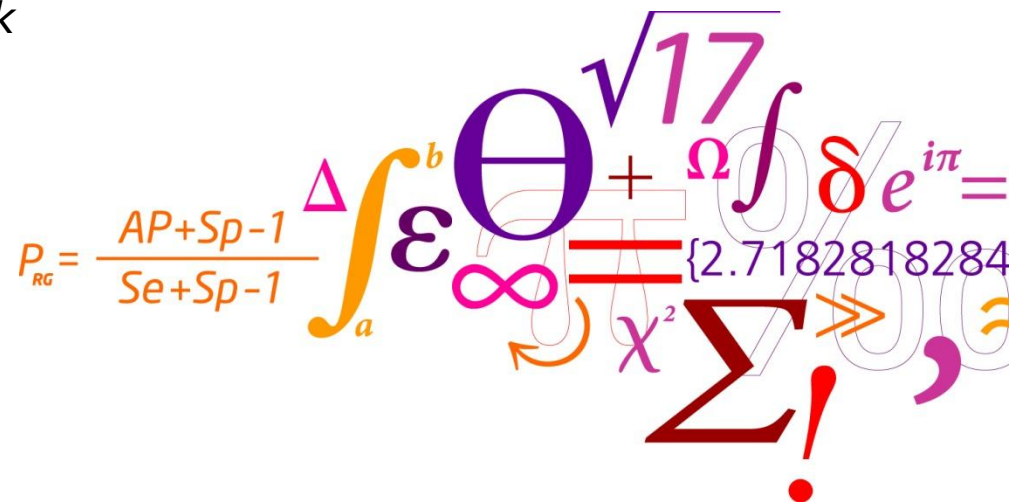


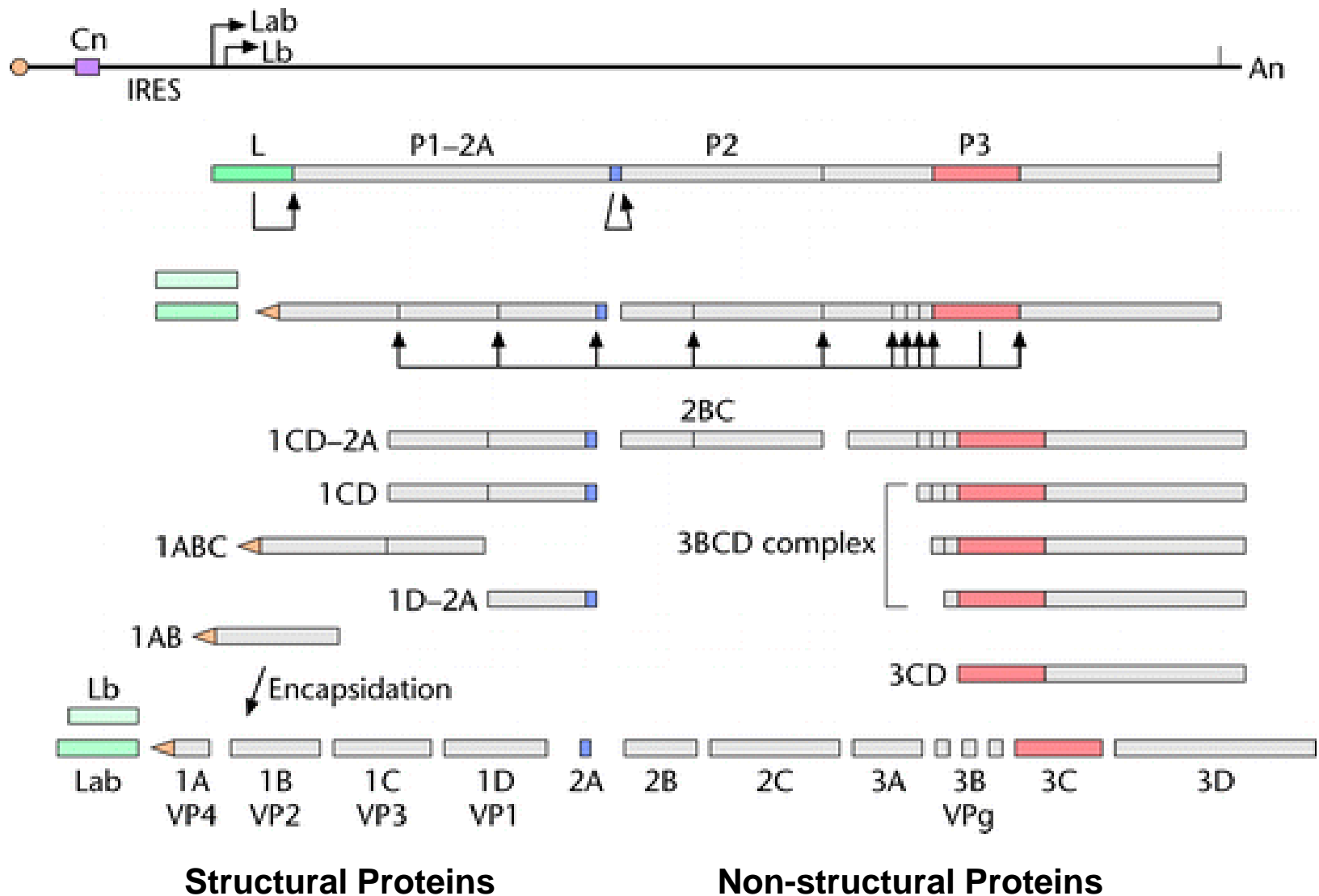
Identification of candidate amino acid substitutions, exposed on the surface of FMDV, leading to antigenic change

