

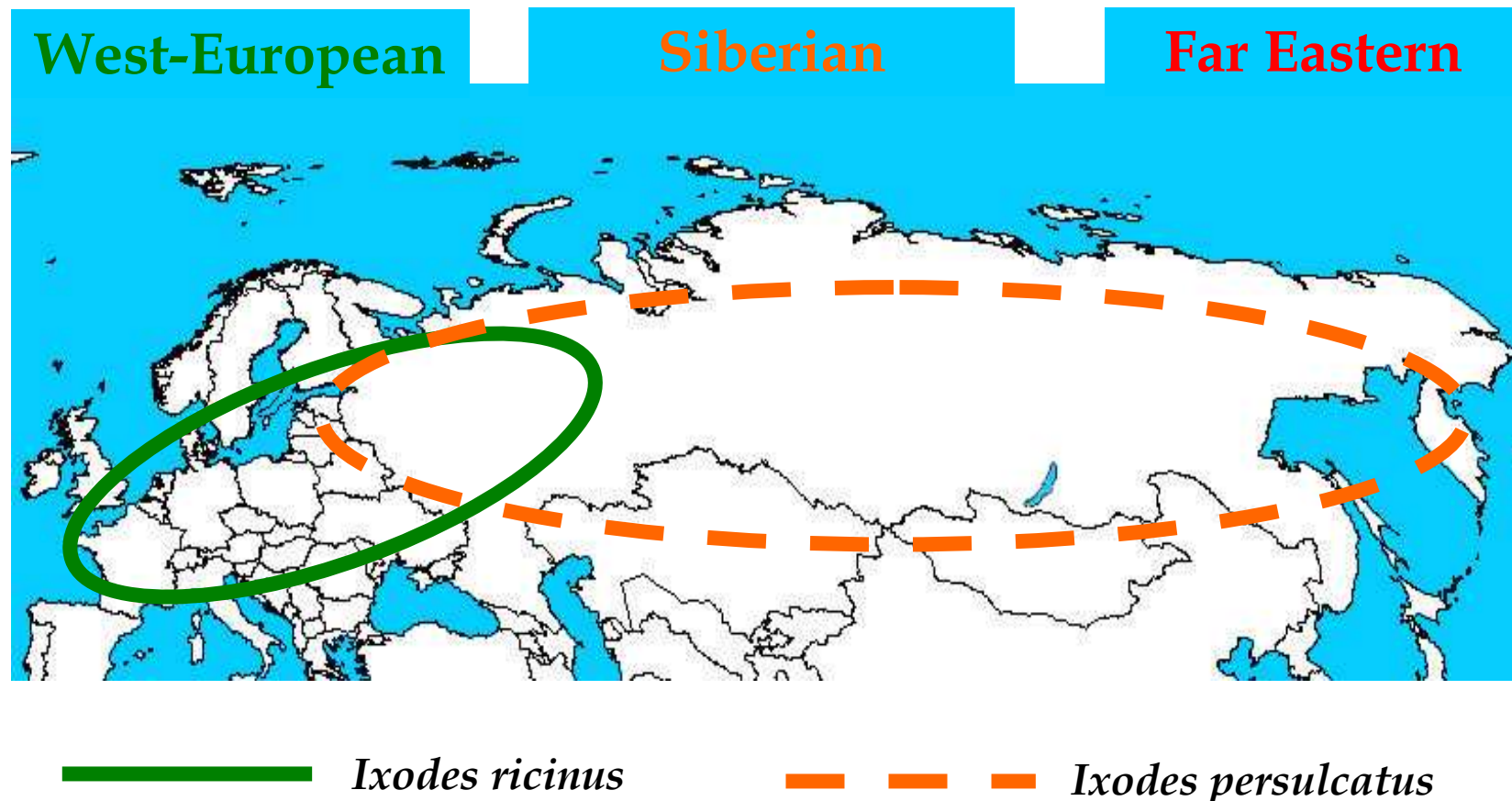
**CERTAIN POINT MUTATIONS IN THE ENVELOPE
PROTEIN OF TICK-BORNE ENCEPHALITIS VIRUS
(TBEV) ENHANCE NON-VIRAEMIC
TRANSMISSION EFFICIENCY IN A TICK VECTOR**

