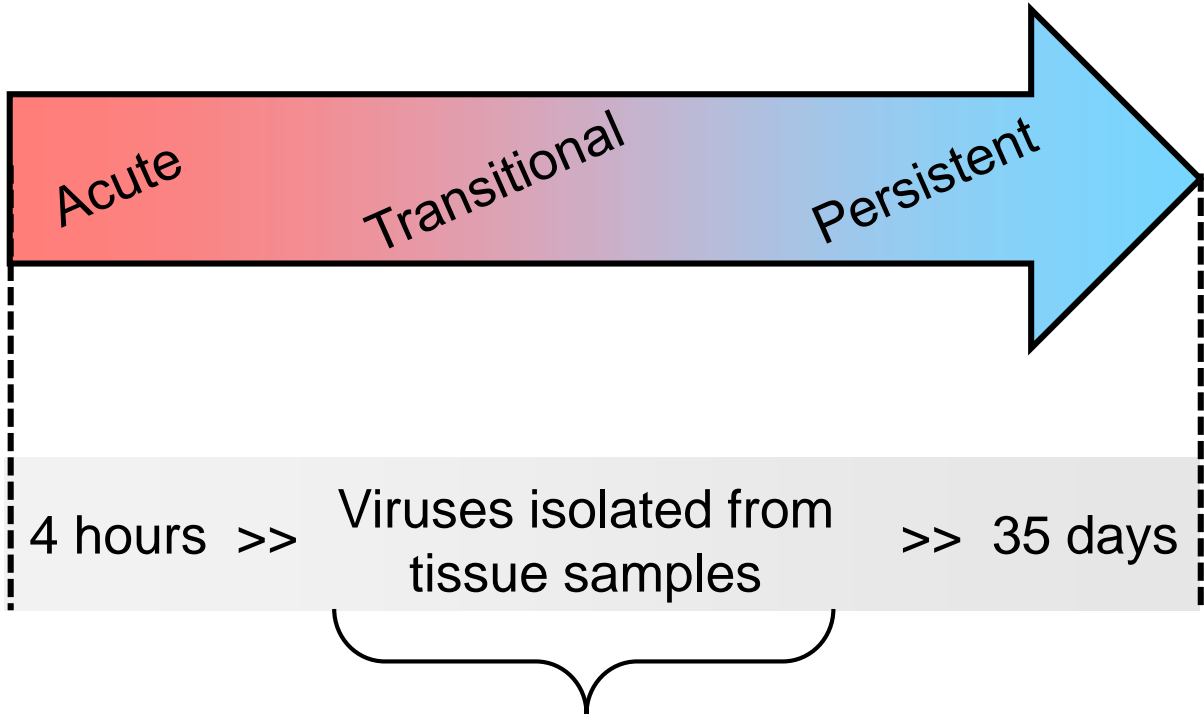
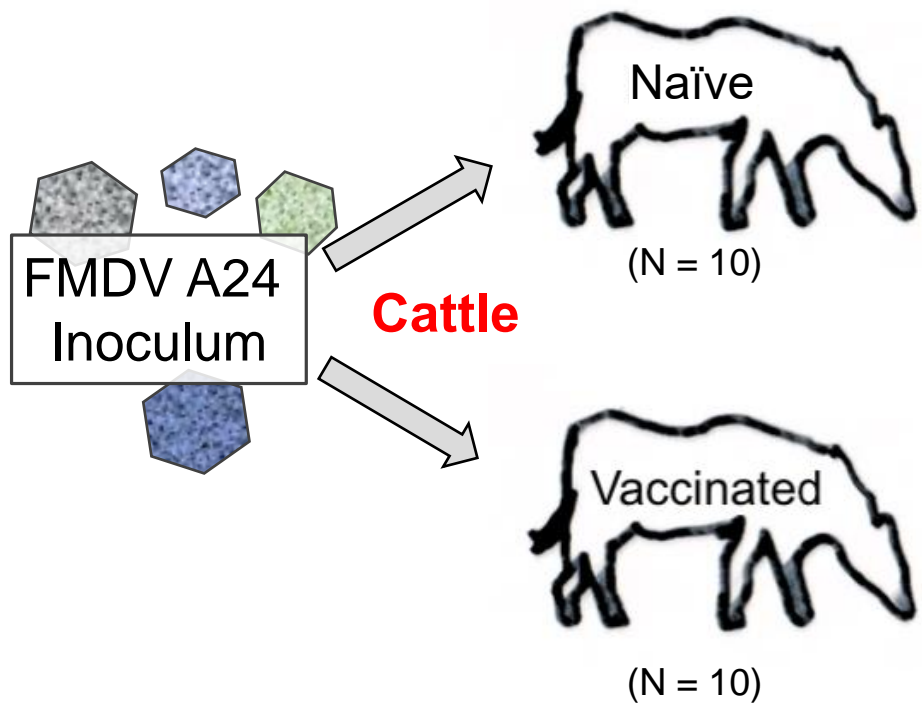
Are there viral factors associated with persistent infection **of cattle**?