Graham J. Belsham

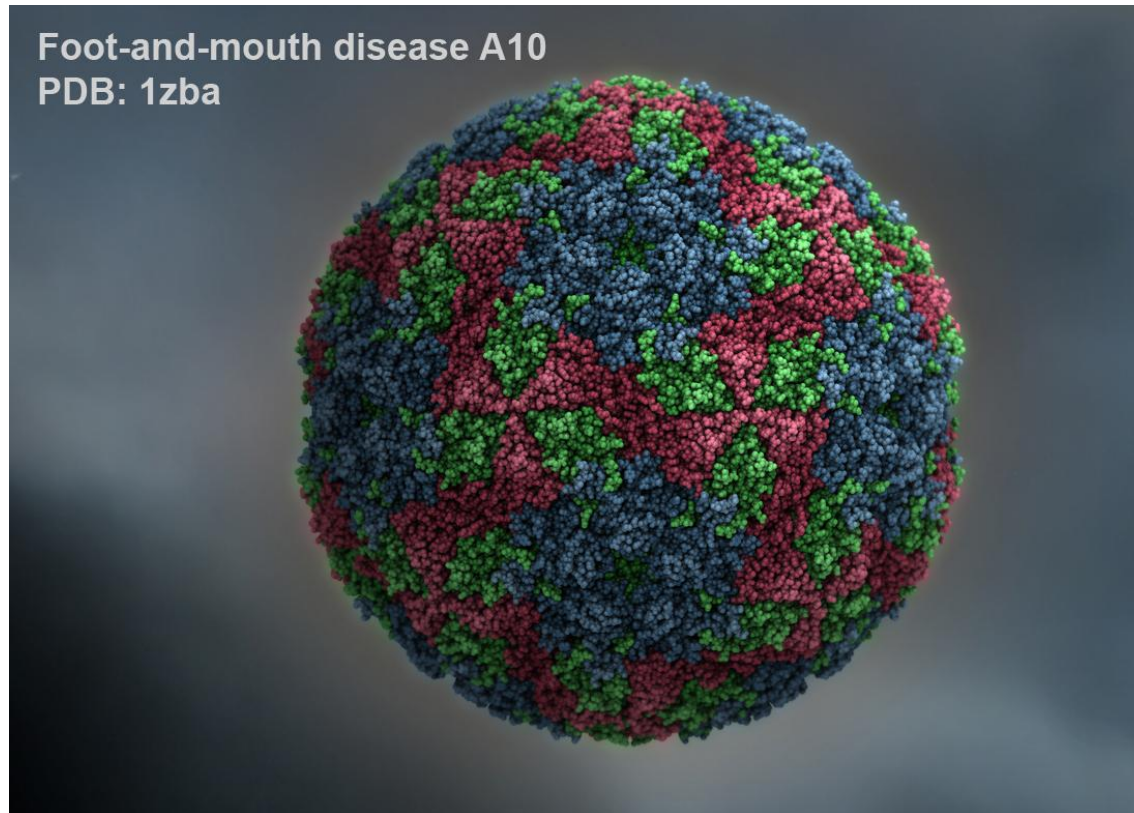
*National Veterinary Institute, Technical University of Denmark,
Lindholm, 4771 Kalvehave, Denmark*



FMDV Genome organization



The FMDV particle



Surface exposed proteins are VP1, VP2 and VP3- these define antigenicity
VP4 is internal