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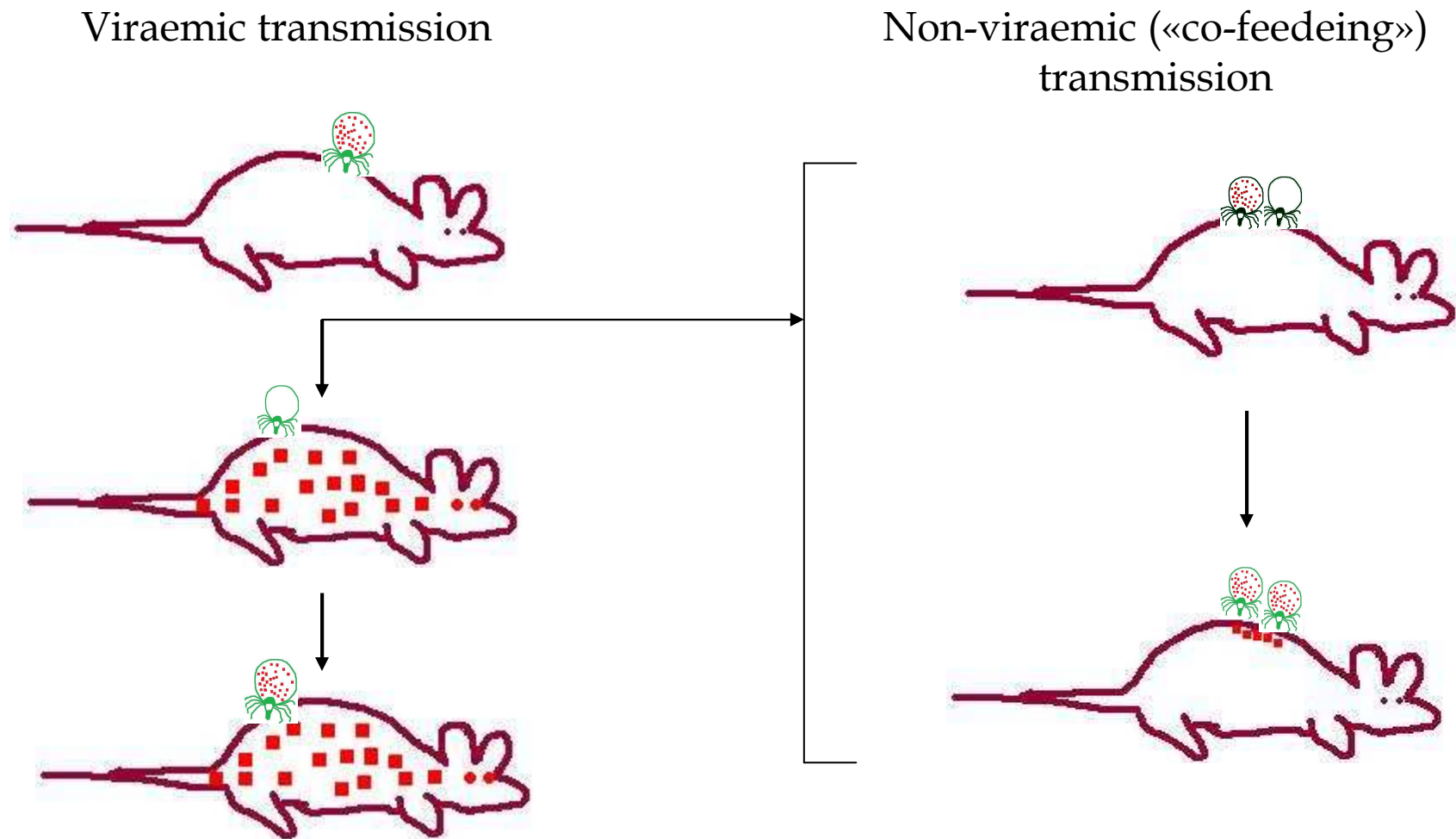
There are 3 subtypes of TBEV distributed across Eurasia.

Ixodes persulcatus is the main vector for Siberian and Far Eastern Subtype;

I. ricinus is the main vector for West-European Subtype



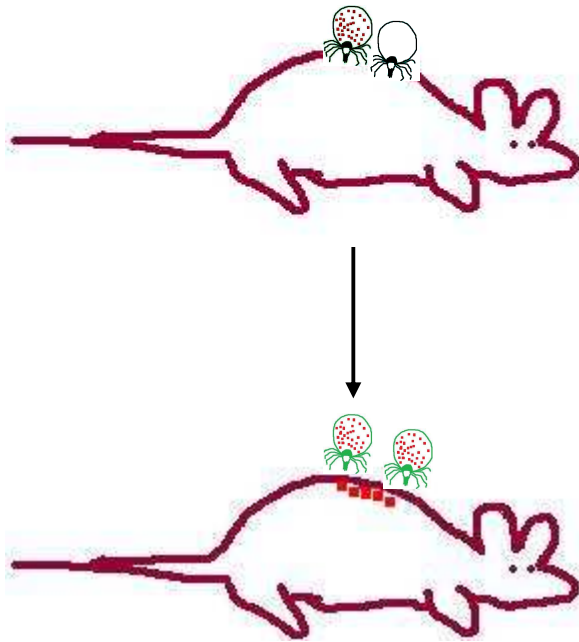
Effective transmission between the hosts is critical for virus survival in nature.



According to: L.Jones et al, 1987; M.Labuda, et al. 1996, 1997

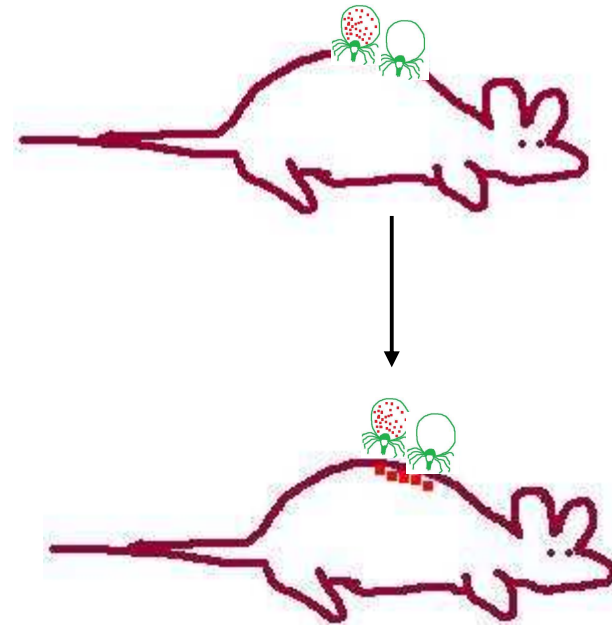
Non-viraemic transmission of different viruses between *I. ricinus* ticks goes with significantly unequal effectiveness.