Experimental Design



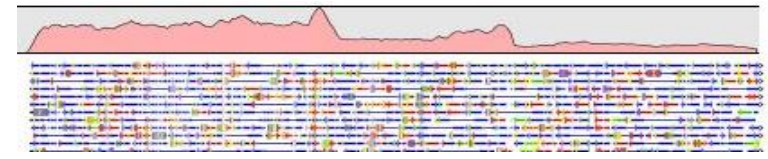
Results



All animals became infected with FMDV
Half of each cohort became persistently infected



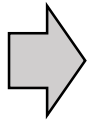
Illumina NextSeq
★ 69 deep-sequenced
★ 33 consensus-only seqs



Results & Analysis

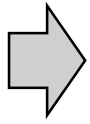
Substitution rates and Shannon entropy

☆ Substitution rates higher in naïve
 ☆ Entropy higher in naïve



☆ Minimal bottleneck in naïve
 ☆ Strong and lasting bottleneck effect in vaccinated

☆ Terminators had diminishing entropy



☆ Evidence of a waning population

	Substitution Rate (subs/st/yr)			Shannon Entropy			
	Early	Transitional	Persistent	CDS	Nonstructural	Structural	Structural / CDS
Inoculum	-	-	-	0.0156	0.0147	0.0181	1.16
Naïve	0.188*	0.127	0.080*	0.0171	0.0160	0.0200	1.17
Vaccinated	0.131	0.089	0.079	0.0120	0.0115	0.0130	1.08

animal	dpi	cds
14-33	1_dpi	0.0158
14-33	2_dpi	0.0154
14-33	3_dpi	0.0176
14-33	4_dpi_pass	0.0151
14-33	6_dpi	0.0152
14-33	10_dpi_Sa	0.0133
14-33	21_dpi_OPF	0.0154
14-33	28_dpi_OPF	0.0173
14-34	1_dpi	0.0151
14-34	2_dpi	0.0146
14-34	3_dpi	0.0183
14-34	4_dpi	0.0145
14-34	5_dpi	0.0160
14-34	6_dpi	0.0169
14-34	7_dpi	0.0175
14-34	8_dpi	0.0160
14-34	9_dpi	0.0176
14-34	28_dpi_raw	0.0187
14-34	28_dpi_pass	0.0179
14-34	31_dpi	0.0171
14-49	2_dpi	0.0261
14-49	6_dpi	0.0215
14-49	10_dpi	0.0210
14-108	0.25_dpi	0.0192
14-108	0.5_dpi	0.0172
14-108	0.75_dpi	0.0149
14-108	1_dpi	0.0203
14-108	3_dpi	0.0200
14-108	5_dpi	0.0122
14-108	8_dpi_pass	0.0143
14-108	8_dpi_raw	0.0160
14-108	10_dpi	0.0135
14-110	0.17_dpi	0.0172
14-110	0.42_dpi	0.0165
14-110	0.75_dpi	0.0195
14-110	0.88_dpi	0.0199
14-110	2_dpi	0.0151
14-110	3_dpi	0.0161
14-110	8_dpi_pass	0.0188
14-110	8_dpi_raw	0.0176
14-110	14_dpi	0.0118
14-110	17_dpi	0.0159
14-110	28_dpi_raw	0.0184
14-110	35_dpi	0.0157
14-111	0.17_dpi	0.0174
14-111	0.42_dpi	0.0182
14-111	0.75_dpi	0.0198
14-111	0.88_dpi	0.0181
14-111	2_dpi	0.0195
14-111	3_dpi_pass	0.0224
14-111	7_dpi	0.0164
14-111	8_dpi	0.0162
14-111	9_dpi	0.0148
14-111	10_dpi	0.0162

