

Molecular epidemiology of EU-genotype PRRSV in Europe: clues to PRRSV emergence, and implications for disease control

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The disease: PRRS

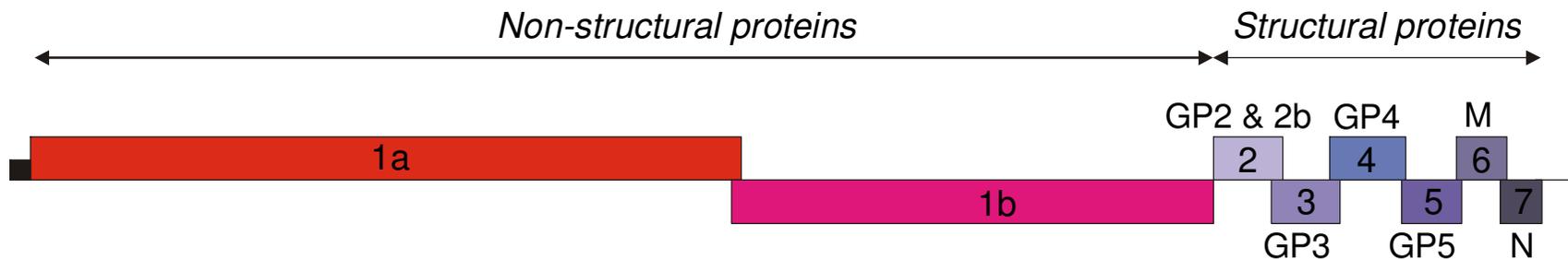
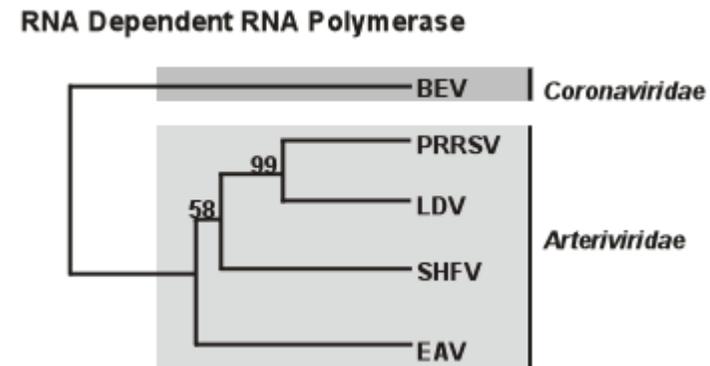
- North America
 - USA – 1987 (Keffaber, 1989)
 - Mystery swine disease
- Asia

Since 1993 in most of the swine producing countries

- Germany – 1990 (Van, 1991)
- Blue ear disease
- South America
 - Venezuela – 1996 (Dewey, 2000)

The virus: PRRSV

- The Netherlands: Lelystad virus (Wensvoort et al. 1991)
- USA: VR-2332 (Collins et al. 1992)
- ss RNA virus
- *Arteriviridae* family
 - *Arterivirus* genus
 - Replication in macrophages
 - Prolonged viremia
 - Persistent infection



Early evidence for PRRSV emergence

- North America
 - 1979 Canada (Carman et al., 1995) (antibodies)

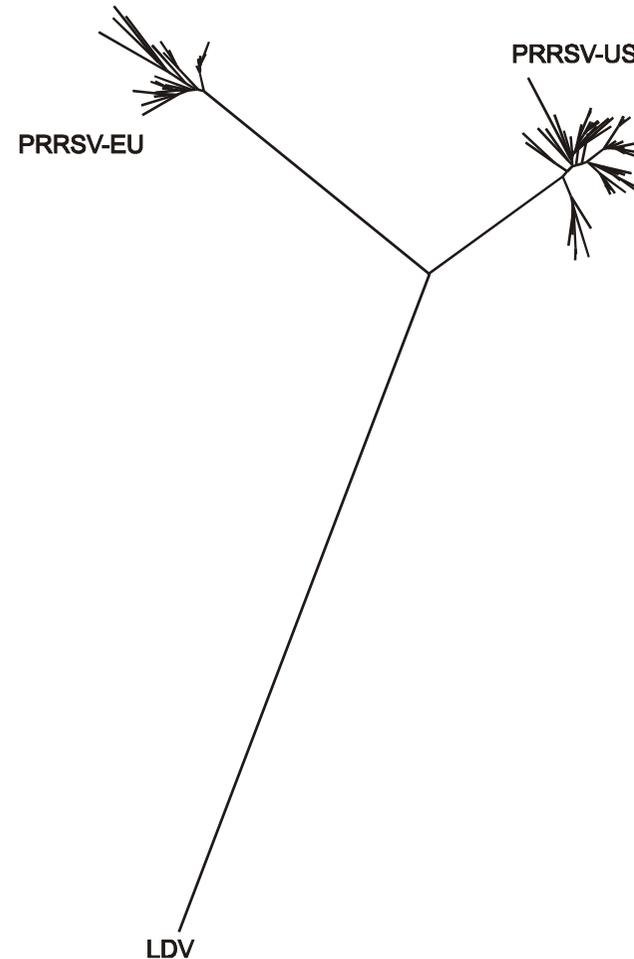
**PRRSV was in pig population
several years before the
disease emerged**

Philippines (Thanawongnuwech et al., 2003)

- Europe
 - 1987 former East Germany (Ohlinger et al., 2000) (antibodies)

Two genotypes of PRRSV

- Only 55-70% nucleotide identity in different genes
- European genotype (PRRSV-EU) – emerged in Europe
 - Mostly in Europe but also in America and Asia
 - Higher diversity in Europe and Asia than in North America
- American genotype (PRRSV-US) – emerged in North America
 - Mostly in America and Asia but also in Europe
 - Higher diversity in America and Asia than in Europe
- New terminology:
 - genotype 1 = EU
 - genotype 2 = US



Short communication

Phylogenetic relationships of European strains of porcine reproductive and respiratory syndrome virus (PRRSV) inferred from DNA sequences of putative ORF-5 and ORF-7 genes.

Paloma Suárez^a, Rafael Zardoya^b, M. Jesús Martín^c, Cinta Prieto^d, Joaquín Dopazo^e,
Alfredo Solana^a, José M^o Castro^{a,*}

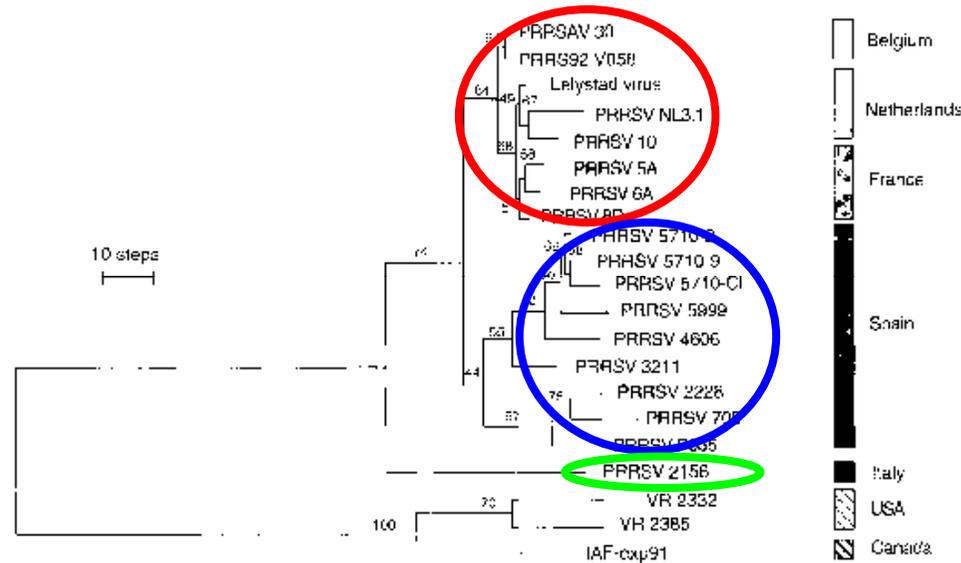
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European strains grouped in clades:

1. French, Dutch and Belgian
2. Spanish

Italian PRRSV - most closely related to the original outbreak of the disease in Europe

Identification of radically different variants of porcine reproductive and respiratory syndrome virus in Eastern Europe: towards a common ancestor for European and American viruses

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Lithuanian sequences expanded the known diversity range of European-type PRRSV:

- PRRSV-EU diversity exceeds PRRSV-US diversity.

Unique sequence markers of Lithuanian strains:

- Lithuanian ORF7 was 378 nucleotides, intermediate between prototypical EU-type (387 nucleotides) and US-type (372 nucleotides) ORF7 lengths.