WE subtype
(Hypr strain)



Transmission rate
60-80%

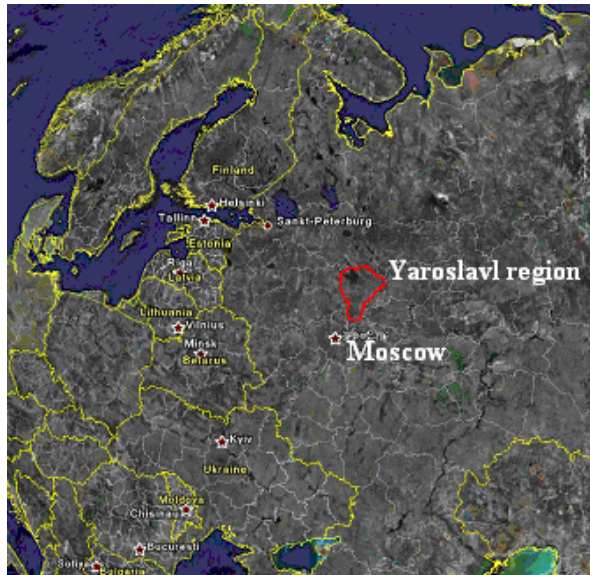
SIB subtype
(Vasilchenko strain)



Transmission rate
0-15%

The aim was to probe the role of envelope protein in the process of viral non-viraemic transmission

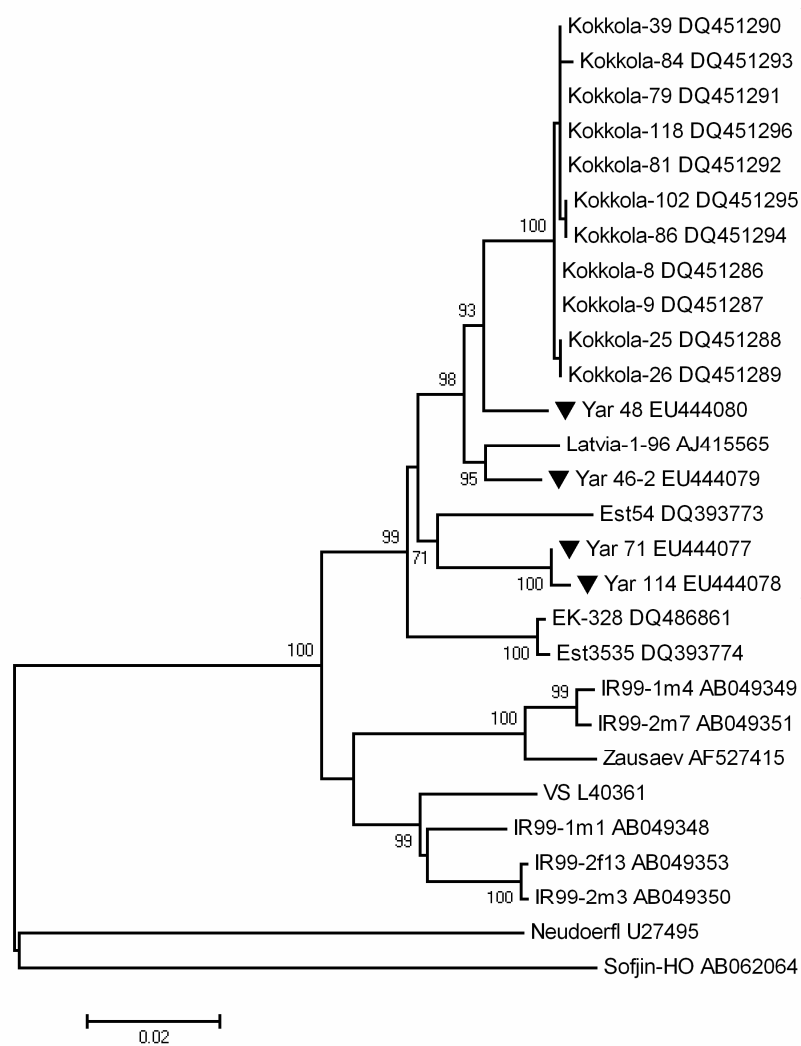
There are several low-passaged TBEV isolates that have unusual phenotype of virion surface.



Virus	Isolation source
Yar 46-2	TBEV patient
Yar 48	<i>Ixodes persulcatus</i>
Yar 71	<i>Ixodes persulcatus</i>
Yar 114	<i>Ixodes persulcatus</i>

- loss of haemagglutinating activity
- Small plaque phenotype in porcine kidney PS cell line
 - Low complement-fixing activity
- Relatively weak neutralisation by specific anti-TBEV serum