FMDV sequence diversity- an example from Pakistan & Afghanistan

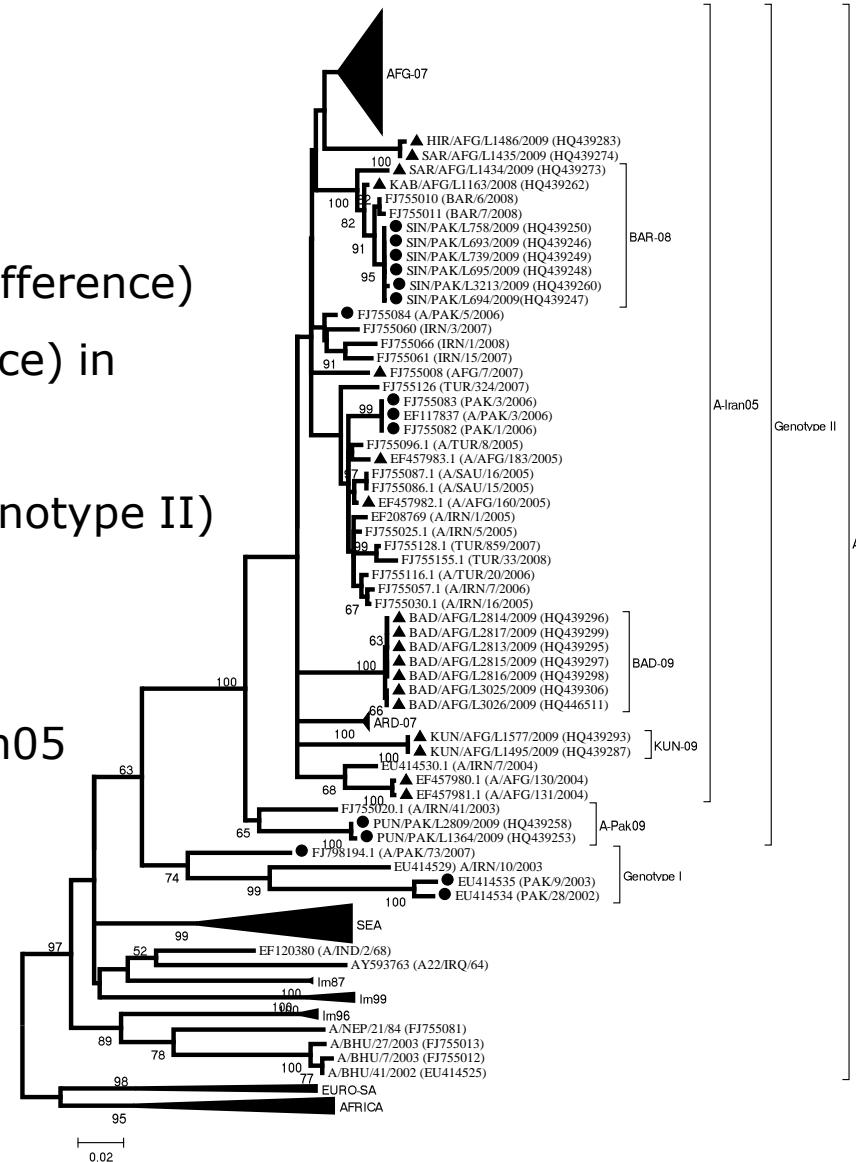
- 157 oral swab and epithelial samples collected within Pakistan & Afghanistan during 2008/2009
- Tested for FMDV RNA using real-time RT-PCR- 135 positive, 119 sequenced (VP1)
- 33 serotype O
- 18 serotype Asia-1
- 69 serotype A (22 fom Pakistan and 47 from Afghanistan)

Sequence diversity of FMDV A-Iran05 (VP1 sequences)