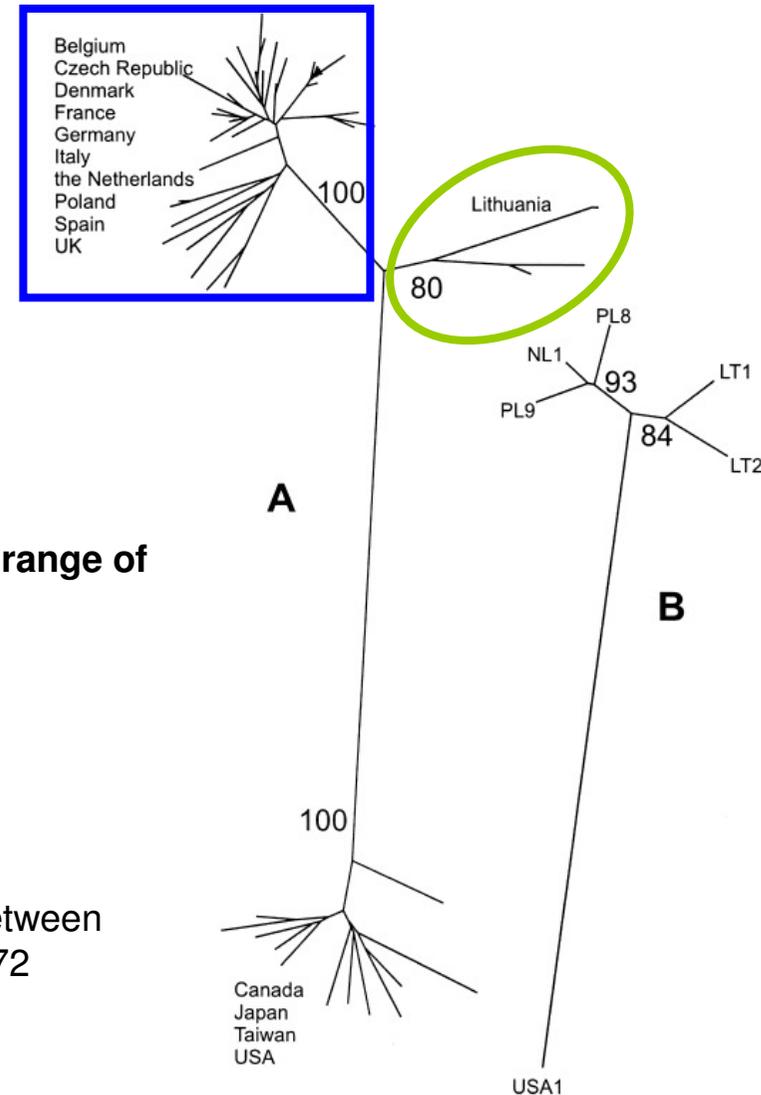


Fig. 2. Phylogenetic trees of EU-type and most diverse of US-type PRRSV sequences. The large tree (A) depicts the phylogenetic relationship of the ORF5 sequences. The small tree (B) was based on the ORF7 sequences.

	140	150	160	370	380	390
Lelystad	GGACAGGCCAA	---AAAGAAAAGCCTG		CTACATCCGCCAGTCAG	GGTGCAAGTTAA	
AuS-LithuaniaT...	TAA.....G....C.		.C.....T.....T..		
vr2332	...A.AAA..	TAAG.....C.G.		.AT.C.T.AGCATGA		

Porcine reproductive and respiratory syndrome virus strains of exceptional diversity in eastern Europe support the definition of new genetic subtypes

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²Novo Nordisk A/S, Virology and Molecular Toxicology, Novo Nordisk Park, 2760 Målev, Denmark

³S. N. Vysheslskij Institute of Experimental Veterinary Medicine, National Academy of Sciences of Belarus, 2 Vysheslskij Street, Minsk 223020, Belarus

- 31 sequences from 11 farms in Belarus
- Four genetic subtypes of PRRSV
- Exceptional diversity of ORF7: 373–378, 387 (prototype), 393 nucleotides long

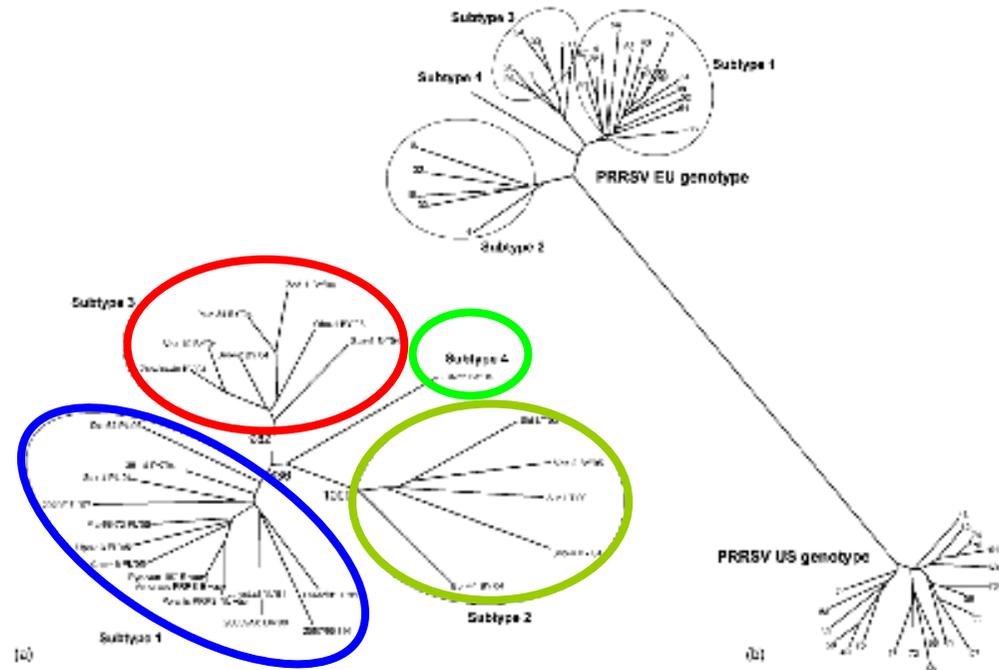


Fig. 2. Belarusian strains expand the diversity of EU genotype PRRSV beyond that of US genotype PRRSV. (a) EU genotype ORF7 tree. The bootstrap values adjacent to the main nodes represent the number of 1000 trees that supported the clustering. The Sid, Aus, Bor and Sio herds had trade links (replacement animals and semen). (b) Pan-PRRSV (EU + US genotype) ORF7 tree. Branch numbers correspond to the first column of Table 1.

	140	150	160	370	380	390
Lelystad	GGACAGGCC	AAAAAGAAAAGC	CTG	CTACATCCGCCAG	TCAG	GGTGCAAGTTAA
AuS-LithuaniaT.AATG.....C.		.C.....T.....T..		
ZaD-BelarusG---C...C.T..T...T..		
BoR-BelarusTCACG.....		.CG..C..AATGA.TT..		..C....C.C..TAG
vr2332	...A..AAAAT	..G.....C..G.		.AT..C..T.AGCATGA		

PRRSV in Russian Federation

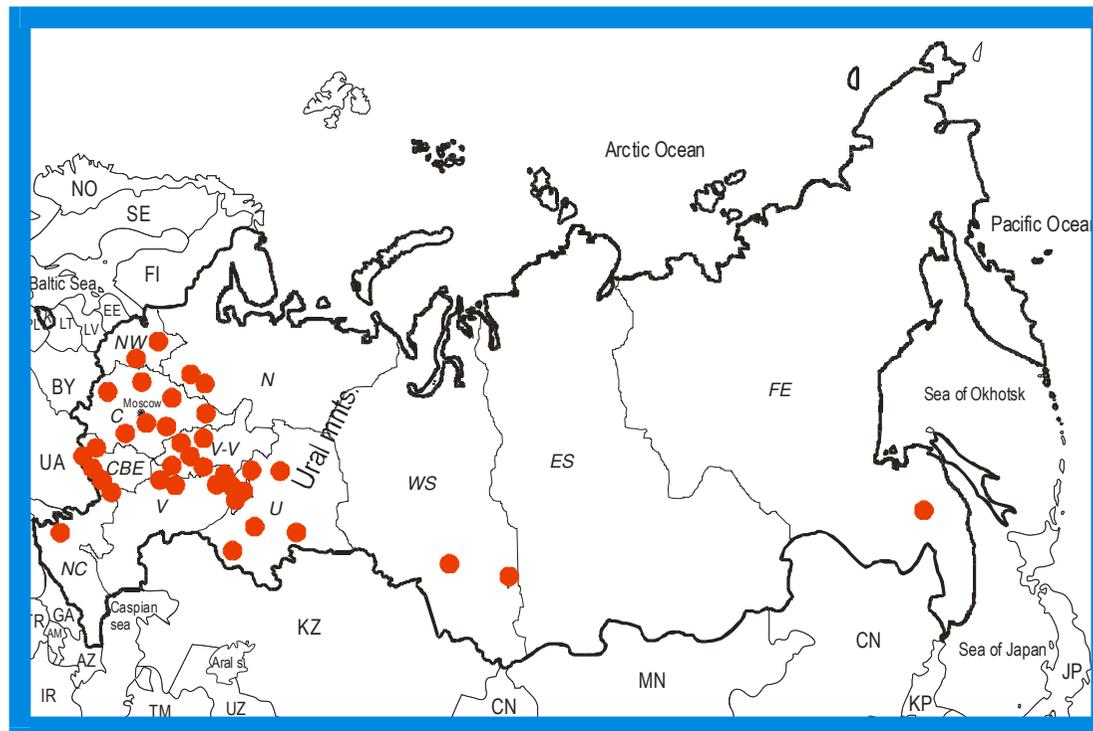
Definition of subtypes in the European genotype of porcine reproductive and respiratory syndrome virus: Nucleocapsid characteristics and geographical distribution in Europe