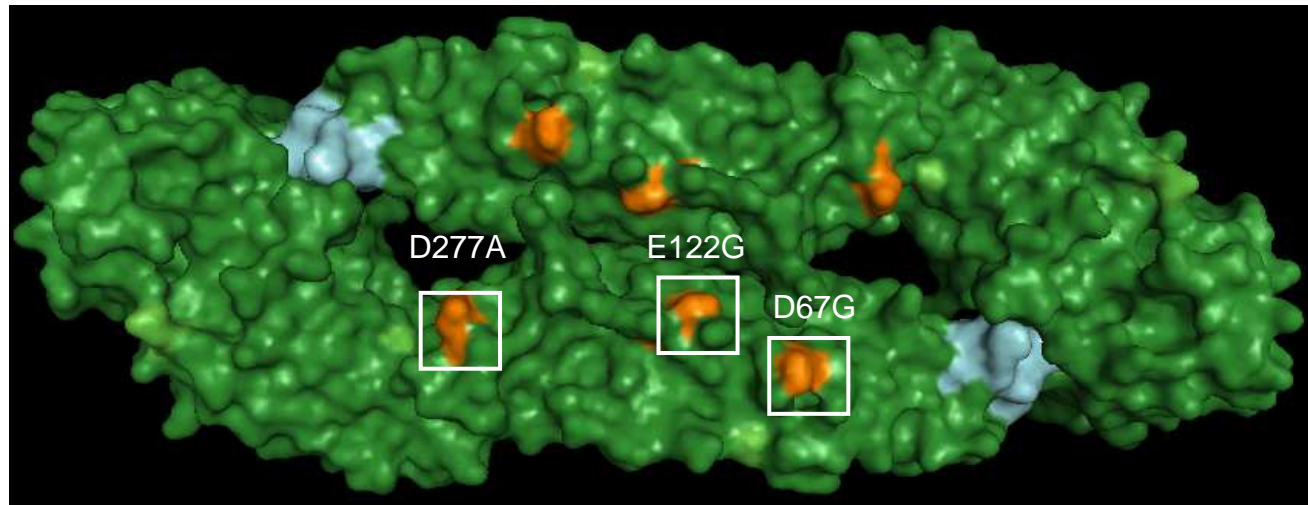
Sequence analysis for envelope protein gene



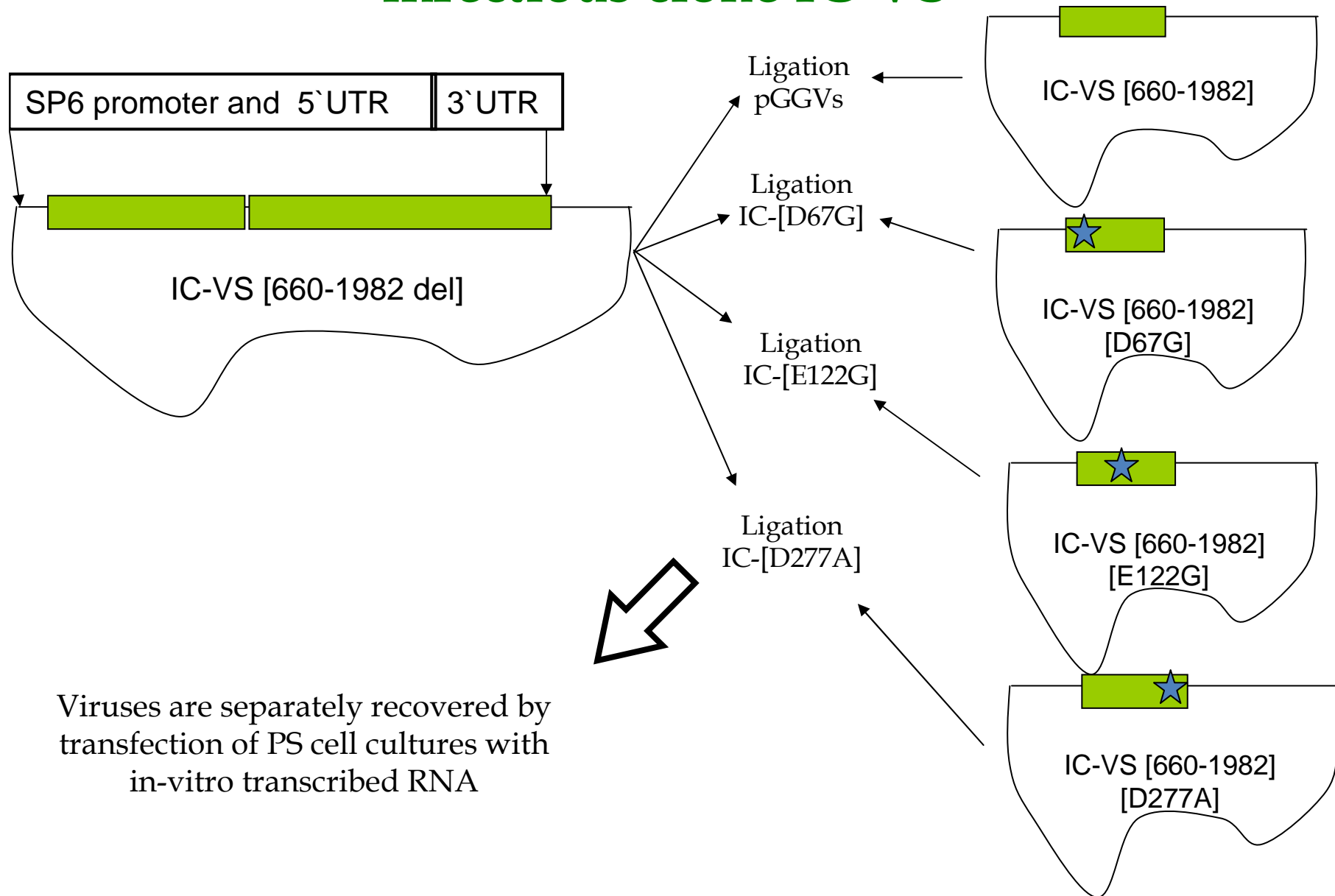
Baltic group

Virus\substitutions	E 67	E 122	E 175	E 277
Yar 71	D	G	N	D
Yar 46-2	G	E	N	D
Yar 48	D	E	N	A
TBE/EK-328	D	E	T	D