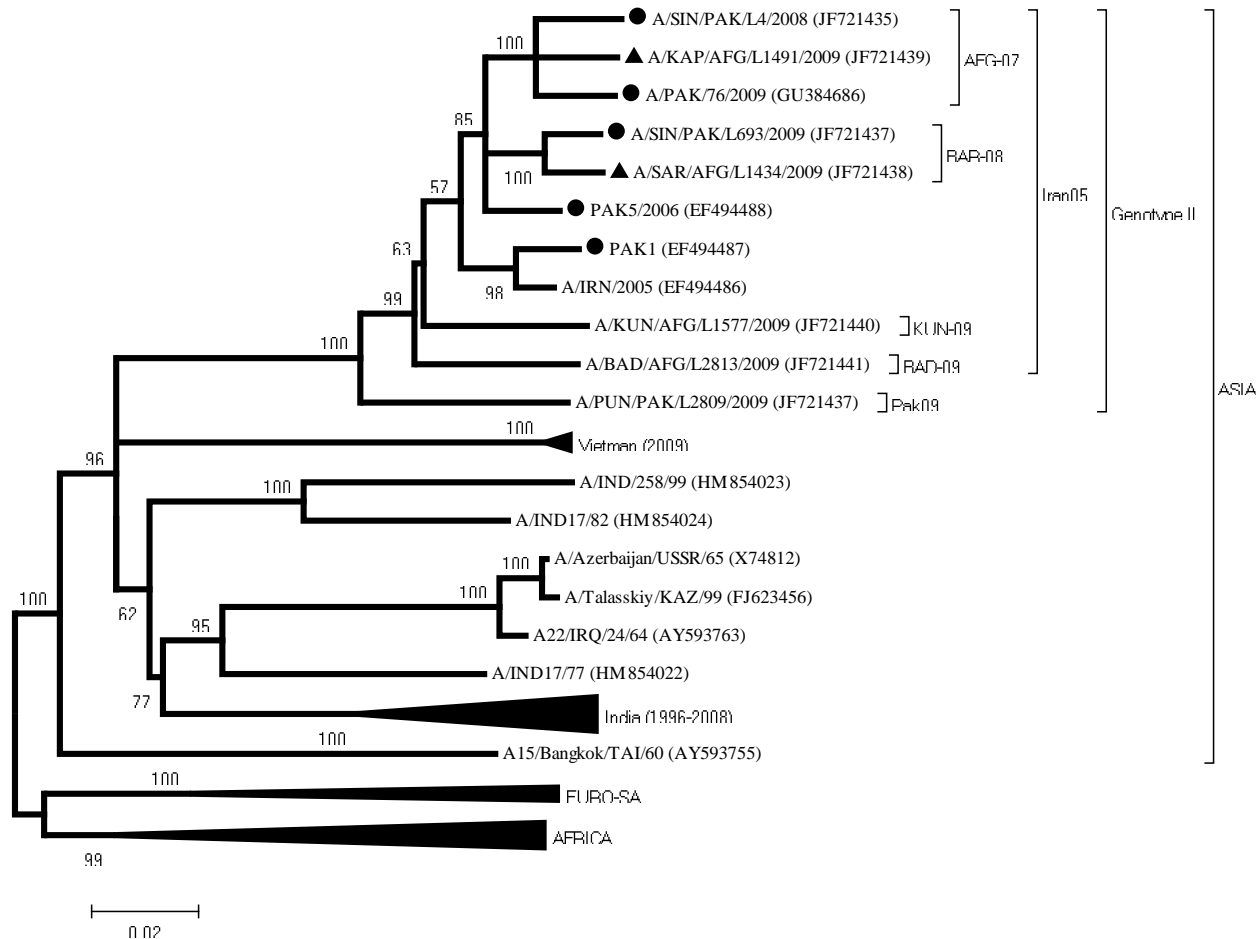
Since 2002, >4 lineages (>7.5% nt difference) within 2 genotypes (>15% nt difference) in Asia toptotype of serotype A
 A-Iran 05 predominant lineage (in genotype II) (all but 2 from >60 in 2008/2009)

4 major current sub-lineages of A-Iran05 (>5% nt difference):

A-Iran05^{BAR08}, A-Iran05^{AFG07},
 A-Iran05^{BAD09}, A-Iran05^{KUN09}



Phylogenetic tree generated using the coding sequences for all four capsid proteins (P1 region) of FMDV serotype A



Antigenic change?

- A-Iran 05 lineage has evolved into multiple sub-lineages including A-Iran05^{AFG07}, A-Iran05^{BAR08}, A-Iran05^{KUN09} and A-Iran05^{BAD09}
- A22 vaccine may not protect against A-Iran05^{BAR08} (WRL reports 2009/2010)
- What are the differences between A-Iran05^{BAR08} and other A-Iran05 viruses?
- Antigenic change can reflect amino acid changes within VP1, VP2 and VP3.

Alignment of VP1 amino acid sequences (residues 1-170)

A-Iran05^{AFG-07}

A/PAN/AFG/L3640/2009 TTTAGESADP VTTTVENYGG ETQAQRHHHT DVGFMIDRFV KINPVSPTHV IDLMQTHQHA LVGALLRAAT YYFSDLEIVV RHDGNLTWVP NGAPVEALAN TSNPTAYHKQ PFTRLALPYT APHRVLATVY NGVSRYSTTG GRRGDLGEL AARVAQQLPS SFNFGAIQAT

A-Iran05^{BAR-08}

A/SIN/PAK/L3213/2009D.....EN..N.....K.....S.....R..
A/SIN/PAK/L758/2009D.....EN..N.....K.....S.....R..
A/SIN/PAK/L739/2009D.....EN..N.....K.....S.....R..
A/SIN/PAK/L695/2009D.....EN..N.....K.....S.....R..
A/SIN/PAK/L693/2009D.....EN..N.....K.....S.....R..
A/SIN/PAK/L694/2009D.....EN..N.....K.....S.....R..
A/KAB/AFG/L1163/2008D.....EK..D.....K.....S.....R..
A/SAR/AFG/L1434/2009D.....EI..D.....K.....S.....R..