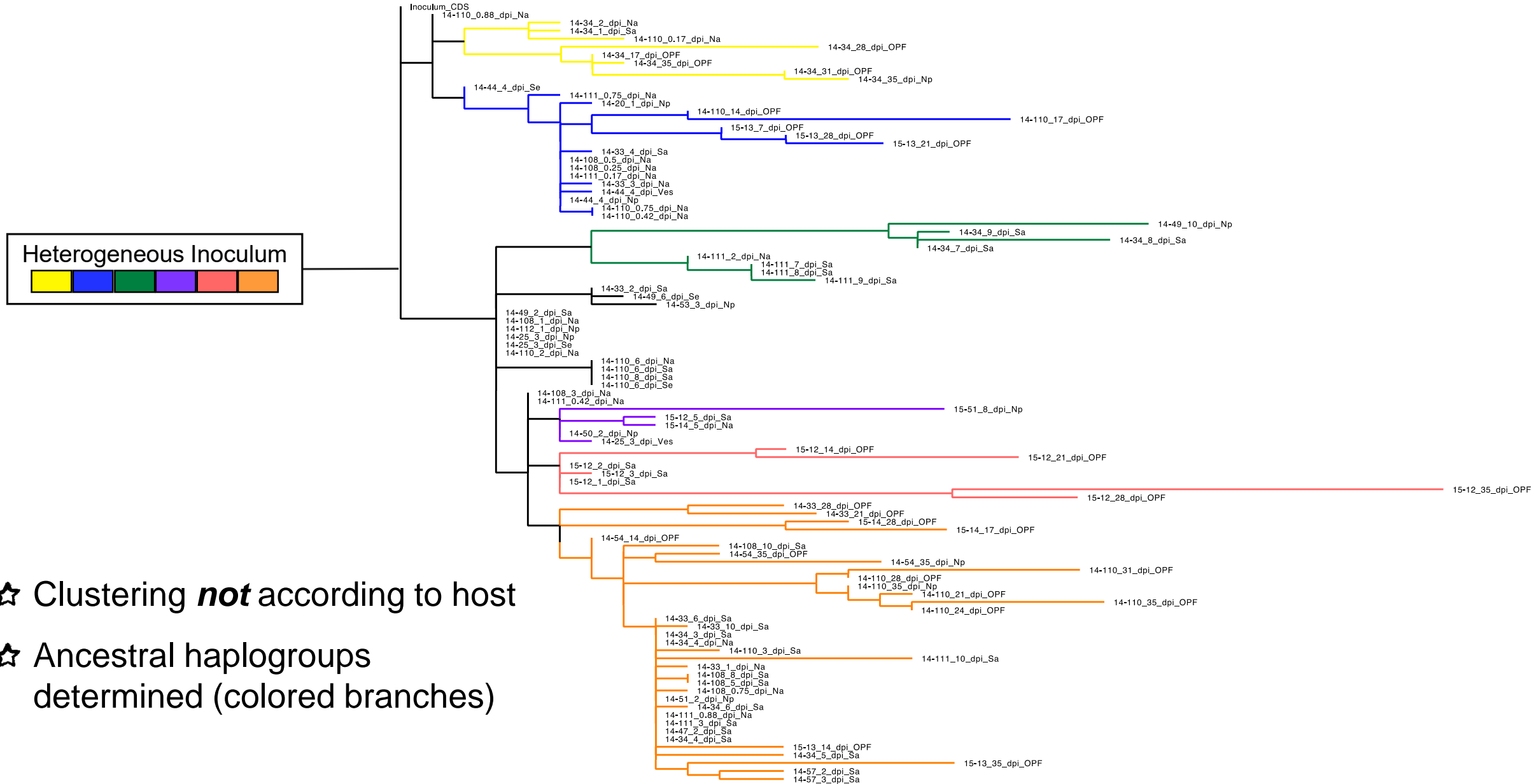
Naïve ↑
 ↓ Vaccinated



1450	2_dpi	0.0083
1451	2_dpi	0.0087
14-57	2_dpi	0.0134
14-57	3_dpi	0.0152
15-12	1_dpi	0.0153
15-12	2_dpi	0.0146
15-12	3_dpi	0.0153
15-12	28_dpi	0.0170
15-12	35_dpi	0.0140
15-13	7_dpi	0.0093
15-13	14_dpi	0.0095
15-14	5_dpi	0.0093
15-14	17_dpi	0.0089
15-14	28_dpi	0.0110
1551	8_dpi	0.0095

Results & Analysis

Consensus-level phylogeny



☆ Clustering *not* according to host

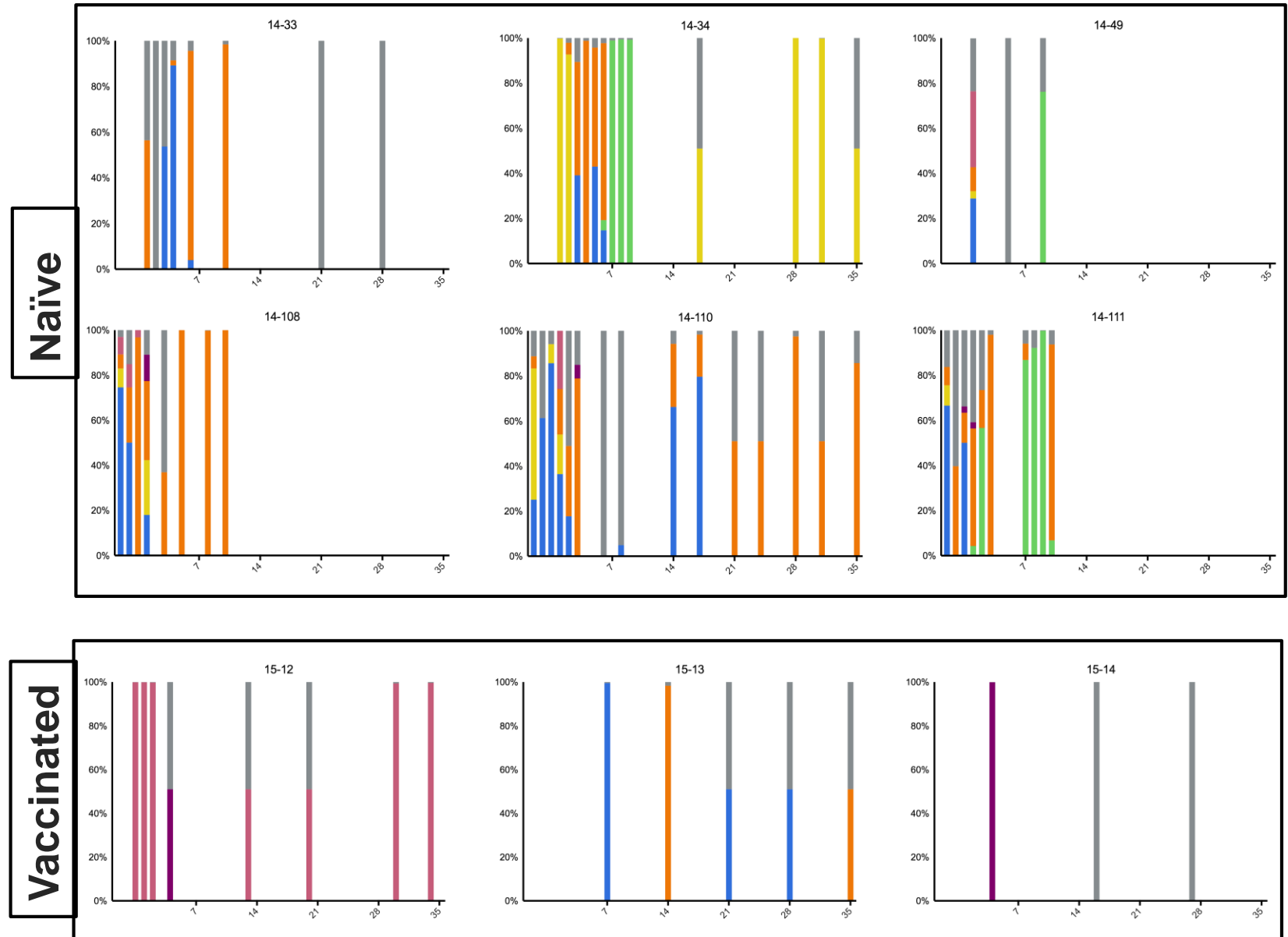
☆ Ancestral haplogroups determined (colored branches)