Tomasz Stadejek, Martin B. Oleksiewicz, Alexei V. Scherbakov, Anna M. Timina, Jonas S. Krabbe, Katarzyna Chabros, Denis Potapchuk

Archives of Virology *in press*

PRRSV in Russian Federation

- 57 sequences from 37 farms from 1996-2006, from EU part, Caucasus, Urals, Siberia and Far East
- Sow herds 15,000-120,000 heads
- Majority used Russian replacement stock (a few DK, CAN, UK, EE, BY, PL)
- Majority used Russian ML or inactivated vaccines (ARRIAH, NARVAC)



Summary on the diversity of PRRSV-EU

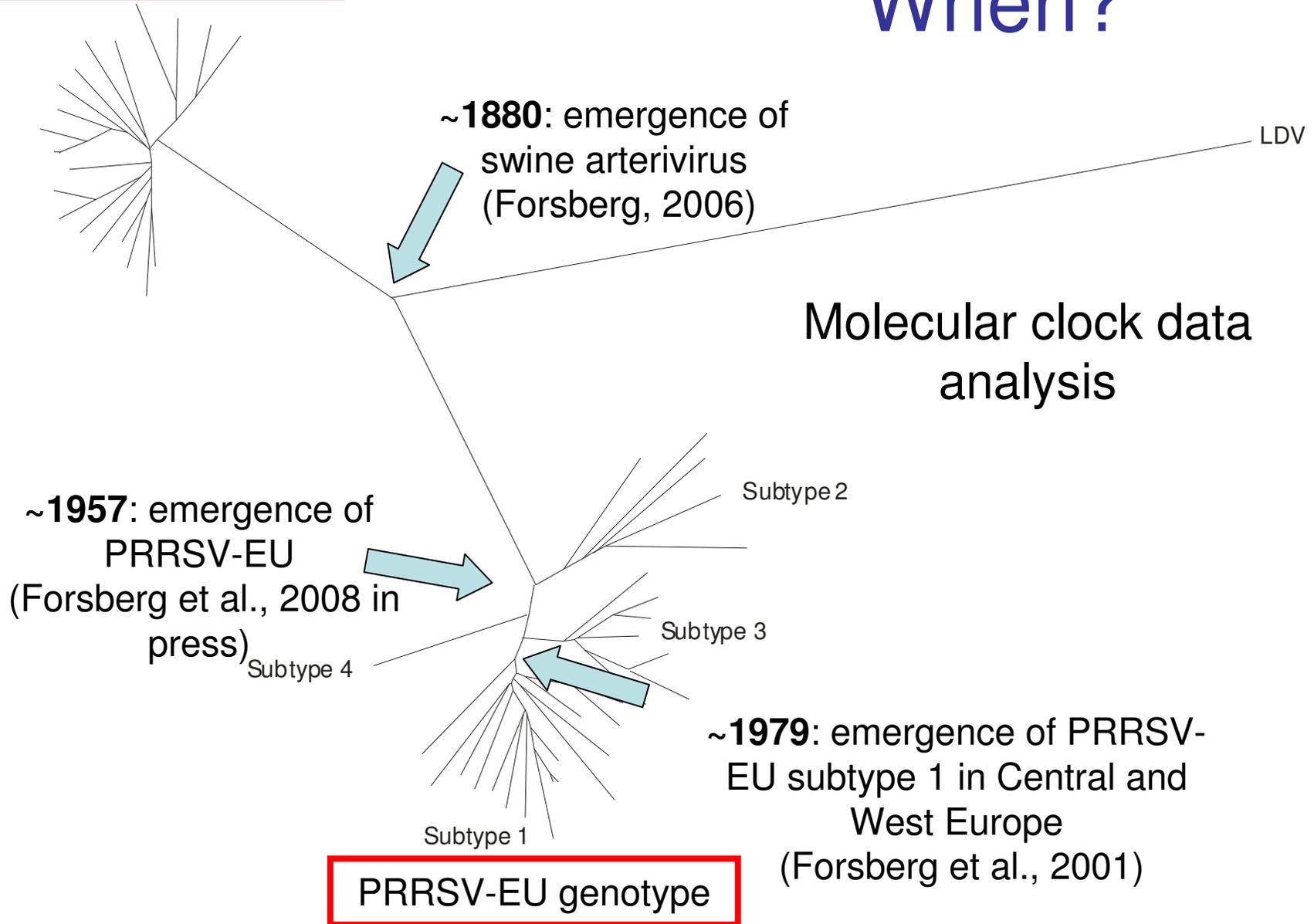
- PRRSV-EU consists of multiple genetic subtypes
 - „**classical**” **subtype 1** (includes all Western and Central European isolates and „Russian” subtype 1 variant(s))
 - **subtype 2** (includes Lithuanian, Belarusian and Russian isolates)
 - **subtype 3** (includes Belarusian and a single Russian isolates)
 - **subtype 4** (includes Belarusian and Latvian isolates)
 - **and possibly others**
- ORF7 size appears to be a unique marker for PRRSV-EU subtype

An outline map of Europe showing national borders. The map is centered on the continent and includes major islands like the British Isles, Ireland, and the Mediterranean islands. The text is overlaid on the central part of the map.

**When, where and how did
PRRSV-EU emerge?**

PRRSV-US genotype

When?



Where?