A-Iran05^{ARD-07}

A/TUR/23/2008Q.....K.....N.....S.....R..
A/TUR/1009/2007Q.....K.....N.....S.....R..
A/TUR/53/2008Q.....K.....N.....S.....R..
A/TUR/1/2008Q.....K.....N.....S.....R..
A/TUR/1018/2007Q.....K.....N.....S.....R..
A/TUR/28/2008Q.....K.....N.....S.....R..
A/TUR/130/2008Q.....K.....N.....S.....R..

A-Iran05^{BAD-09}

A/BAD/AFG/L3025/2009Q.....E.....K.....N.....S.....R..
A/BAD/AFG/L3026/2009Q.....E.....K.....N.....S.....R..
A/BAD/AFG/L2813/2009Q.....E.....K.....N.....S.....R..
A/BAD/AFG/L2814/2009Q.....E.....K.....N.....S.....R..
A/BAD/AFG/L2815/2009Q.....E.....K.....N.....S.....R..
A/BAD/AFG/L2816/2009Q.....E.....K.....N.....S.....R..
A/BAD/AFG/L2817/2009Q.....E.....K.....N.....S.....R..

Different sub-lineages of A-Iran05 have unique amino acid substitutions within VP1

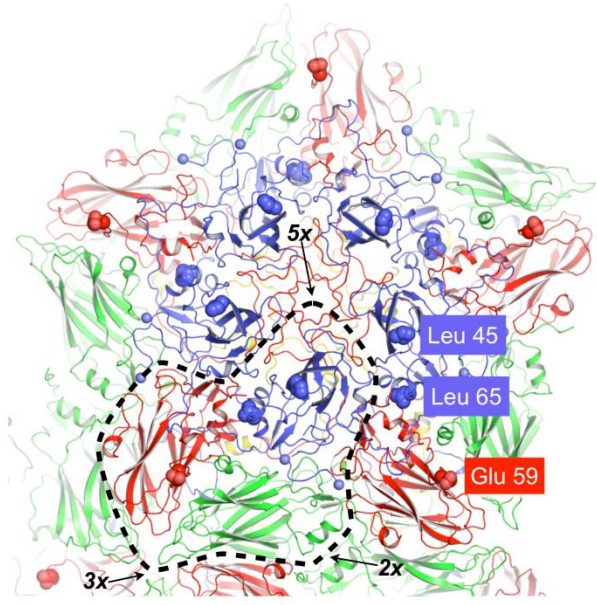
Capsid Protein	Site	A22/Iraq/64	A-Iran05			A-Pak09		
			Parental	AFG-07	BAR-08	KUN-09	BAD-09	
VP1	4	T	A	A	A	A	A	A
	33	T	G	G	G	G	G	G
	43	Q	S	N	N	S	S	N
	44	N	P	P	P	P	P	P
	45	L	V (G ¹)	V	A	V	V	V
	46	N	S	S (G ²)	S (G ³)	S	S	S
	60	G	A	A	A	A	A	A
	65	L	L	L	F	L	L	L
	83	D	D	D	E	E	D	E
	102	G	S	S	S	S	S	S
	108	L	H	H	H	H	H	H
	110	A	Q	Q	Q	Q	Q	Q
	133	T	V	V	V	V	V	V
	135	K	K	R	K	R	K	K
	138	A	T	T	T	T	T	M
	139	G	T	T	T	T	T	T
160	A	S	S	S	S	S	S	
168	Q	R	Q (R ⁴)	R	R	R	Q	
VP2	39	Q	G (R ⁵)	G	G	G	G	G
	71	P	T	T	T	T	T	T
	86	D	E	E	E	E	E	E
	110	S	T	T	T	T	T	T
	149	S	N	N	N	N	N	N
	159	V	T	T	T	T	T	T
	173	K	Q	Q	Q	Q	Q	Q
	190	N	S	S	S	S	S	S
	191	T	S	S	S	S	S	N
	192	V	I	I	I	I	I	I
	193	S	G	G	G	G	G	G
	195	G	S	S	S	S	S	S
	207	H	F	F	F/S ⁶	F	F	F
VP3	59	E	D	D	N	D	D	D
	99	A	T	T	T	T	A	A
	116	D	E	E	E	E	E	D
	130	V	M	M	M	M	M	M
	131	E	D	D	D	D	A	D

Amino acid substitutions in the surface exposed capsid proteins between viruses from Pakistan, Afghanistan and Iran and the A22/Iraq/64 vaccine strain

