

**Preliminary phylogenetic analysis of some
Italian equine infectious anemia virus strains
and geographic structuring within global
EIAV isolates**

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EIAV in Europe

2000-2004: 8 outbreaks in France
74 outbreaks in Italy
9953 outbreaks (30.132 cases) in Romania



2006: 29 outbreaks (first report in a sport horse)

2007: 356 outbreaks



OIE (Office International des Epizooties) classification:

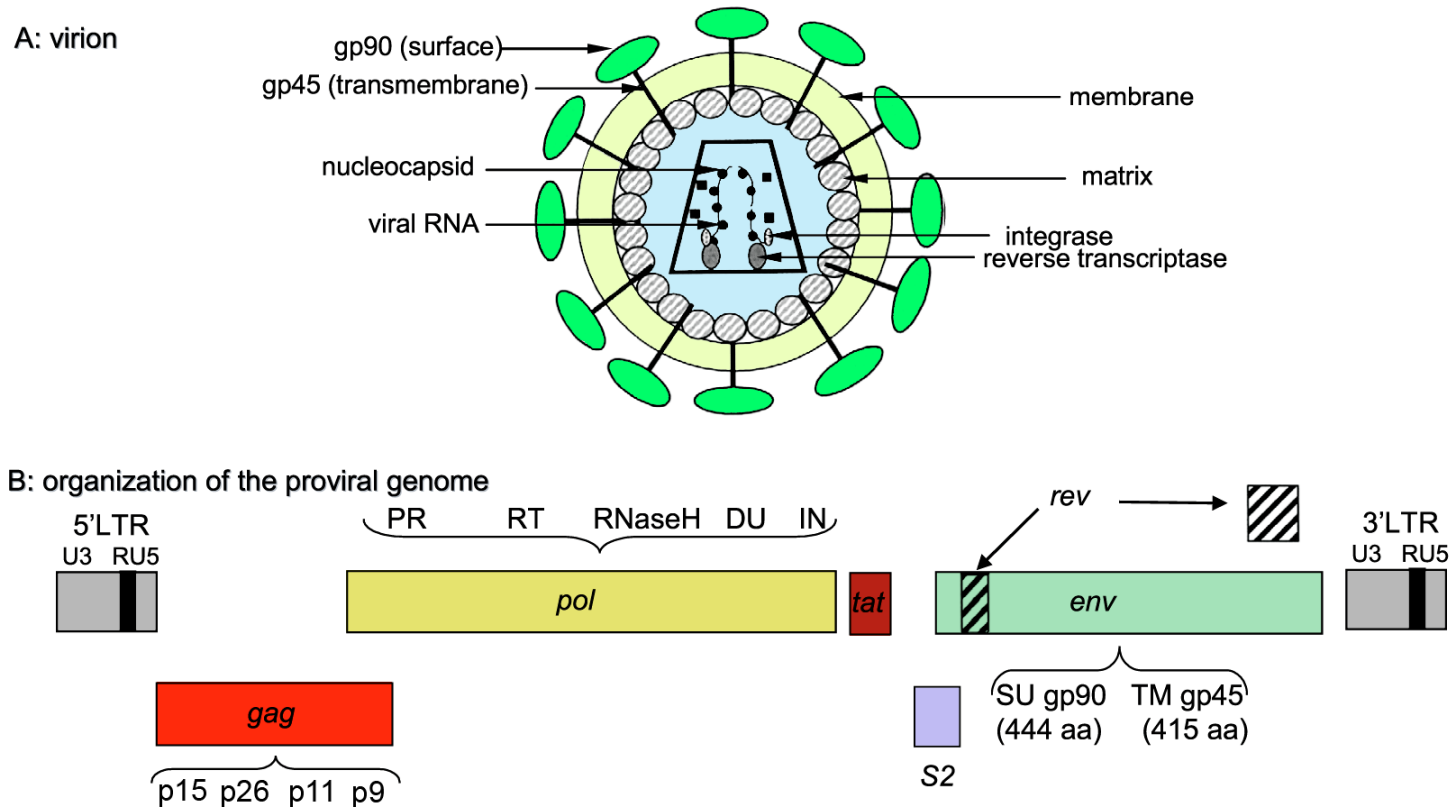
a **relevant disease** for the public health

and economic aspect too



Equine Infectious Anemia Virus

Simplest genome organization of all the lentiviruses
8.2kbp: only 2/3 the length of the HIV-1 genome