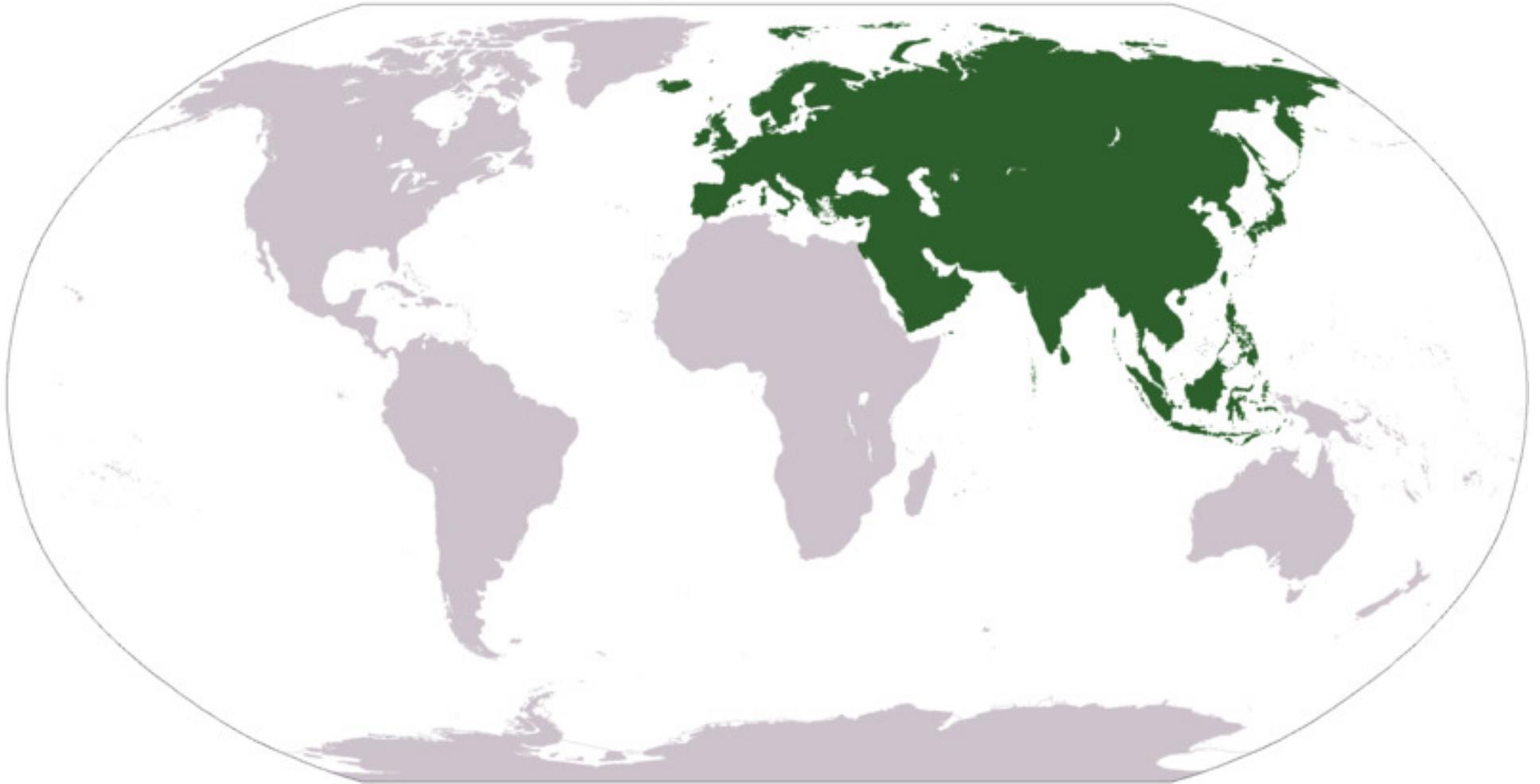
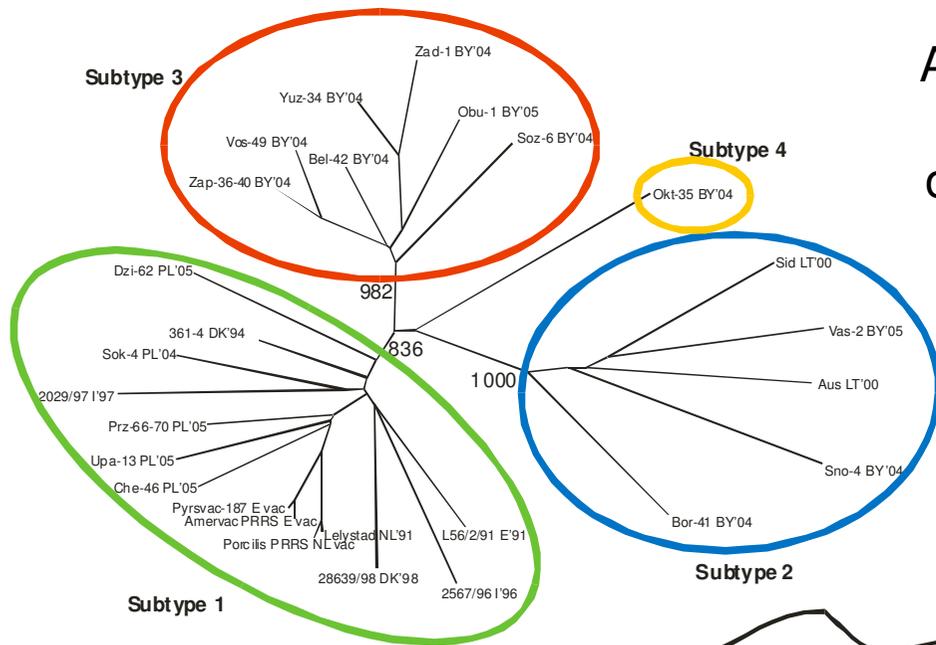
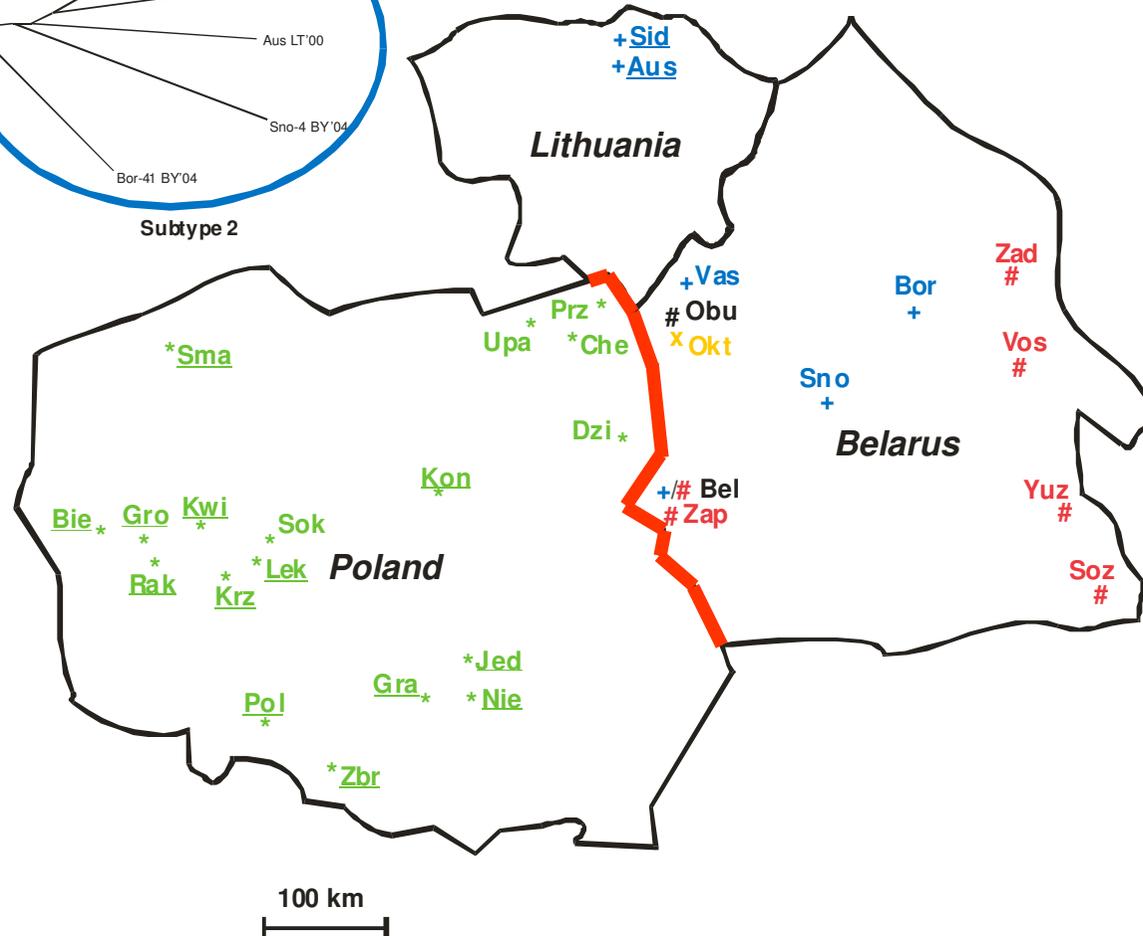


Figure 16. Independent States of the Former Soviet Union





A very sharp geographical demarcation of highly diverse EU genotype PRRSV is observed along the eastern Polish border (formerly the western border of Soviet Union)



Ancestral populations are expected to be more diverse than descendant populations

Observed diversity pattern supports hypothesis of PRRSV emergence in former Soviet Union

PRRSV strains were introduced by pig movement and not by emergence from some natural host

How?