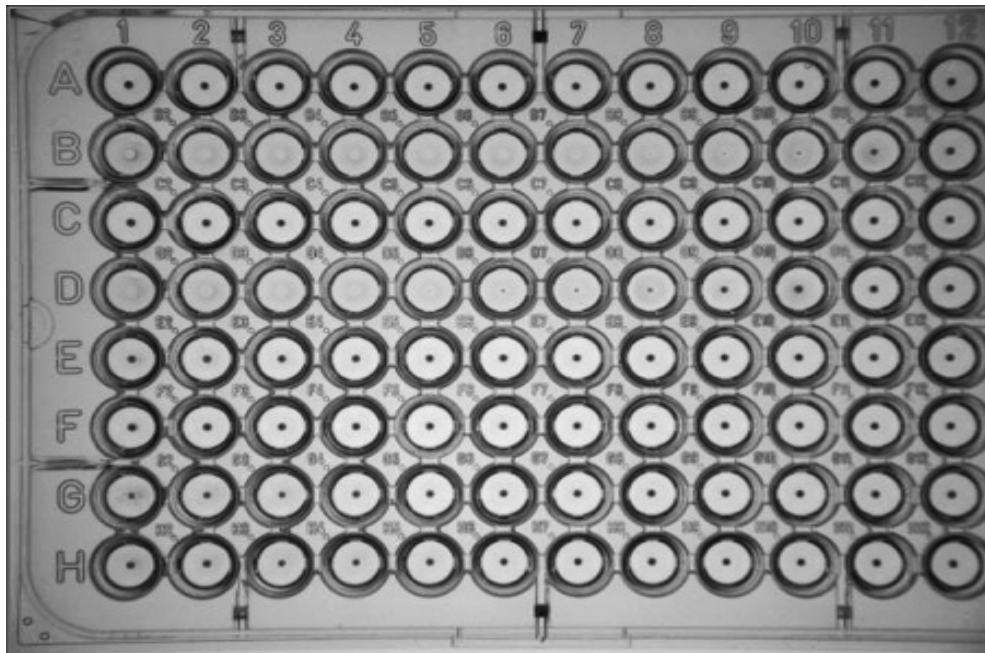
Localization of residues 67, 122 and 277 in crystal structure of TBEV envelope protein dimer



Reverse genomic procedure on the base of infectious clone IC-VS



Haemagglutinating activity of mutant viruses



Uninfected cell culture supernatant

HA control

Yar-71 (E122G)

pGGVsH

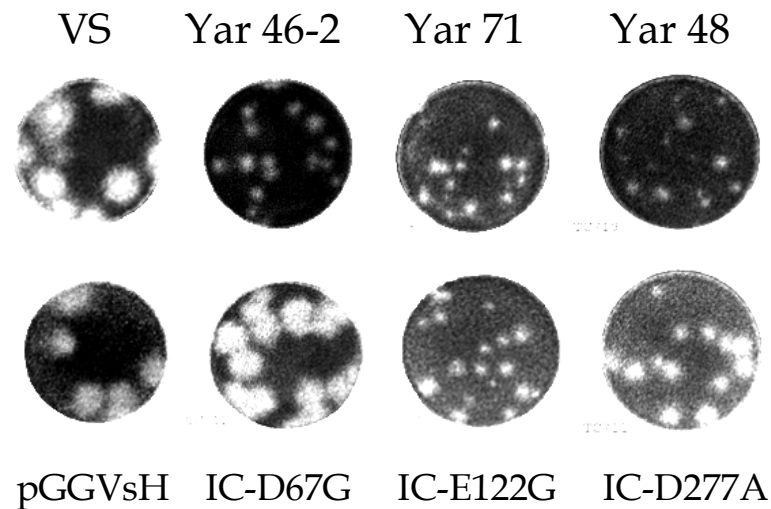
IC-E122G

IC-D277A

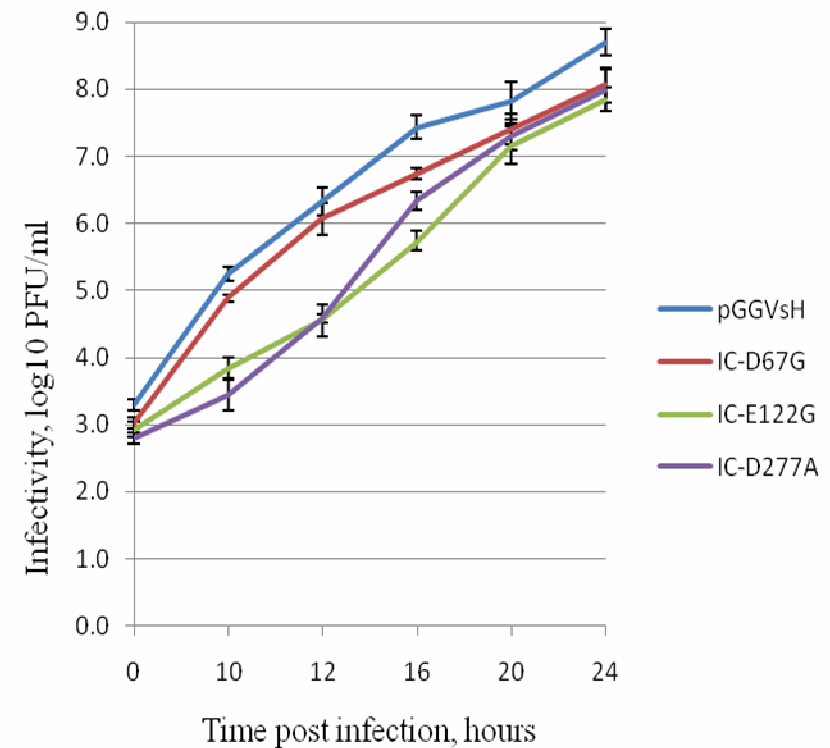
IC-D67G

Reproduction of mutant viruses in mammal cells.