Results & Analysis

Haplotypic composition of FMDV populations

☆ **Naïve:** Haplotypically diverse and in flux through acute phase > no evidence of positive selection

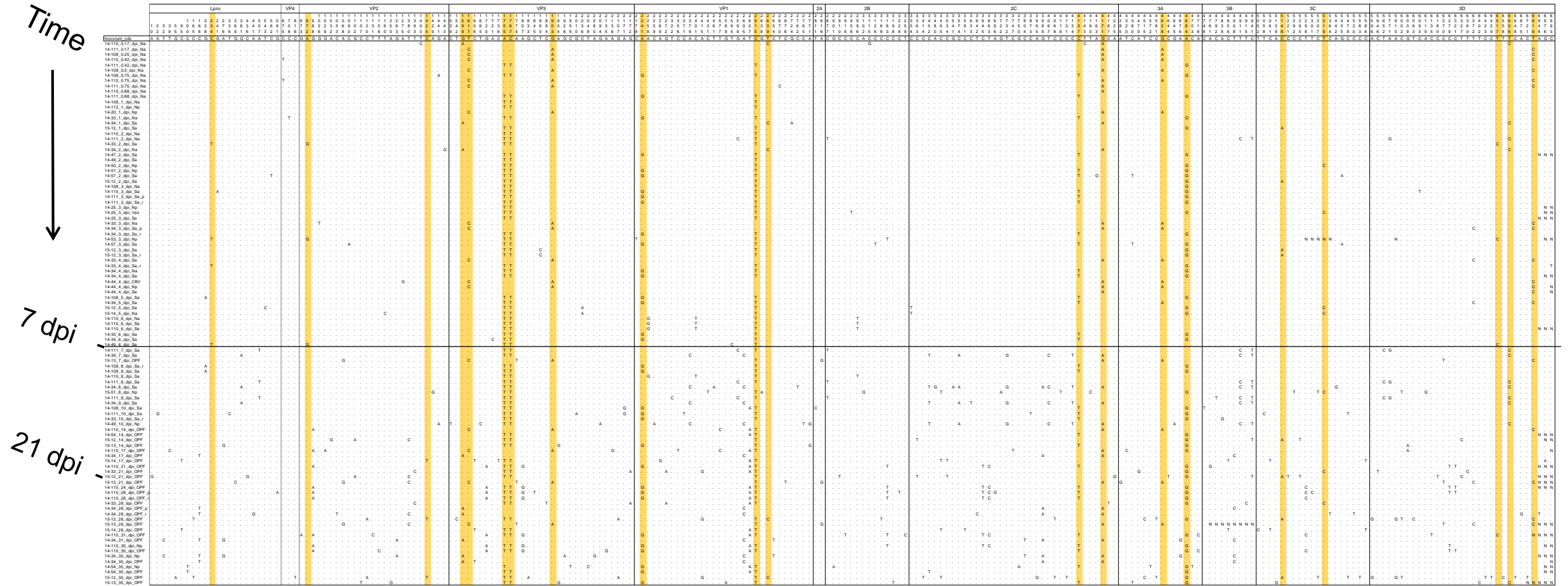
☆ **Vaccinated:** minimal heterogeneity > narrow bottleneck



Analysis

Alignment of all sample consensus sequences

■ = SNPs present in the inoculum (>0.5%)