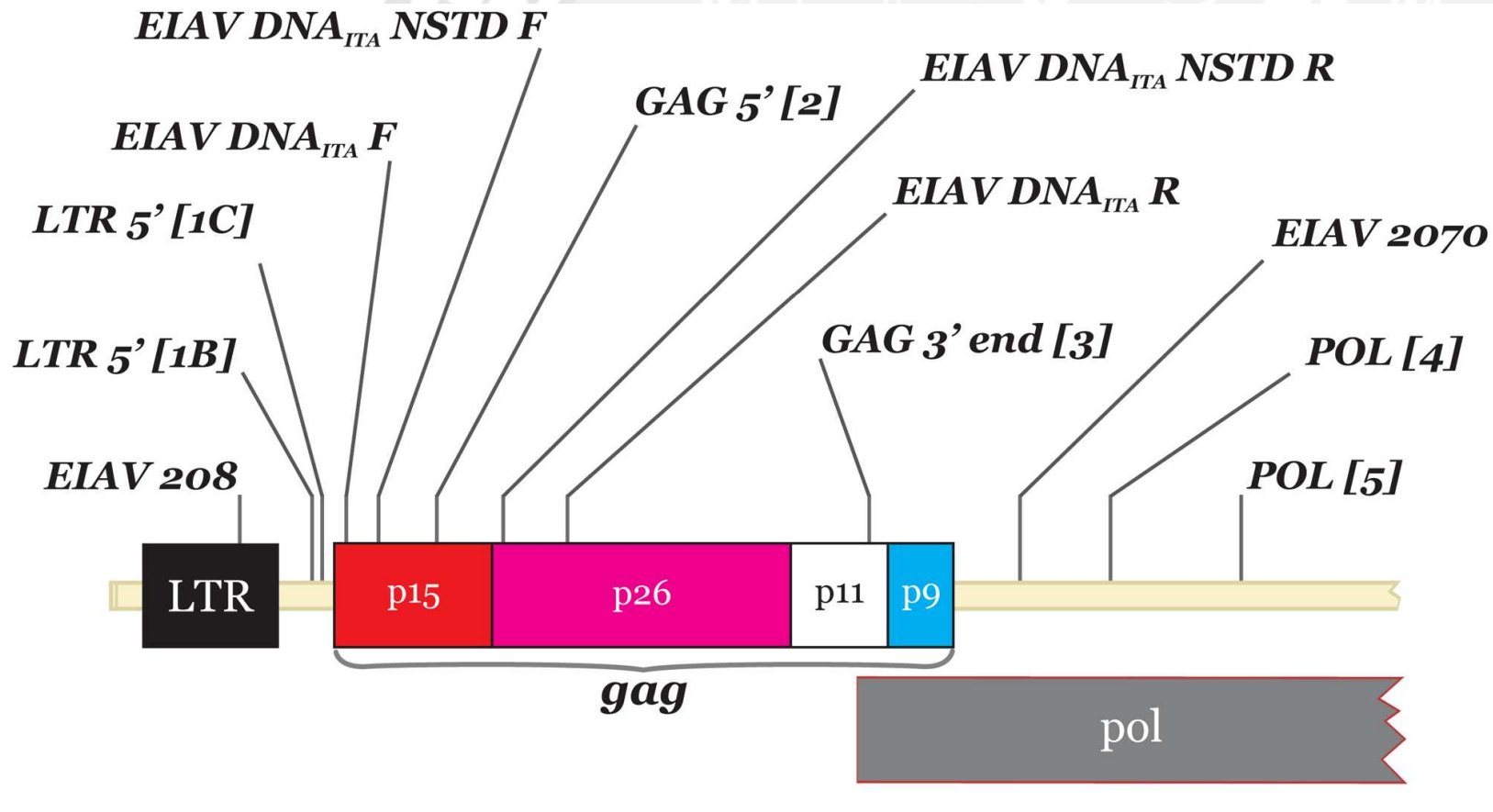
Leroux et al., Vet. Res. 35 (2004) 485-512