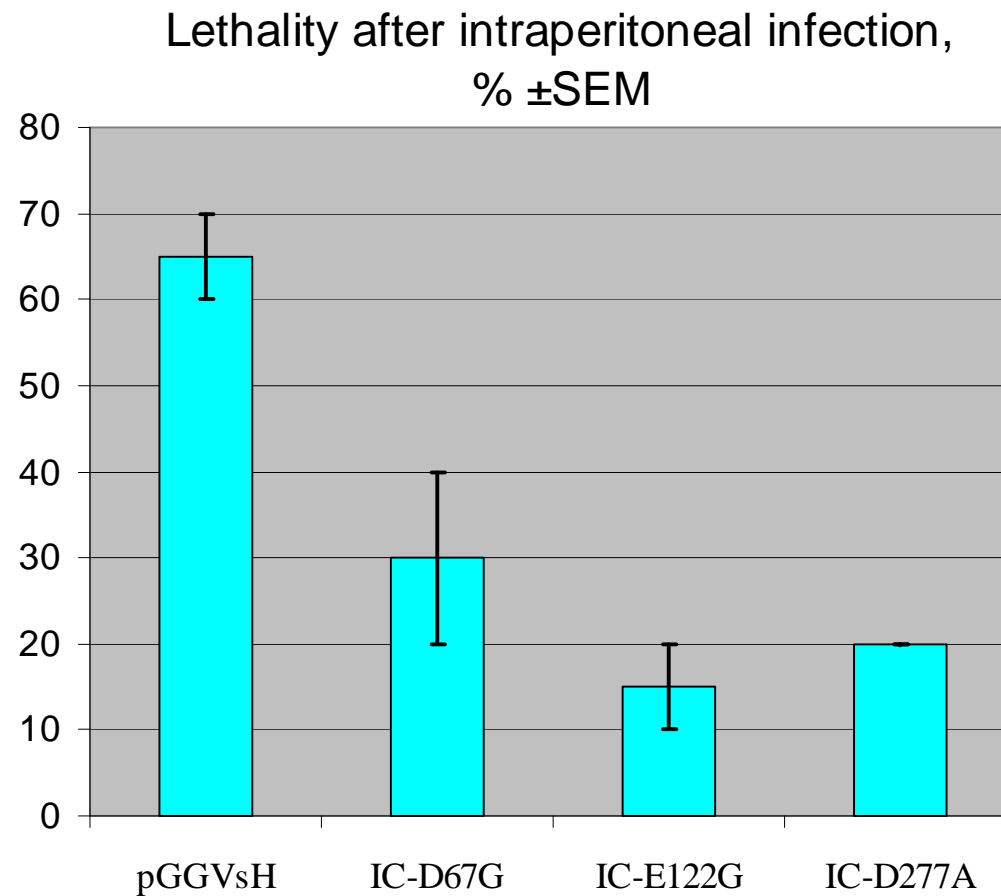
Plaque morphology in porcine kidney PS cell line