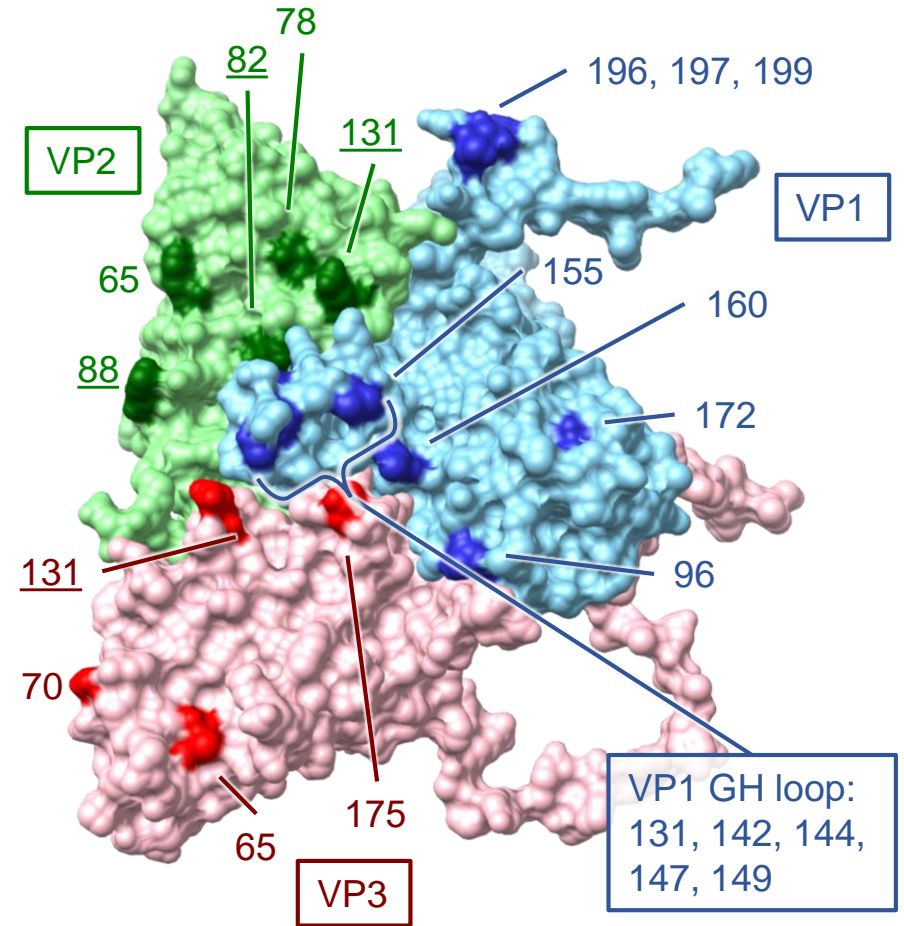


Acute stage variation is inherited and transient >> Neutral and purifying selection dominate

Analysis

Capsid Variation: Selection and Replacement

	site	inoculum consensus	variant 1	variant 2	variant 3	variant freq. in inoculum	MEME, $P \leq 0.10$ n = 9	naïve n = 10	vaccinated n = 10
VP2	44	A	T				1	1	
	65	Y	C	S			1		
	78	L	M						1
	82	E	K						1
	88	H	N				1		1
	131	E	G						1
VP3	65	T	N				1		
	70	D	A	N	G		2	2	
	99	T	A				1	1	
	111	F	V				1		
	131	E	K	G		23 (K)		7	1
	175	T	K						2
220	Q	R					2		
VP1	96	S	T					1	
	131	N	S				1		
	142	G	R				1	1	
	144	S	R	G		☆	3	5	2
	147	T	M			48.2	1	9	9
	149	S	A	Y	P		4		
	155	V	A	M		3.2 (A)		2	2
	160	A	V				2	1	
	172	I	V				1		
	196	S	F					1	
197	S	L					1		
199	D	G					1	1	

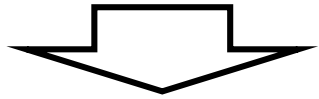


Analysis

Convergent evolution and 2 rebels

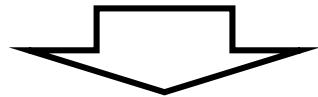
By 35 days, all infected cattle **except 2** encoded VP1-144 serine to arginine mutation