ITA G T C T G T G G G C G C T A A G T T T G G
P1 G T A A T T G G G C G C T A A G T C T A G



EIAV GAG isolation strategy

- RNA and DNA extraction (spleen, liver, bone marrow, and buffy coat)
- Amplification (RT-PCR, PCR)
- Sequencing (39 obtained)



EIAV gag gene amplification ad diagnostic pcr primer

Assay	Primer Name Sequence	Amplicon length (bp)	Template	Reference
Full length #1 First Round	EIAV 208 CGGTCTGAGTCCCTTCTCTG	1862	cDNA / DNA	Quinlivan et al. 2007 (23)
	EIAV 2070 TTCCACATTTCTCCCACTC			
Full length #1 Second Round	EIAV 450 ATGGGAGACCCAGTGACATGGAGCAAA	1429	cDNA / DNA	Quinlivan et al. 2007 (23)
	EIAV 1879 CTCCACAAACTGTTCAAATTGAGATCCT			
Full Length #2 First Round	LTR 5' [1B] GGACAGCAGAGGAGAACTTACAGA	2234	cDNA / DNA	Consensus sequence derived
	POL [5] GTGAAAGCTGTATATGGTCTAAACTCTGGA			
Full Length #2 5' Second Round	LTR 5' [1C] GTCTTCTGGAGGTGTTCTGGCCA	1325	cDNA / DNA	Consensus sequence derived
	GAG 3' end [3] TGAGCCCTTGYYTCCCGTTTTTTGG			
Full Length #2 3' Second Round	GAG 5' [2] AACATGGTGGCAATTKYTGCTGT	1625	cDNA / DNA	Consensus sequence derived
	POL [4] GTGAGTGGCCATTGAGGAATTTTTGGCC			
Diagnostic PCR First Round	EIAV DNA_{ITA} F GACATGGAGCAAAGCGCTCA	547	DNA	Consensus sequence derived
	EIAV DNA_{ITA} R CTGCCAGGCACCATCTA			
Diagnostic PCR Second Round	EIAV DNA_{ITA} NSTD F TGTGGCGCTAAGTTTGGTG	313	DNA	Consensus sequence derived
	EIAV DNA_{ITA} NSTD R TTTCTGTTCCAGCCCATC			
cDNA check	BACTINE F GAGCAAGAGGGGCATCCTGA	184	cDNA	Rieder et al. 2001 (24)
	BACTINE R GGTCATCTTCTCGGTTGG			

Italian samples 2006-2009

	N° Samples	AGID +	Diagnostic PCR +	Symptomatics
Arabian horse	9	1	3	0
Donkey	4	1	1	0
Heavy horse	78	8	8	0
Mule	48	7	7	0
Standard breed	72	19	22	13
Thoroughbred	41	5	5	4
Warm blood	148	12	24	11
Grand Total	400	53	70	28

Cappelli, K. et al. Molecular Detection, Epidemiology, and Genetic Characterization of Novel European Field Isolates of Equine Infectious Anemia Virus. *Journal of Clinical Microbiology* 49, 27–33 (2010).