- Pig production in Soviet Union/Russia
 - Pig production dropped from 27.7 mln pigs in 1928 to 4.4. mln in 1946 due to WWII
 - Restoration of pig production by import of breeding stock from Western and Central Europe – return to pre-WWII values in 1953
 - Breeding programs to cross Western European and local breeds
 - Construction of very big industrial farms (100 000 sows) populated by Soviet made breeds in the 1970-ies
 - Mass import of gilts and breeding stock from Western and Central Europe, North America since 2004

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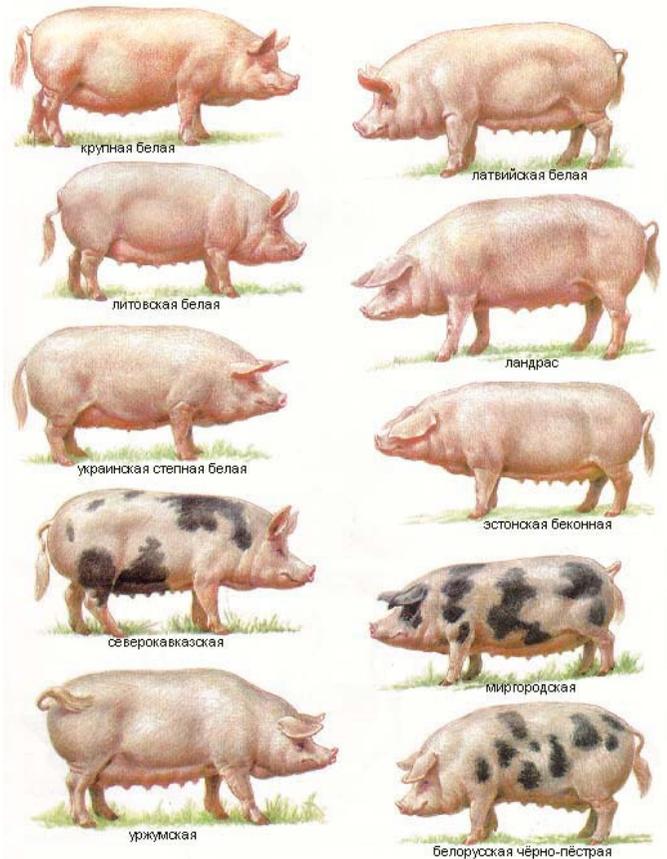
P.O.Box 71 11 80, D-30545 Hannover, Germany

email: [Joern.Wrede \(at\) tiho-hannover.de](mailto:Joern.Wrede@tiho-hannover.de) , Fax: +49/511-953-8582

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Pig breeds in former Soviet Union



North Caucasus (est. 1955) – Large White x Berkshire x Edelschwein x **local Kuban**

Urzhum (est. 1957) – Large White x **local**

Kemerovo (est. 1961) – Berkshire x Large White x Large Black x **Siberian Black Pied**

Semirechensk (est. ?) – Large White x **Kemerovo x Asiatic Wild**

Siberian (est. ?) – Large White x **local Siberian**

Tsivilsk (est. ?) – Large White x **local Chuvash**

Crossbreeding with local East European, Asian or Caucasian breeds and construction of big pig farms created an environment allowing a new virus to emerge, or an already emerged virus to spread

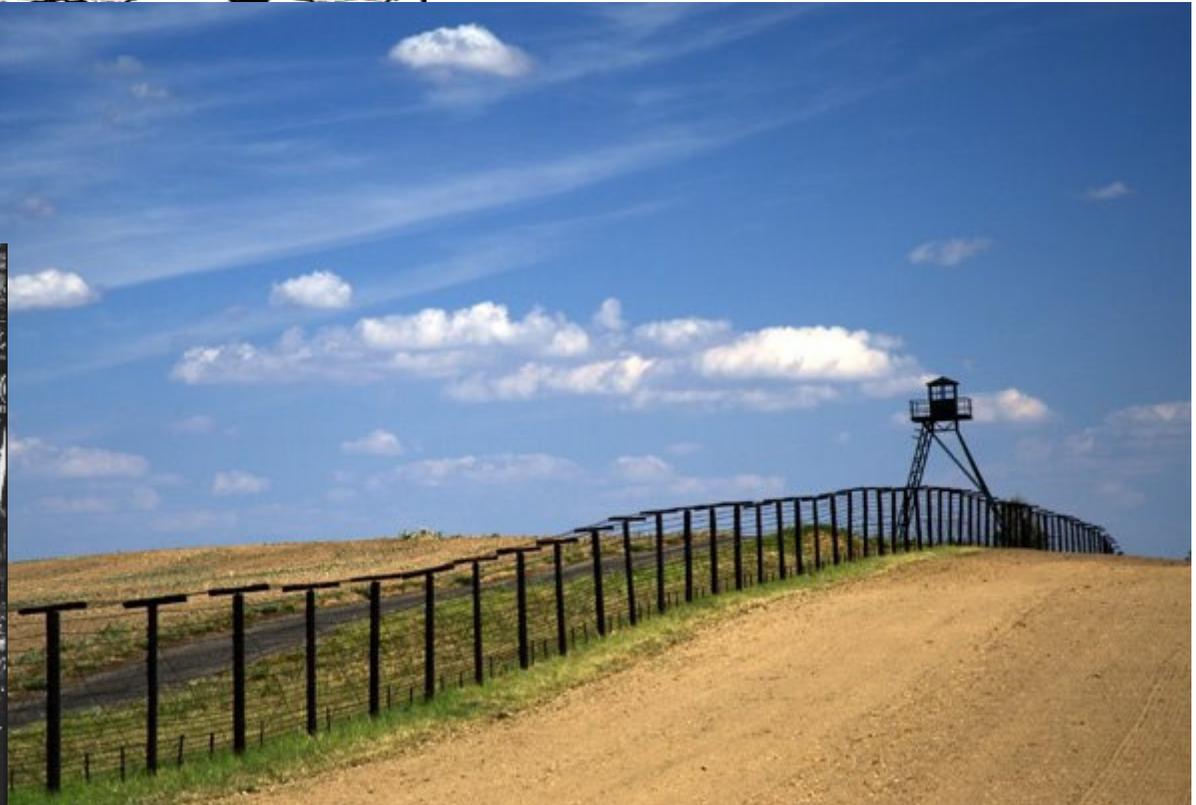
Figure 16. Independent States of the Former Soviet Union



How was PRRSV transmitted
to Western Europe?



Europe 1945-1989



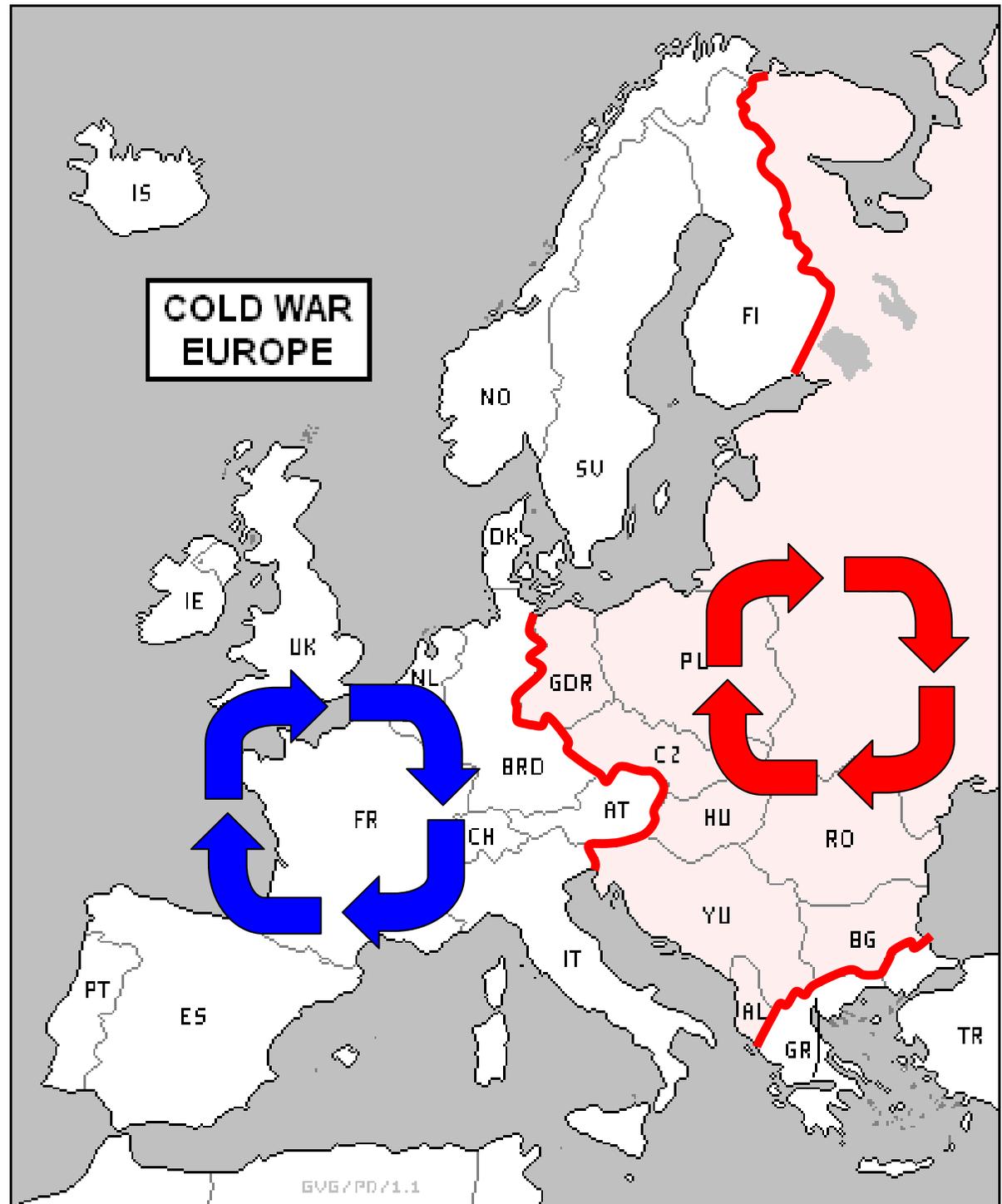
Pig movements

Limited imports of breeding stock from the West to the East due to economical reasons

Very limited export from the East to the West due to sanitary reasons

Often exchange of the breeding material between countries in the West and in the East

If porcine arterivirus existed in Soviet Union it could spread in Easter block





Germany reunification could possibly merge two completely different pig populations

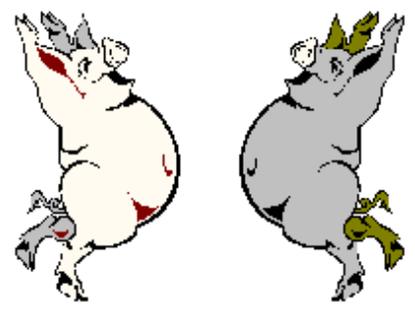
DR



What about pigs from East and West Germany?