☆ SGD becoming fixed as RGD

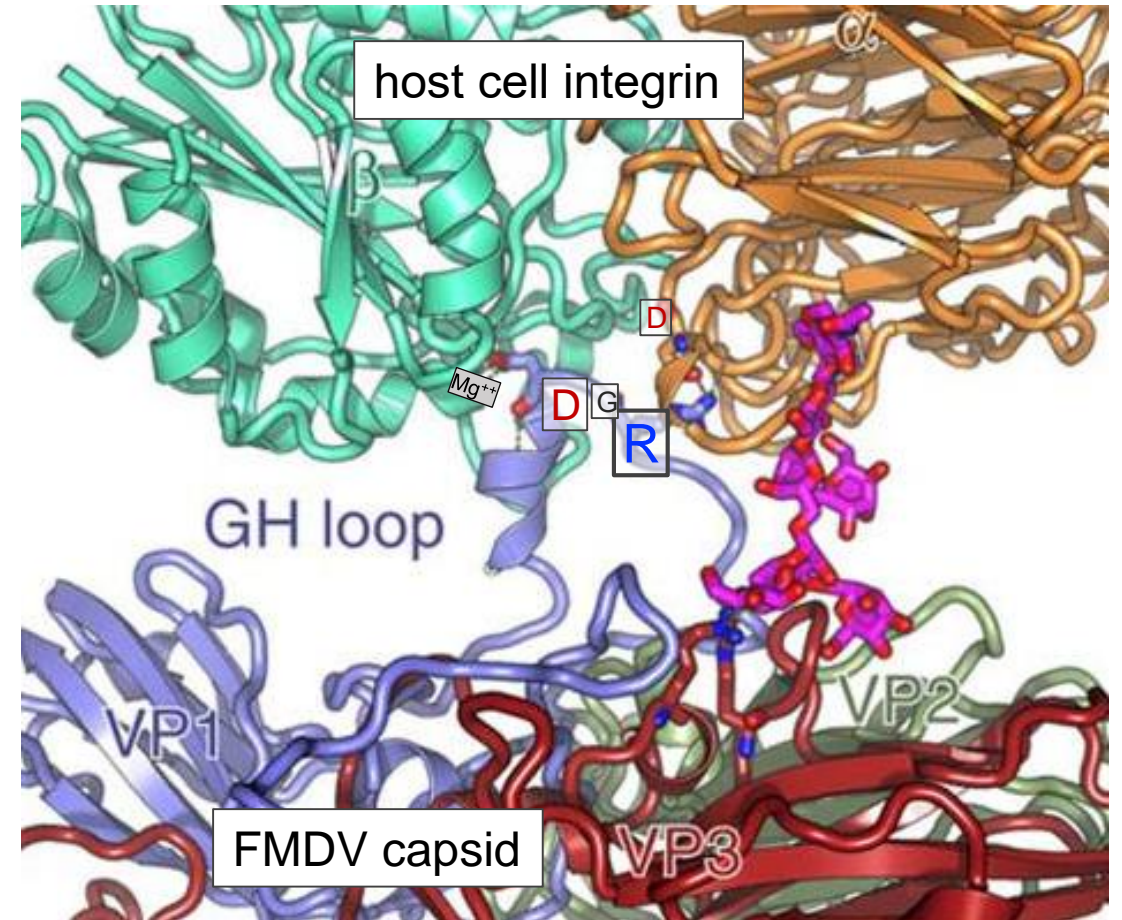


Selective sweeps after acute phase

☆ The **2 unchanged** were vaccinated, suggesting an added benefit of vaccination



Removal of potentially adaptive mutation



Analysis

Convergent evolution and 2 rebels

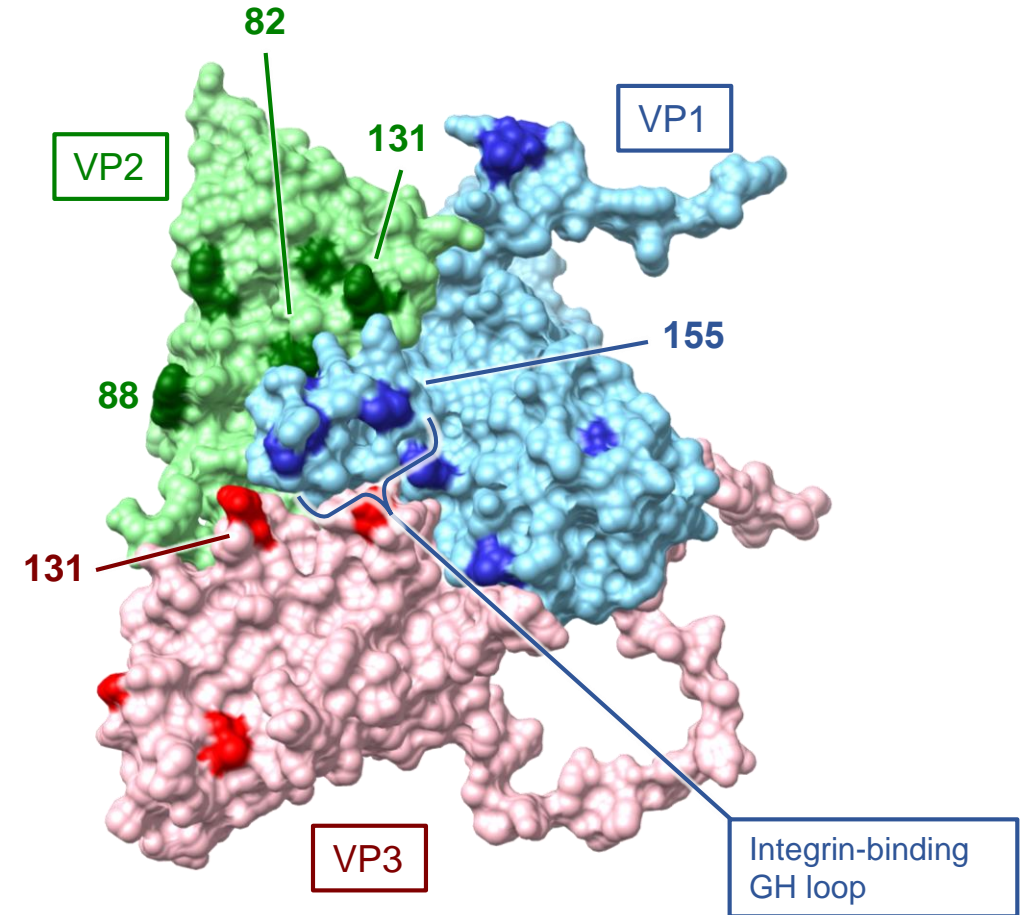
By 35 days, all infected cattle **except 2** encoded VP1-144 serine to arginine mutation