Equine Infectious Anemia

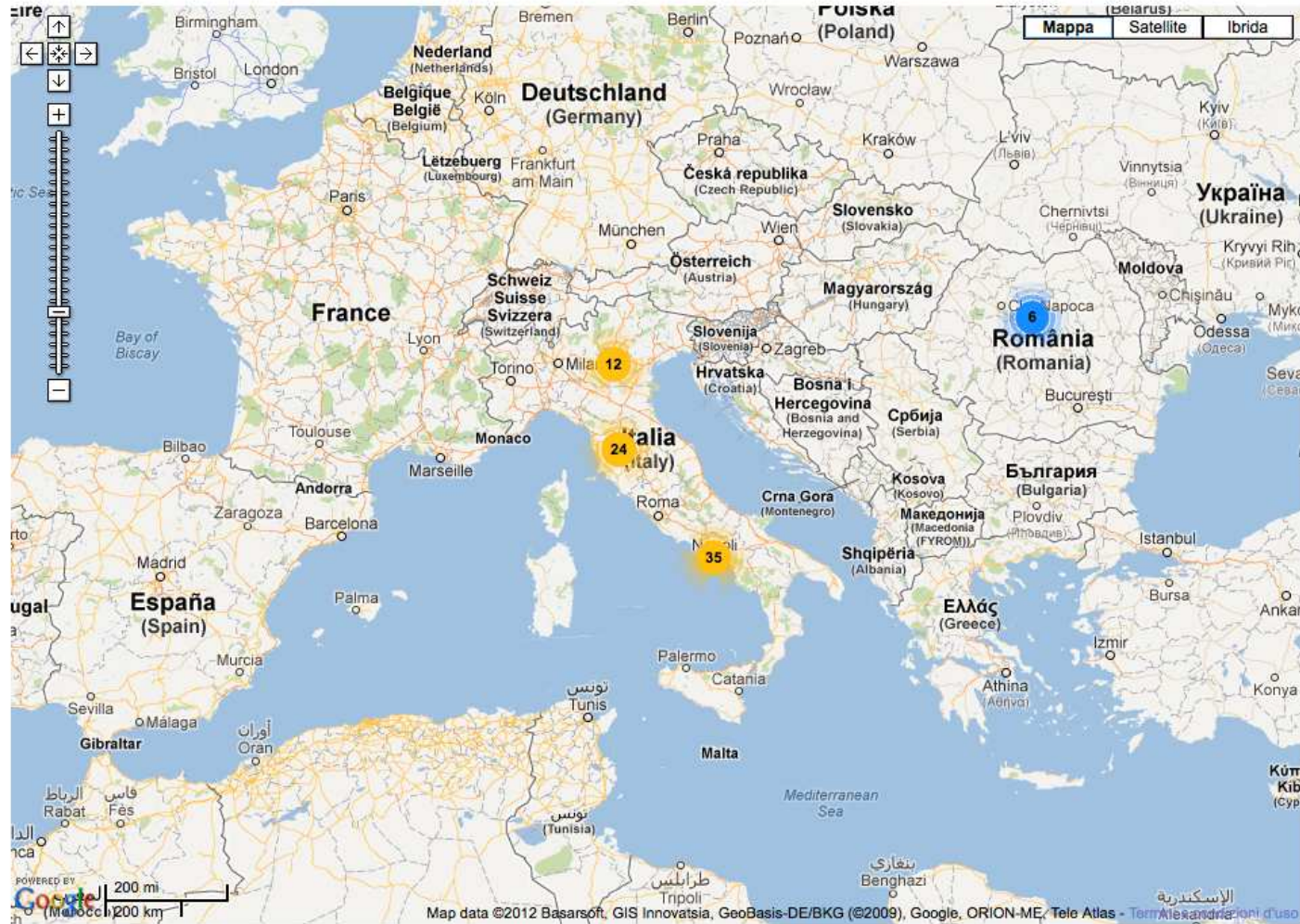
Options...

N° Lab	Type	Name	GenBank	AGID	PCR	DNA	Notes	
1	Symptomatic		EU240733	+	+			
2	Symptomatic		EU375543	+	+			
3	Symptomatic		EU375544	+	+			
4	Symptomatic			+	+			
5	Asymptomatic		EU741609	+	+	Si (TriZ)		
8	Symptomatic			+	+			
9	Symptomatic		GQ927486	-	+	Si (kit)		
10	Asymptomatic		GQ265785	+	+	Si (TriZ)		
11	Asymptomatic		GQ927501	+	+	Si (TriZ)		
12	Asymptomatic			+	-	Si (kit)		
13	Asymptomatic		GQ927505	+	+	Si (kit)		
14	Asymptomatic		GQ927483	+	+	Si (kit)		
15	Symptomatic		GQ927489	+	+	Si (Luisa)		
16	Symptomatic		GQ927506	-	+	Si (kit)		
17	Symptomatic		GQ927502	-	+	Si (kit)		
18	Symptomatic			-	+	Si (kit)		
19	Symptomatic		GQ927484	-	+	Si (kit)		
20	Symptomatic			-	-	Si (kit)		
21	Asymptomatic			-	-	Si (kit)		
22	Asymptomatic			+	-	Si (kit)		

← HOME
 New Sample
 Geo Localization

- ← HOME
- New Sample
- Geo Localization

All samples / Symptomatics / Asymptomatics



Center Latitude: Center Longitude:

Reset View



Samples geo-localization



Background information for EIAV gag gene sequences

Farm	GenBank	Age(years)	F	T	L	A	E	P	AGID	Tissue	Test
F-1	EU240733	14	✓	✓	✓				-	Spleen	RT-PCR
F-1	GQ927489	55 days	✓	✓	✓	✓		✓	+	Blood	PCR
F-2	EU375544	16	✓	✓	✓	✓	✓	✓	+	Bone marrow	RT-PCR
F-3	EU375543	5 months	✓	✓	✓	✓	✓	✓	+	Liver	RT-PCR
F-4	EU741609	15			Asymptomatic				+	Buffly Coat	RT-PCR
F-5	GQ927486	8	✓	✓	✓	✓	✓	✓	-	Blood	PCR
F-6	GQ265785	16			Asymptomatic				+	Buffly Coat	RT-PCR
F-7	GQ927501	10			Asymptomatic				+	Blood	PCR
F-8	GQ927505	22			Asymptomatic				+	Blood	PCR
F-9	GQ927483	10			Asymptomatic				+	Blood	PCR
F-10	GQ927506	11	✓	✓		✓			-	Blood	PCR
F-11a	GQ927502	10	✓	✓	✓	✓		✓	-	Blood	PCR
F-11b	GQ927484	5	✓	✓	✓	✓	✓	✓	-	Blood	PCR
F-12a	GQ927500	15			Asymptomatic				+	Blood	PCR
F-12b	GQ927492	14			Asymptomatic				+	Blood	PCR
F-13	GQ927482	10	✓	✓	✓	✓		✓	-	Blood	PCR
F-14	GQ927503	17	✓	✓		✓			-	Blood	PCR
F-15	GQ927485	3	✓	✓	✓	✓	✓	✓	-	Blood	PCR
F-16	GU060664	30			Asymptomatic				+	Blood	PCR
F-17	GQ927497	14			Asymptomatic				+	Blood	PCR
F-18	GQ927495	18	✓	✓	✓	✓			-	Blood	PCR
F-19	GQ927487	5	✓	✓		✓		✓	-	Blood	PCR
F-20	GQ927504	9			Asymptomatic				+	Blood	PCR
F-21a	GQ927498	8			Asymptomatic				+	Blood	PCR
F-21b	GQ927496	12			Asymptomatic				+	Blood	PCR
F-22a	GQ927494	8 months	✓	✓	✓	✓			-	Blood	PCR
F-22b	GQ927493	19			Asymptomatic				+	Blood	PCR
F-23	GQ927490	8	✓	✓		✓			-	Blood	PCR
F-24	GQ927488	3	✓	✓	✓	✓			-	Blood	PCR
F _{ROM} -3	GQ927499	6			Asymptomatic				+	Blood	PCR
F _{ROM} -1	GQ229581	16			Asymptomatic				+	Blood	PCR
F _{ROM} -2	GQ923952	12			Asymptomatic				+	Blood	PCR
F _{ROM} -4	GU060662	10			Asymptomatic				+	Blood	PCR
F _{ROM} -4	GU060663	8			Asymptomatic				+	Blood	PCR

F: fever (> 39°C); T: thrombocytopenia ($16 \times 10^3/\mu\text{l} < T < 40 \times 10^3/\mu\text{l}$); L: lethargy; A: anemia ($2 \times 10^{12}/\mu\text{l} < A < 5 \times 10^{12}/\mu\text{l}$); E: oedema; P: petechial hemorrhagies