Reproduction dynamic in porcine kidney PS cell line lg PFU/ml \pm SEM

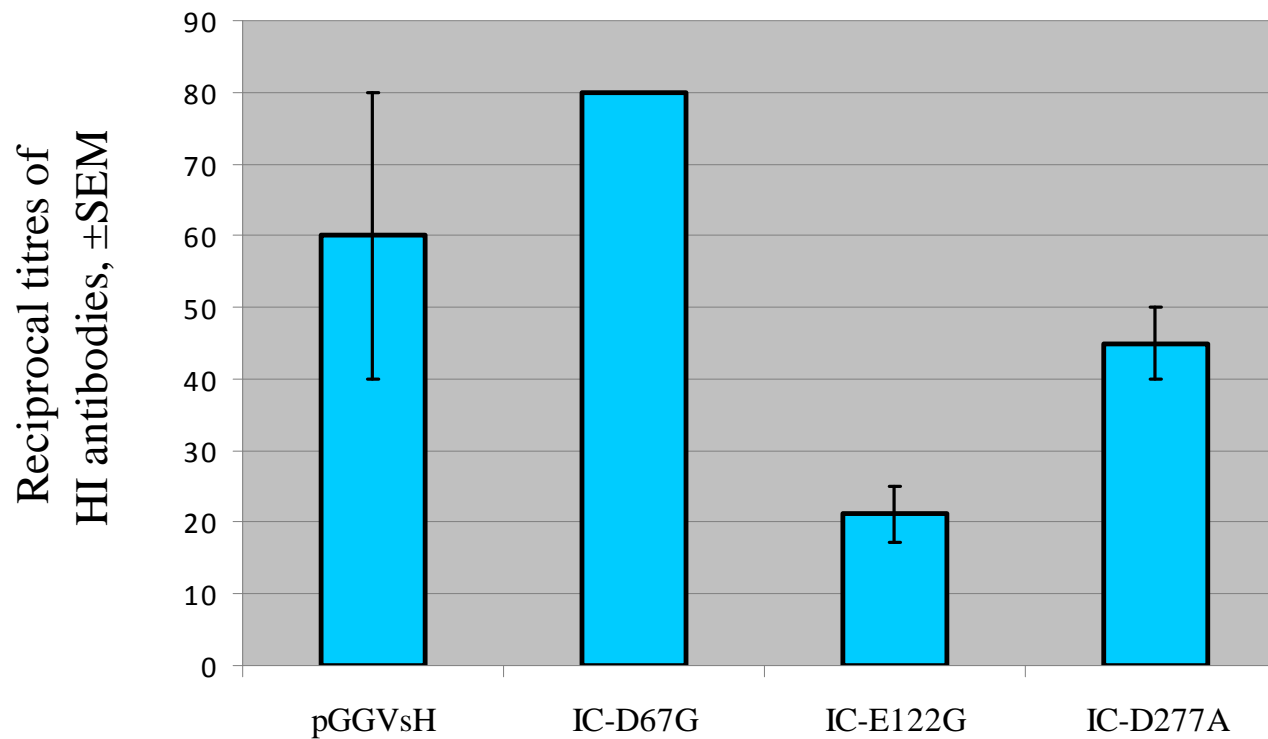


Virulence of the mutant viruses for mice

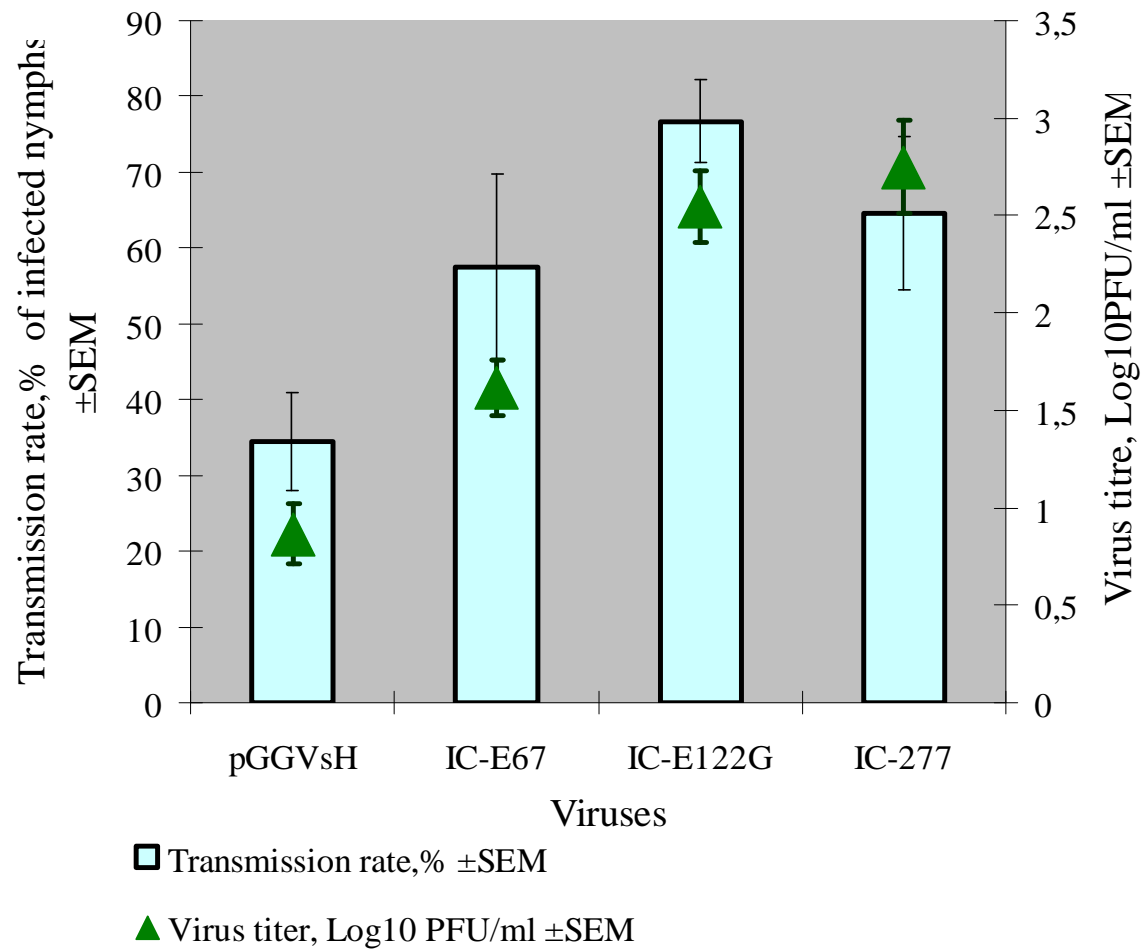


Virulence of the mutant viruses for mice

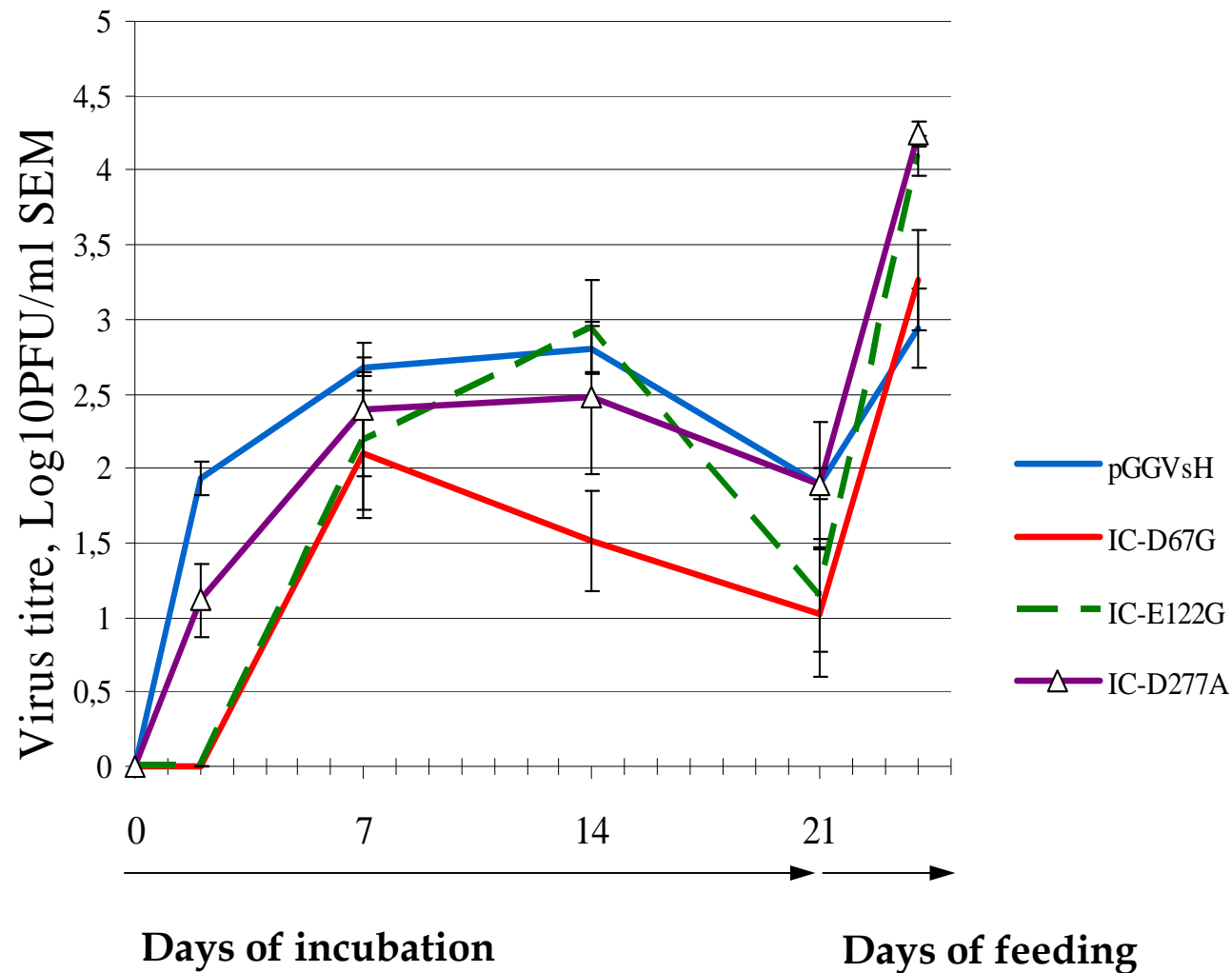
Production of HI antibodies after intraperitoneal infection



Co-feeding transmission and reproduction effectiveness in *I. ricinus* nymphs



Reproduction in salivary glands of *I. ricinus* females



Virus	Specific mutation	Isolation source
Yar 46-2	D67G	<i>Homo sapiens</i> TBE patient
Yar 48	D277A	<i>Ixodes persulcatus</i>
Yar 71	E122G	<i>Ixodes persulcatus</i>
Yar 114	E122G	<i>Ixodes persulcatus</i>

Analysis of 290 TBEV strains for presence of amino acid substitutions in E protein that result in the change of net charge and/or hydrophobicity of surface exposed residues.

In total, about 6% of TBEV exhibit similar mutations.

Mutation	TBEV strain	Accession No	TBEV subtype	Source of isolation	Region of isolation	Clinical manifestations (if known)
E67 (D/G)	Est2546	DQ393779	FE	Field mouse	Estonia	
	T-blood	AF091019	FE	TBE patient	Ural	Meningoencephalitis
	Ural-Nina	FJ214119	FE	TBE patient	Ural	Meningoencephalitis
	Ural-Ponomarev	FJ214118	FE	TBE patient	Ural	
	Ural-Belyaeva	FJ214117	FE	TBE patient	Ural	
	Ural-Antipov	FJ214115	FE	TBE patient	Ural	