☆ However, these 2 were the only cattle that acquired **escape mutations**

	VP2				VP3					VP1										
Antigenic sites	4	8	8	3	7	7	9	3	3	7	9	0	3	3	9	4	4	4	5	6
Inoculum	A	E	H	E	D	A	T	E	R	I	I	K	I	G	S	G	S	T	V	A
14-110_17_dpi	T							K									R	M		
14-34_17_dpi																	R	M		
15-14_17_dpi					V								V				R	M		
14-110_21_dpi	T				N	A							S				R	M		
14-33_21_dpi																	R	M		
15-12_21_dpi		K						K										M		
15-13_21_dpi									K									M		
14-110_24_dpi	T				N	A											R	M		
14-110_28_dpi	T				N	A											R	M		
14-110_28_dpi	T				N	A											R	M		
14-33_28_dpi													S				R	M		
14-34_28_dpi																	R	M		
14-34_28_dpi																	R	M		
15-12_28_dpi		N																M	A	
15-13_28_dpi								K										M		
15-14_28_dpi																	R	M		
14-110_31_dpi	T				N	A											R	M		
14-34_31_dpi																	R	M		V
14-110_35_dpi	T				N	A											R	M		
14-110_35_dpi	T				N	A											R	M		
14-34_35_dpi								K		V	R						R	M		V
14-34_35_dpi																	R	M		
14-54_35_dpi																	R	M		
14-54_35_dpi									T								R	M		
15-12_35_dpi		N																M	A	
15-13_35_dpi		G						G										M		