GAG Aminoacidic alignment

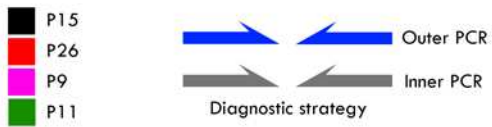
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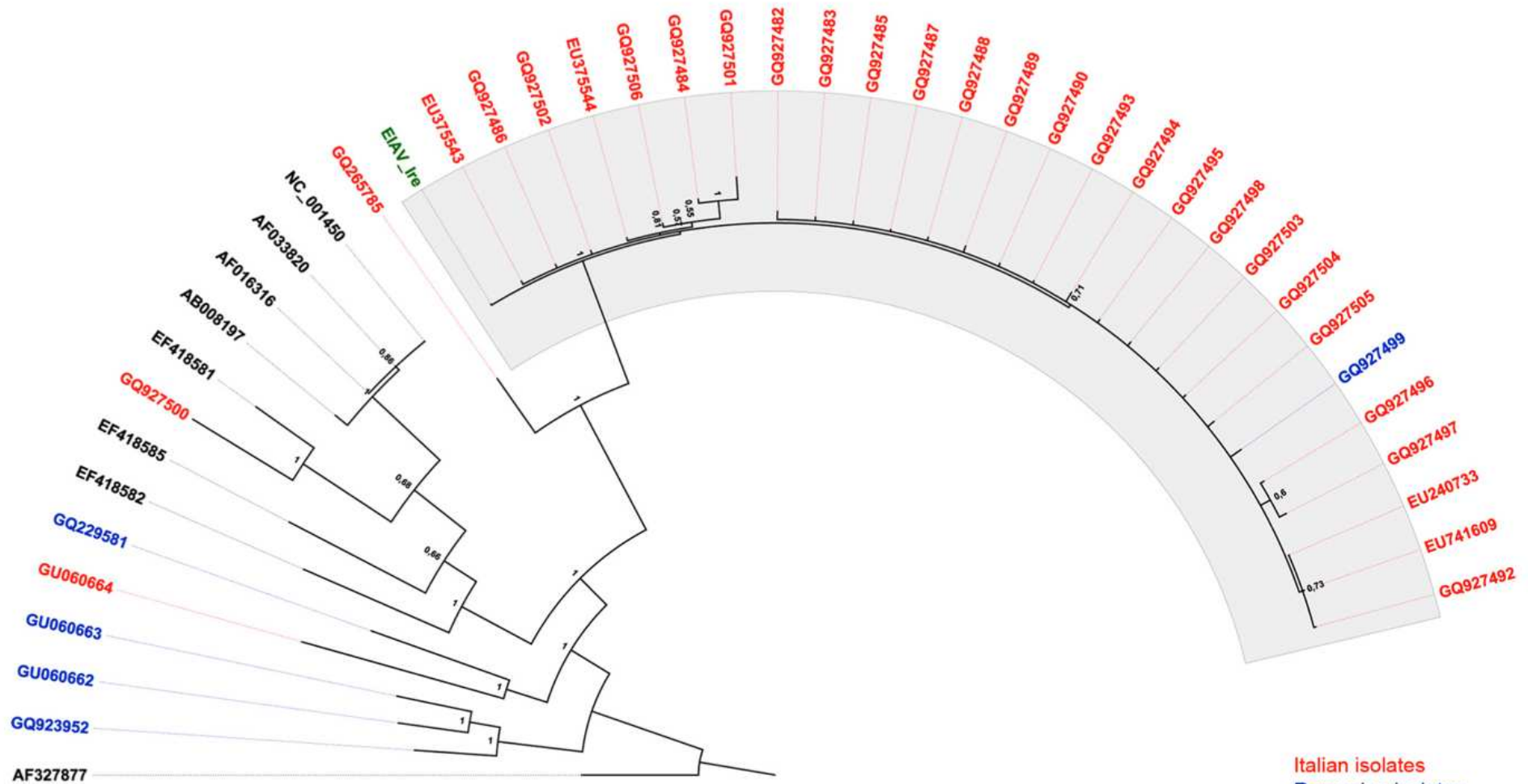
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EIAV phylogenetic tree



The highlighted zone indicates the main Italian *gag* gene subtype. International sequences are the following: NC_001450, reference genome; AF033820, Wyoming; AF016316, United Kingdom; AF327877, Liaoning; AB008197, Japan; EF418582, Canada-1; EF418585, Canada-10; and EF418581, Argentina 1.