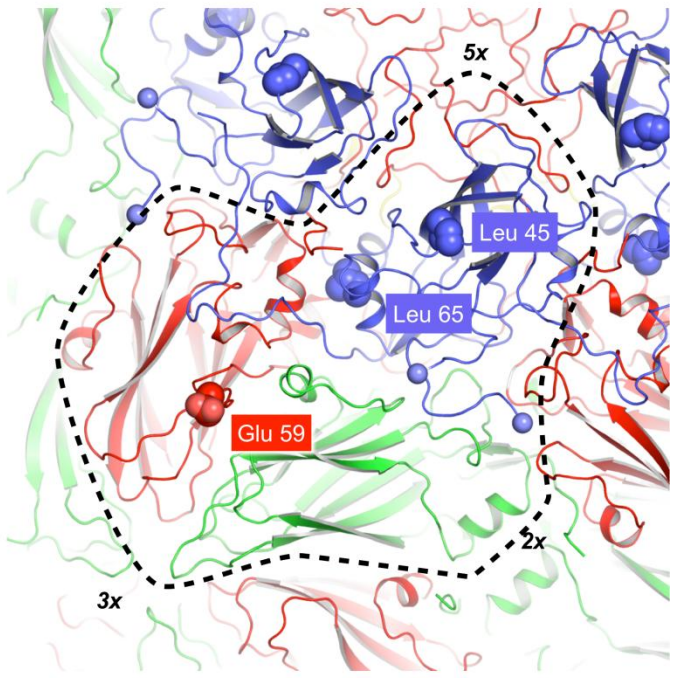
Mapping selected capsid protein differences between A-Iran05^{BAR08} and the A22/Iraq vaccine strain on the structure of the vaccine virus