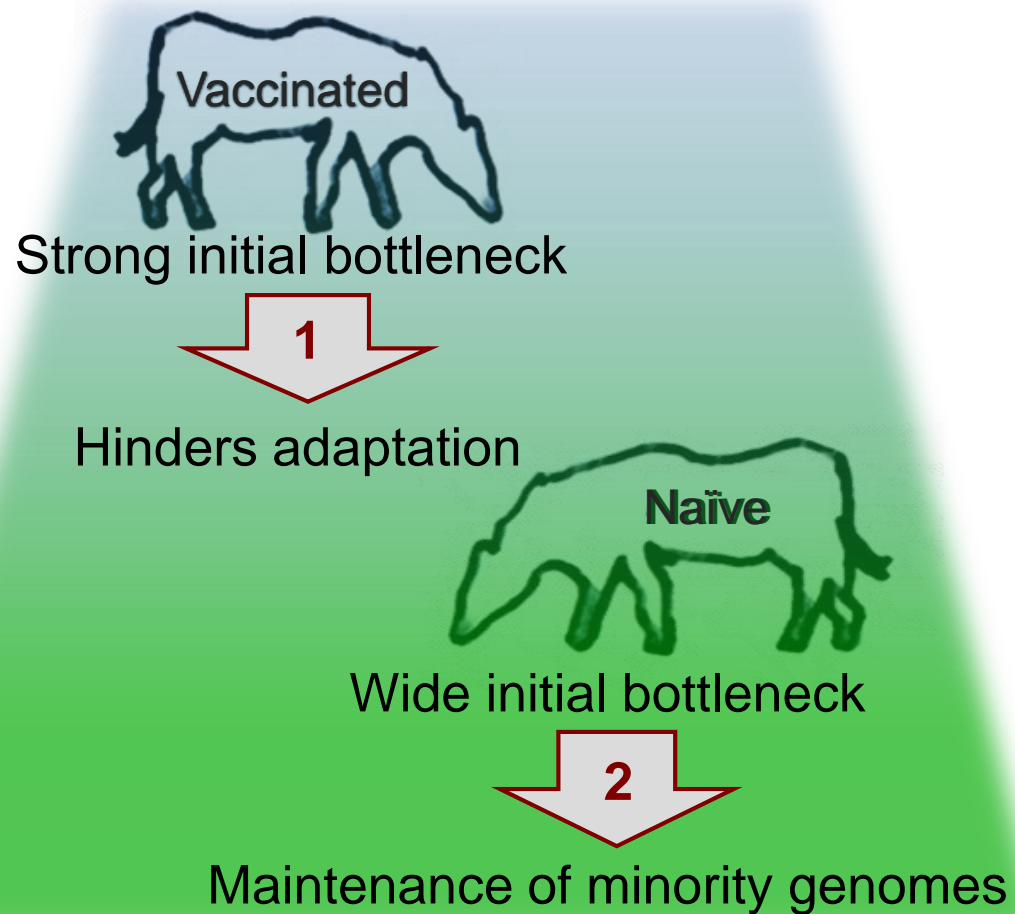
17 dpi

35 dpi

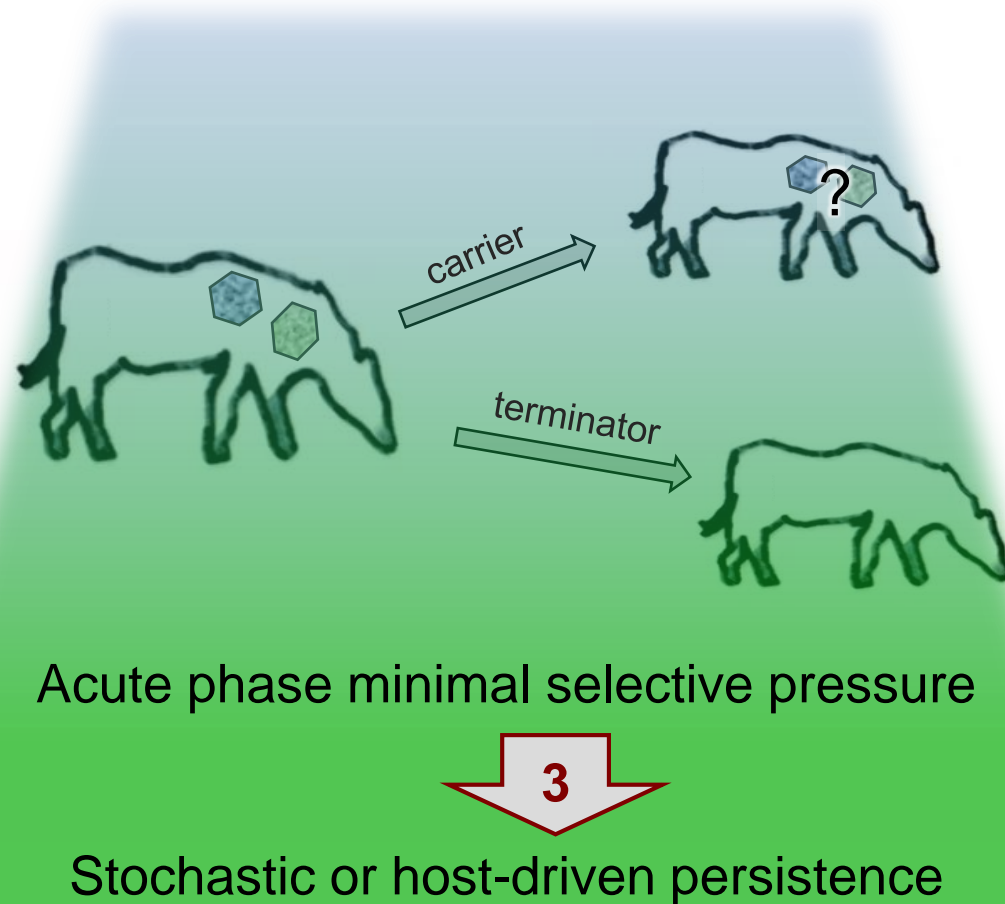


Conclusions

☆ What effect does vaccination have on the FMDV population in vivo?

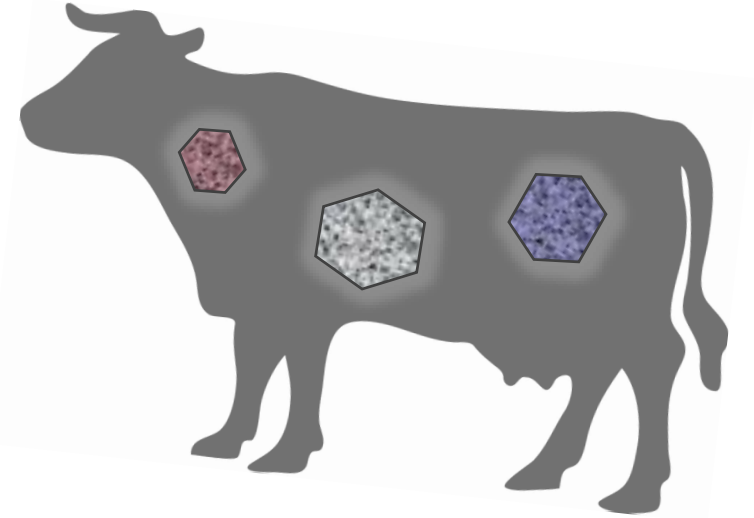


☆ What viral factors are associated with persistent FMD infection?



But how does this intra-host population stuff affect my research?

1. Consider **the subconsensus**. Co-dominant viruses can affect
 - Phylogenetic trees
 - Transmission networks
 - Evolutionary rates
2. Immunity-evading viruses arise *after* the window of transmission has passed, so **FMDV carriers are important**
3. Vaccination significantly decreases FMDV diversity*



*just a nice thing to throw in your grant applications



Thank
moo!

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and mitigating the impact of FMDV
persistence in cattle subsequent to Ad5-FMD
vaccination” and **USDA-ARS** base funds



United States Department of Agriculture
Agricultural Research Service