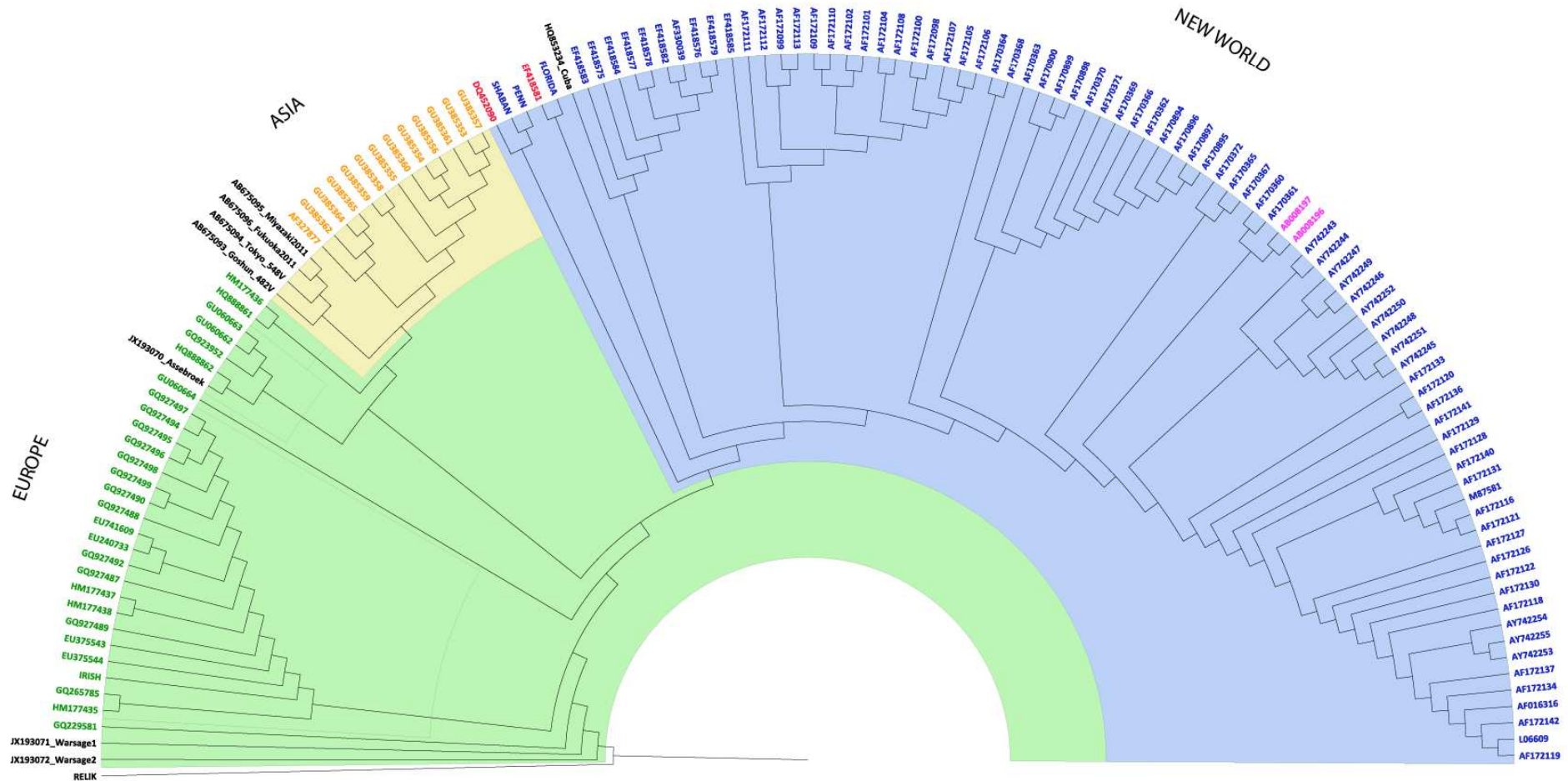
Italian isolates
 Romanian isolates
 Irish isolate
 International isolates



Italian circulating strains



EIAV phylogeography



Based on
Capomaccio, S. *et al.* Geographic structuring of global EIAV isolates:
A single origin for New World strains? *Virus Research* **163**, 656–659 (2012).



CONCLUSIONS

- **Obtained a “pro-virus oriented assay”**
- **There are at least 7 circulating strains of EIAV in Italy; one cluster corresponds to the EIAV outbreak in Europe**
- **Horse and EIAV seem to share an Eurasian origin and may have differentiated locally following human migration**
- **A single origin of New World strain is consistent with the the re-introduction of horses by European settlers**



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