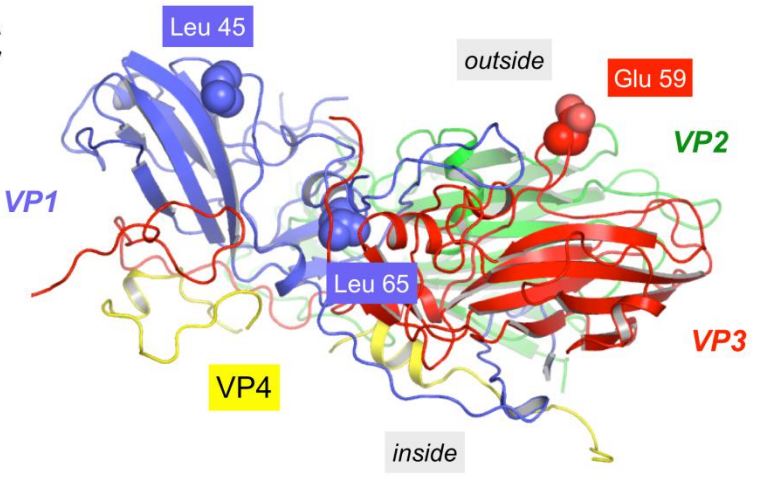
A



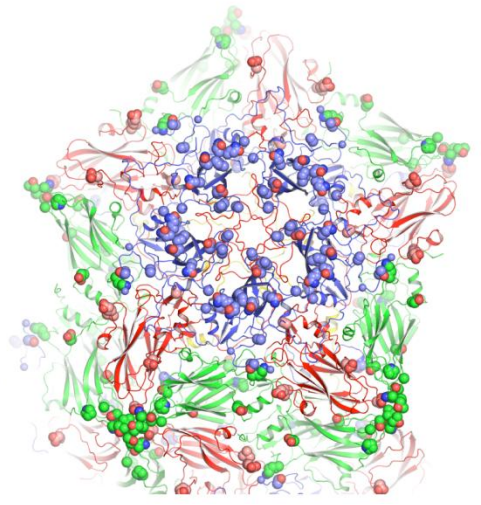
B



C



D

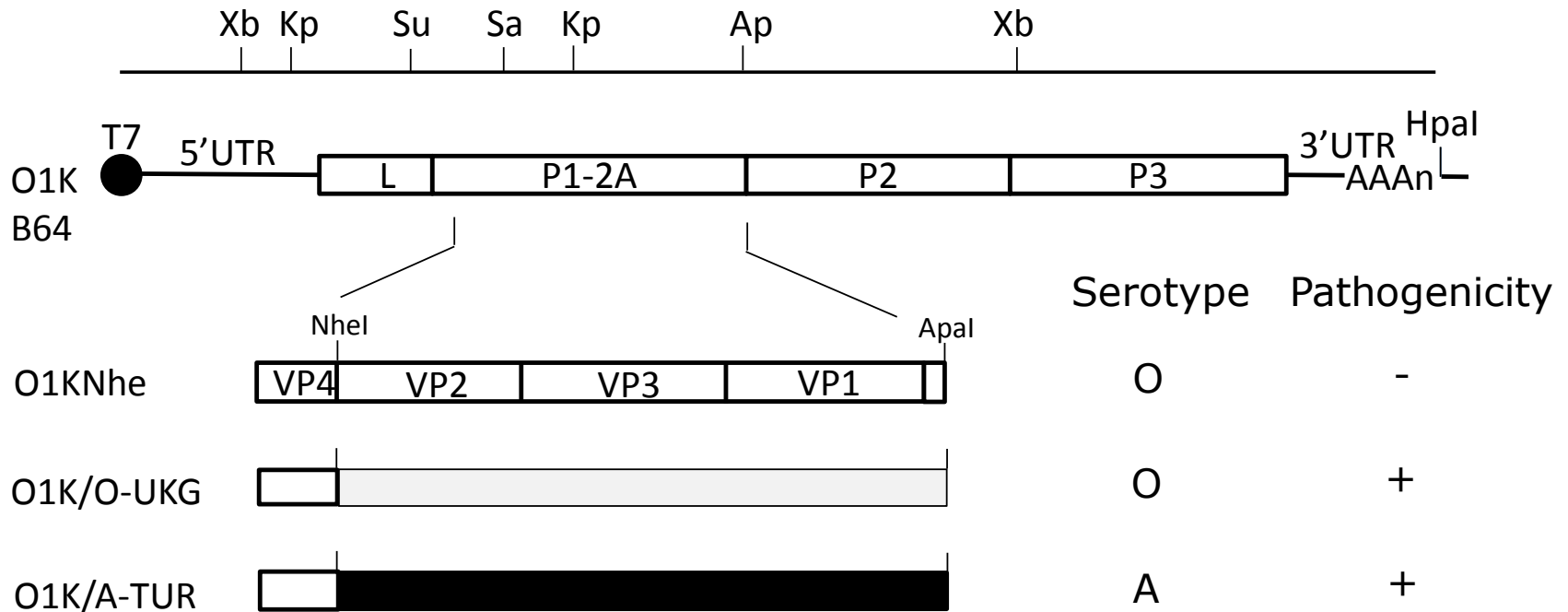


Candidate residues conferring antigenic change

Residues changes at positions 45 and 65 in VP1 and 59 in VP3 are unique to A-Iran05^{BAR08}

- Surface exposed
 - Non-conservative changes (L45A, L65F & E59N)
 - Candidates for antigenic change
-
- To prove..... use reverse genetics

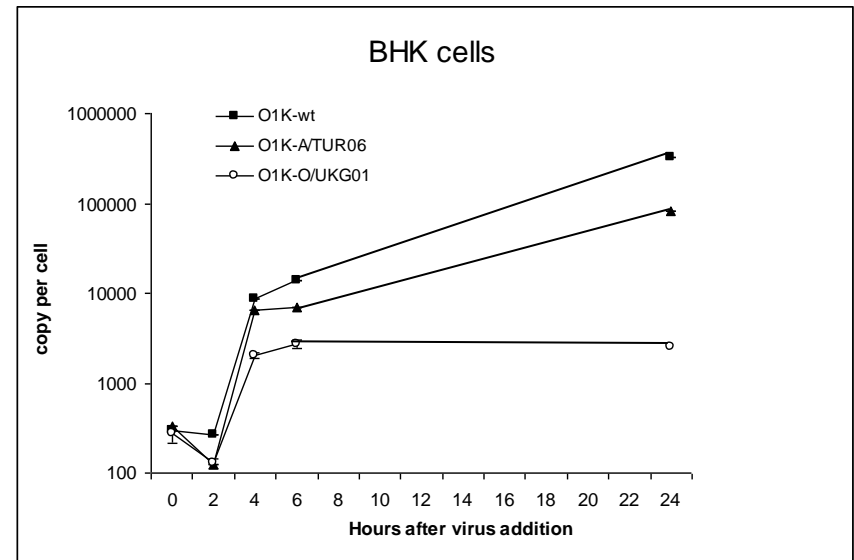
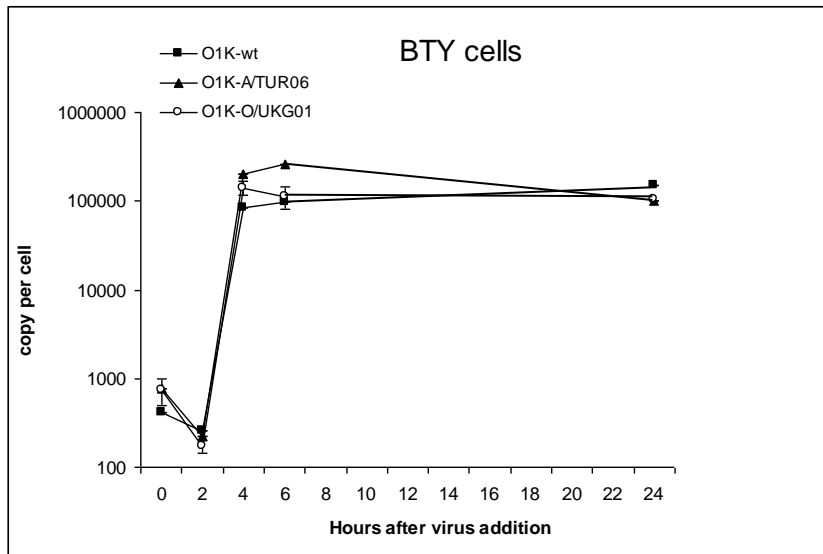
Chimeric viruses as a tool