1949-1990



1990



Pigs in the former German Democratic Republic

General information:

International breed name: **German Large White**

Local breed name: **Deutsches Edelschwein**

Breed status: **carried over by another breed**

Group of similar breeds: Main group **White breeds with erect ears**; Subgroup [Large White / Yorkshire descendants](#)

Main location: **Mecklenburg, Thüringen, Sachsen-Anhalt**

Breed society: **Zentralverband der Deutschen Schweineproduktion e.V., Germany**

Origin and development:

Composite of Large White types: [German Large White \(Germany\)](#), [Large White \(Russian Federation\)](#), Large White (Hungary); introgression since 1980 from [Latvian White \(Latvia\)](#), Yorkshire (Hungary), Yorkshire (Hungary)

Herdbook established: **1954**



Pigs in Germany

General information:

International breed name: **German Large White**

Local breed name: **Deutsches Edelschwein**

Breed status: **acknowledged**

Group of similar breeds: Main group **White breeds with erect ears**; Subgroup [Large White / Yorkshire descendants](#)

Main location: **country-wide**

Breed society: **Hannoversche**

Erzeugergemeinschaft für Zuchtschweine (HEZ), Wilhelm-Seedorf-Straße 1, D-29525 Uelzen, Germany, Fax: +49-581-18563 , Internet:

<http://www.hez-uelzen.de>

Origin and development:

Since 1993 East German breed was included;

composite of local landraces, Large White, [Middle White \(United Kingdom\)](#),

[German Large White \(The Former German Democratic Republic\)](#); introgression since 1980 from Groot Yorkshire (Netherlands), [Large White \(United Kingdom\)](#), Yorkshire (Scandinavia)

Herdbook established: **1904**

Summary

- Following evidence supports hypothesis of Eastern European origin of PRRSV:
 - Presence of PRRSV specific antibodies in East Germany from 1987 (Ohlinger et al. 2000)
 - Presence of PRRSV in Primorskiy Krai of former Soviet Union (Far East Asia) in 1986 (Grebennikova et al., 2005)
 - Extremely high genetic diversity of PRRSV and presence of phylogenetically oldest strains in countries formerly being part of Soviet Union
 - PRRSV epidemic in West Germany that coincided with Germany reunification

Consequences of PRRSV-EU diversity

	10	20	30	40	50	60	70	80	90	100													
Lelystad NL 1991	ATG	GCC	GTAAA	ACCAG	CAGCC	AGAAA	AGAA	---AAAG	TACAG	TCCGAT	GGGGAA	ATGG	CCAG	CCAG	TCA	TA	CTACT	GTGCC	AGTTG	CTGG	GTG	CAA	
Prz PL 2005	GG	G	---	T	A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	C
KT CZ 2006	G	---	---	A	T	A	---	---	---	---	---	---	---	---	---	---	---	G	---	C	A	AT	---
FR-2000	G	---	AC	---	A	T	GAG	---	G	---	---	---	---	---	---	---	---	C	---	T	---	---	GAA
RV-2003	G	---	C	---	GA	---	---	---	A	---	---	---	---	---	---	---	---	---	---	---	---	---	CAAG
KM-1999	GG	C	A	G	---	G	C	---	A	---	---	---	---	---	---	---	---	C	A	---	---	---	AAG
DZ-2000	G	T	C	C	---	A	G	---	T	---	AC	---	A	---	---	---	---	G	---	T	---	---	CAA
Bor BY 2004	G	---	C	---	G	T	---	AC	---	---	---	---	---	---	---	---	---	G	---	T	---	---	AA
Soz-F3-19 BY 2006	G	---	C	---	GTT	G	---	AC	---	---	---	---	---	---	---	---	---	G	---	T	---	---	CAA
KR-2000	G	---	C	---	CTTC	G	---	---	A	---	---	---	---	---	---	---	---	G	---	T	---	---	AA
Okt BY 2004	T	---	C	---	C	G	---	---	A	GT	---	---	---	---	---	---	---	---	---	---	---	---	AAG
VL-2006	C	---	C	---	A	C	A	TCA	---	AC	---	---	---	---	---	---	---	G	---	---	---	---	CAA

	110	120	130	140	150	160	170	180	190	200															
Lelystad NL 1991	TGATA	AAAGT	CCAGC	GC	CAAC	CTAG	GGG	AGG	CAGGC	---	CAAAA	AGAAA	AGCC	CTG	AGAG	CCAC	ATT	TTCC	CCCT	GGT	GTCT	GAA	GAT	GAC	ATCG
Prz PL 2005	G	---	CT	---	G	TT	G	AA	---	---	G	---	---	---	---	---	---	C	---	T	A	---	---	---	
KT CZ 2006	G	---	---	---	G	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	G
FR-2000	A	---	T	---	A	---	G	A	---	A	---	G	---	A	---	---	---	---	---	---	---	---	---	---	T
RV-2003	T	T	---	A	---	C	---	G	---	A	G	G	---	G	---	A	---	---	---	---	---	---	---	---	T
KM-1999	G	AA	---	G	---	G	---	CCT	---	CAT	---	GA	G	---	A	---	---	---	---	---	---	---	---	---	TG
DZ-2000	---	---	---	---	A	---	---	CAA	---	G	---	---	---	---	---	---	---	---	---	---	---	---	---	---	G
Bor BY 2004	G	A	---	C	---	---	---	TCA	---	G	---	---	---	A	---	---	---	---	---	---	---	---	---	---	G
Soz-F3-19 BY 2006	C	GAA	---	C	---	A	---	G	---	A	---	T	---	A	---	---	---	---	---	---	---	---	---	---	TG
KR-2000	T	AA	---	C	---	---	---	G	---	G	---	---	---	---	---	---	---	---	---	---	---	---	---	---	TG
Okt BY 2004	G	---	---	A	---	T	---	---	---	G	---	---	---	---	---	---	---	---	---	---	---	---	---	---	T
VL-2006	G	---	T	---	T	---	GC	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	TG

	210	220	230	240	250	260	270	280	290	300																
Lelystad NL 1991	GCACC	ACTC	ACCC	GACT	GAAC	CTCC	CTCT	GTG	CAAT	CGAT	CCAG	ACGG	CCCTT	TCA	TCA	ATCA	AGGC	CAGG	AACT	CGT	CGCT	TTT	CAT	CCAG	GGG	GAAG
Prz PL 2005	T	---	C	---	T	---	T	---	T	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	T
KT CZ 2006	T	T	---	T	---	C	---	G	---	T	---	T	---	C	---	---	---	---	---	---	---	---	---	---	---	---
FR-2000	T	T	---	A	---	G	---	T	---	TC	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	TG
RV-2003	T	---	---	T	---	T	---	C	---	---	---	C	---	T	---	---	---	---	---	---	---	---	---	---	---	AA
KM-1999	T	---	---	TCA	---	G	---	T	---	T	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	C
DZ-2000	T	---	---	TCA	---	G	---	T	---	TC	---	A	---	C	---	---	---	---	---	---	---	---	---	---	---	A
Bor BY 2004	T	---	---	TCA	---	G	---	T	---	TC	---	A	---	C	---	---	---	---	---	---	---	---	---	---	---	G
Soz-F3-19 BY 2006	T	---	---	A	---	AG	---	T	---	T	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	GAA
KR-2000	---	---	---	GGA	---	T	---	T	---	TC	---	A	---	C	---	---	---	---	---	---	---	---	---	---	---	C
Okt BY 2004	A	---	---	T	---	G	---	A	---	T	---	TC	---	A	---	---	---	---	---	---	---	---	---	---	---	A
VL-2006	T	---	---	A	---	---	---	T	---	T	---	TC	---	C	---	---	---	---	---	---	---	---	---	---	---	G

	310	320	330	340	350	360	370	380	390													
Lelystad NL 1991	GTC	AGTTT	CAG	TTG	AGTTT	ATG	TGCC	GGT	TGCT	CATAC	AGTGC	CGCT	GATTC	CGCT	GACTT	CTAC	ATCC	GCAG	TCCAG	GGTG	CAAG	TTAA
Prz PL 2005	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
KT CZ 2006	A	---	G	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
FR-2000	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
RV-2003	T	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
KM-1999	---	---	---	A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DZ-2000	---	---	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Bor BY 2004	A	---	T	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Soz-F3-19 BY 2006	C	---	---	C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
KR-2000	---	---	---	T	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Okt BY 2004	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
VL-2006	---	---	---	A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

PCR?