	Volkhov-Khromov	FJ214114	FE	TBE patient	European Russia	Chronic encephalitis, lethal outcome
E67 (D/N)	RK 1424	AF091016	FE	<i>I. persulcatus</i>	Latvia	
E84 (E/K or E/G)	1486	EF469755	SIB	<i>I. persulcatus</i>	Siberia	
	4387/7	X76608	WE	<i>I. ricinus</i>	Slovakia	
E122 (E/G)	DXAL5	AY178833	FE	Not specified	China	
	KrM219	DQ988684	WE	Rodents	South Korea	
E155 (E/G)	272-75	AF231806	SIB	Vole	Siberia	
E170 (E/G)	Koltsovo-29	AF540032	FE	TBE patient	Siberia	
E203 (D/G)	DXAL-12 DXAL-13 DXAL-21	EU089977 EU089976 EU089980	FE	Not specified Not specified Not specified	China	

Discussion:

The mechanism of adaptation of TBEV to host organism utilizes the shift of charge/hydrophobicity at several aminoacid residues exposed on virion surface.

That shift result in different biological consequences depending of localisation of certain aminoacid residue.

Associated with efficient occupation
of tick organism

E122G and **D277A**

Virus reproduction in
ticks



viral transmission
between co-feeding ticks



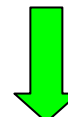
reproduction of TBEV
in mammal models



Associated with invasion into
mammalian organism

D67G

virus reproduction
in adult ticks



Association with
TBEV infection in mammals



reproduction of TBEV
in mammal models



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