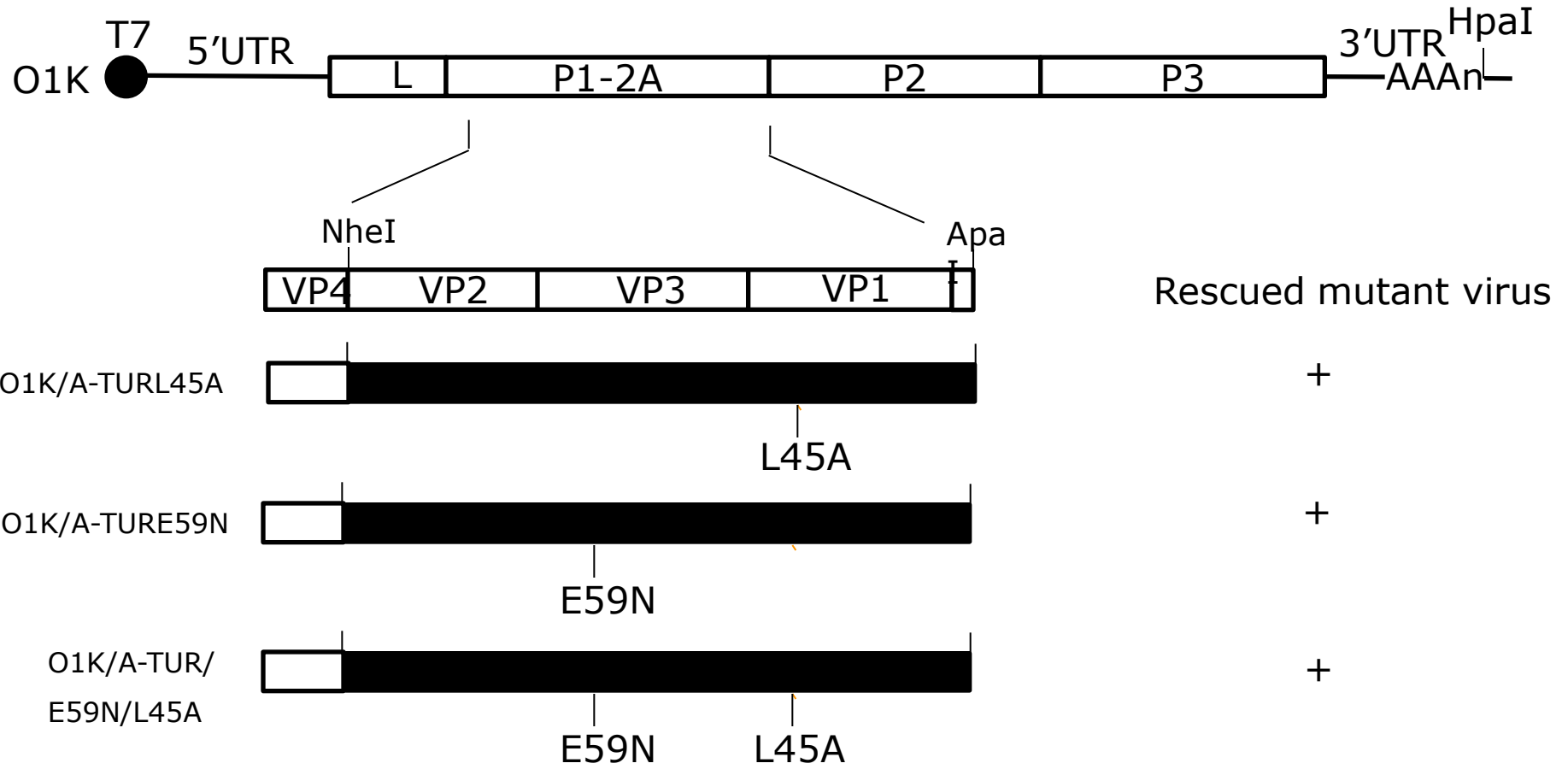
O1K B64 is completely attenuated in cattle

O1K/O-UKG and O1K/A-TUR (A-Iran05 lineage) are pathogenic for cattle

Chimeric virus growth curves



Rescue of mutant A-Iran05 viruses



Rescued mutant virus

Antigenicity ????
(serotype A)

Summary & Conclusions

Sequence analysis of FMDV P1 coding sequence can allow identification of candidate residues which can confer antigenic change

Candidates residues identified as:

-non-conservative amino acid substitutions

-surface exposed residues

Proof requires construction of mutant viruses and analysis of their antigenic properties

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