Very high nt diversity of ORF7 affects diagnostic RT-PCR primer binding sites

May influence sensitivity of diagnostic RT-PCR assays

To be determined

Vaccines?

Impact of genetic diversity of European-type porcine reproductive and respiratory syndrome virus strains on vaccine efficacy

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¹ Laboratory of Prionology, Faculty of Veterinary Medicine, Ghent University, Coupure links 653, 9000 Ghent, Belgium

² Biological Research Department, Interior International BV, P.O. Box 21, 5820 LL Breda, The Netherlands

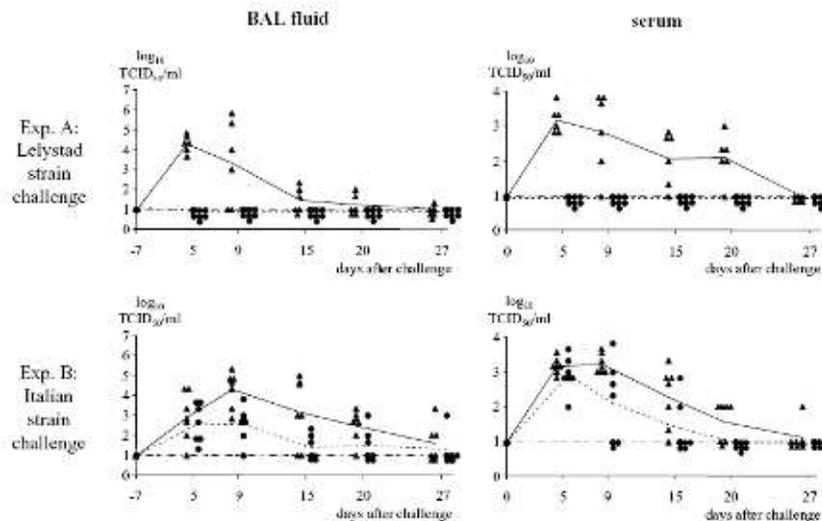


Fig. 2. Course of PRRSV titres in broncho-alveolar lavage (BAL) fluids and in serum samples after challenge with either the Lelystad strain or the Italian strain. Triangles represent individual virus titres of non-vaccinated control pigs. Dots represent individual virus titres of vaccinated pigs. Lines represent group means (—) non-vaccinated pigs; (---) vaccinated pigs. The horizontal dotted line represents the detection limit.

- All vaccinated pigs remained **free of virus** after Lelystad-like challenge (**98% identity of vaccine and challenge virus in ORF5**)
- All vaccinated **pigs had virus** in BAL and serum after Italian-like challenge (**84% identity of vaccine and challenge virus in ORF5**)

Genetic diversity within EU-type PRRSV may affect the efficacy of the current EU-type vaccines

The most dissimilar ORF5 sequences of PRRSV-EU can share only ~75% identity!!!

What is the geographical distribution of
PRRSV-EU subtypes ?

Studies on genetic diversity of PRRSV in Europe

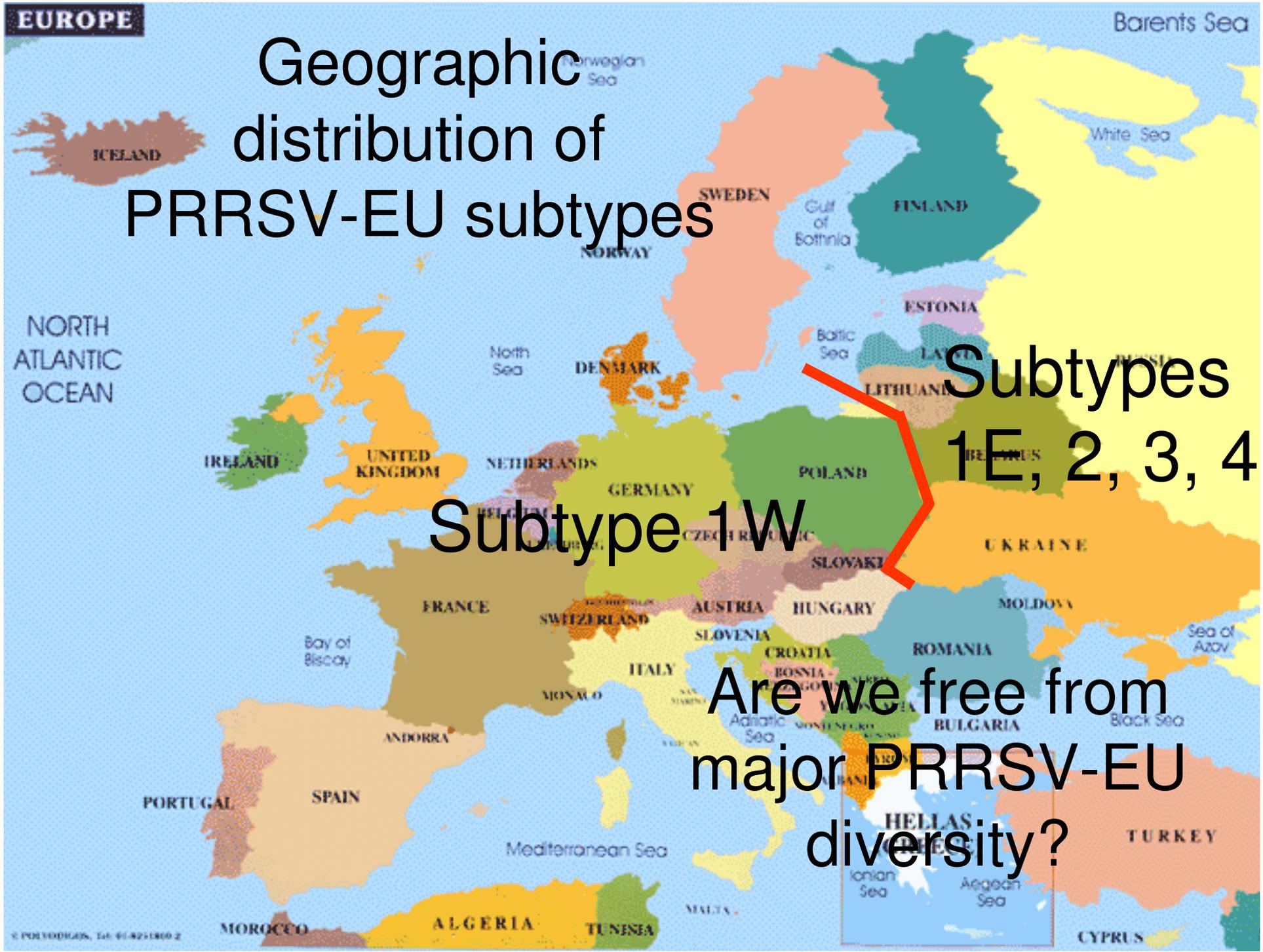
1. **Suarez, P., Zardoya, R., Martin, M. J., Prieto, C., Dopazo, J., Solana, A. & Castro, J. M. (1996).** Phylogenetic relationships of European strains of porcine reproductive and respiratory syndrome virus (PRRSV) inferred from DNA sequences of putative ORF-5 and ORF-7 genes. *Virus Res* **42**, 159-165
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We may not have the complete picture, as sensitivity of existing RT-PCR assays for highly divergent PRRSV-EU strains is unknown

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EUROPE

Geographic distribution of PRRSV-EU subtypes



Subtypes 1E, 2, 3, 4

Subtype 1W

Are we free from major PRRSV-EU diversity?



New insights into the genetic diversity of European porcine reproductive and respiratory syndrome virus (PRRSV)

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- 66 strains from 1991–2002
- There is no stringent correlation between date and location of virus detection
- The phylogenetic analysis of 66 European PRRS strains from 1991–2002 revealed a continuous genetic drift
- More isolates like 24717 is expected to evolve in the future

24717 D 1999

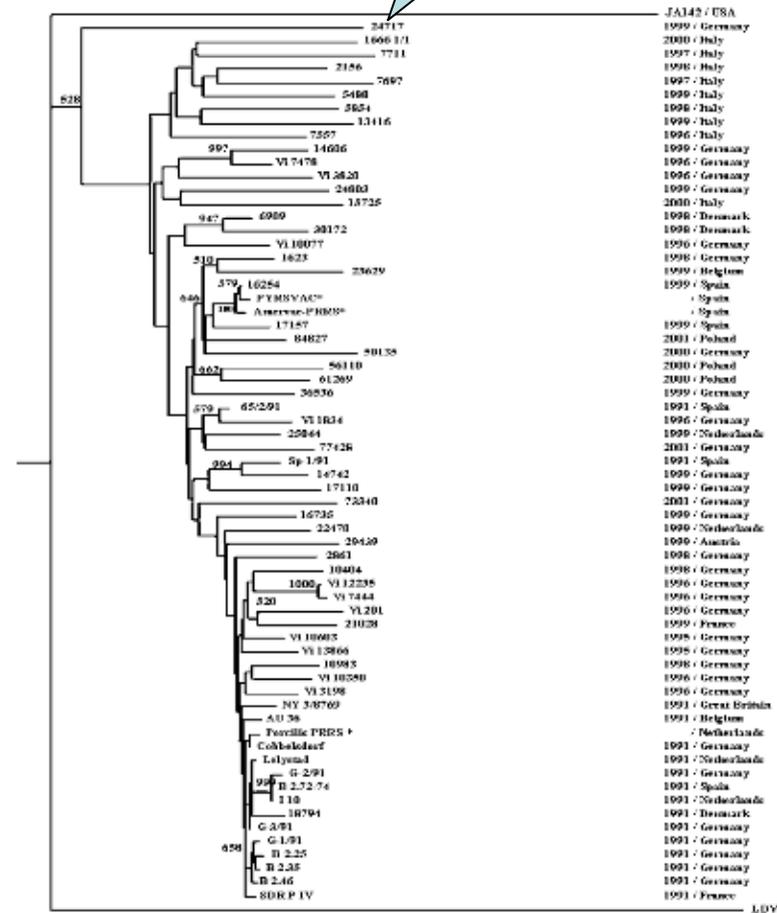
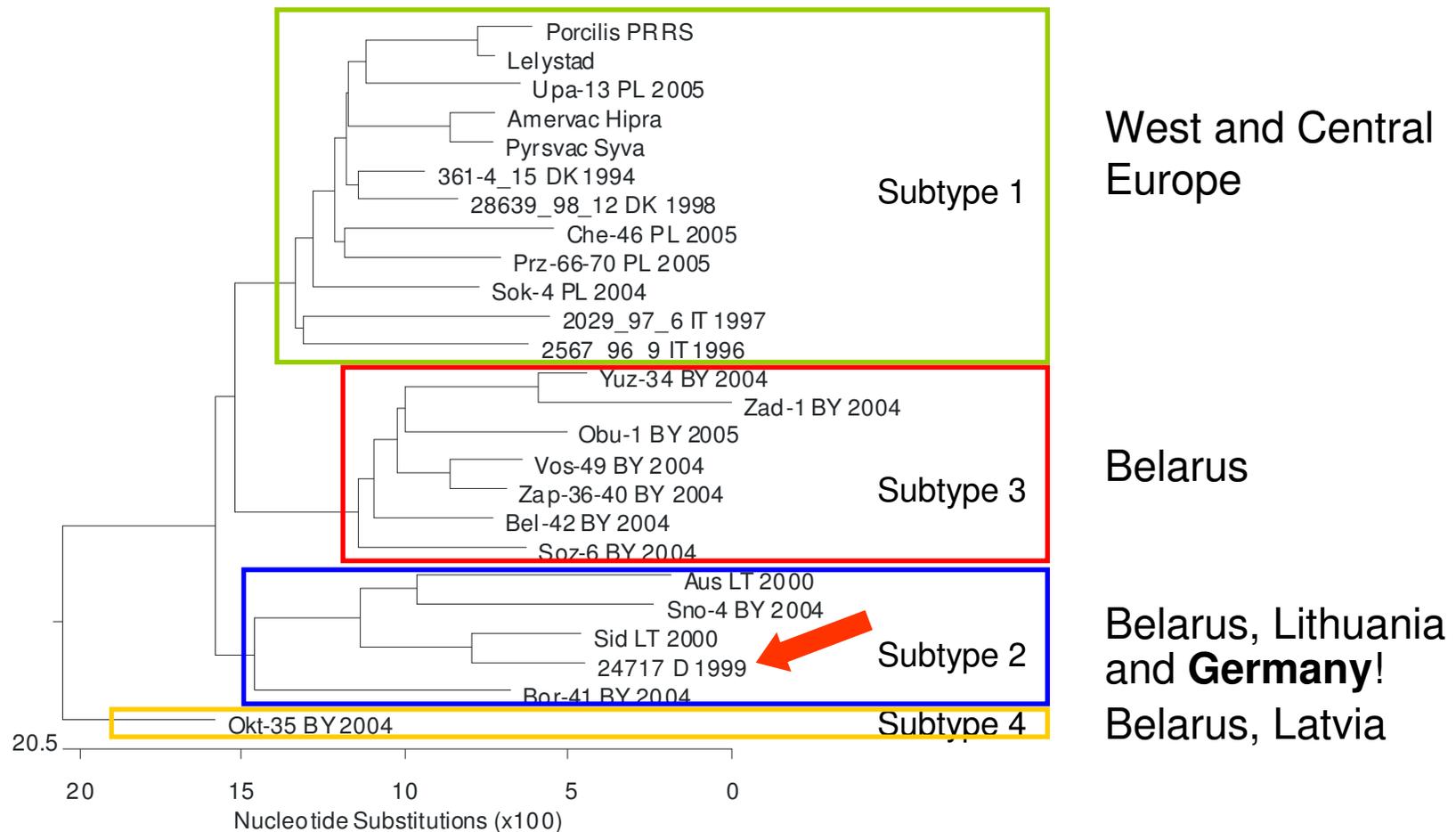


Fig. 3. Phylogenetic analysis of 65 European PRRSV isolates as well as of isolate JA142 from USA and Lactate Dehydrogenase Elevating Virus

Diverse East European PRRSV strains are likely to be present also in West Europe

Phylogenetic tree of ORF5 amino acid sequences including German sequence 24717 from Pesch et al., 2005



Summary

- PRRSV-EU emerged in Eastern Europe or Central Asia
- Spread likely linked to geopolitical factors
- Most recent common ancestor for PRRSV-EU existed 23 – 44 years before the disease emerged
- Diversity of PRRSV-EU is extremely high in comparison to PRRSV-US
- Sensitivity of current RT-PCR assays to divergent PRRSV-EU not known: We may not have the complete picture!
- Currently available vaccines have moderate to good efficacy against subtype 1 (West European) PRRSV but their efficacy against diverse East European strains needs examination

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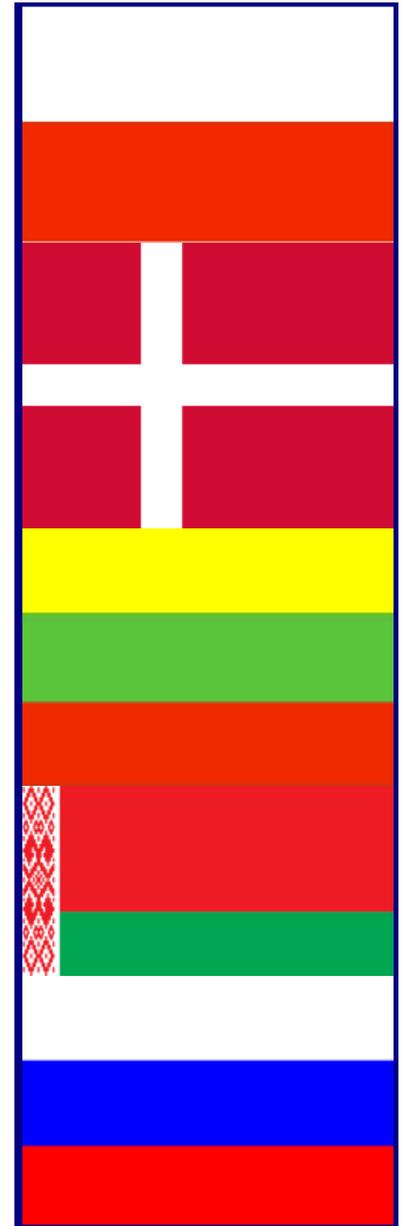
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Thank you for your attention!!!