

□ Analisi filogenetica preliminare di alcuni stipiti del virus dell'anemia infettiva equina (EIAV) circolanti in Italia e caratterizzazione filogeografica rispetto ai genomi virali mondiali

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EIAV in Europa

2000-2004: 8 focolai in Francia
74 focolai in Italia
9953 focolai (30.132 casi) in
Romania



2006: 29 focolai in Italia (primi casi in cavalli sportivi)

2007: 356 focolai in Italia



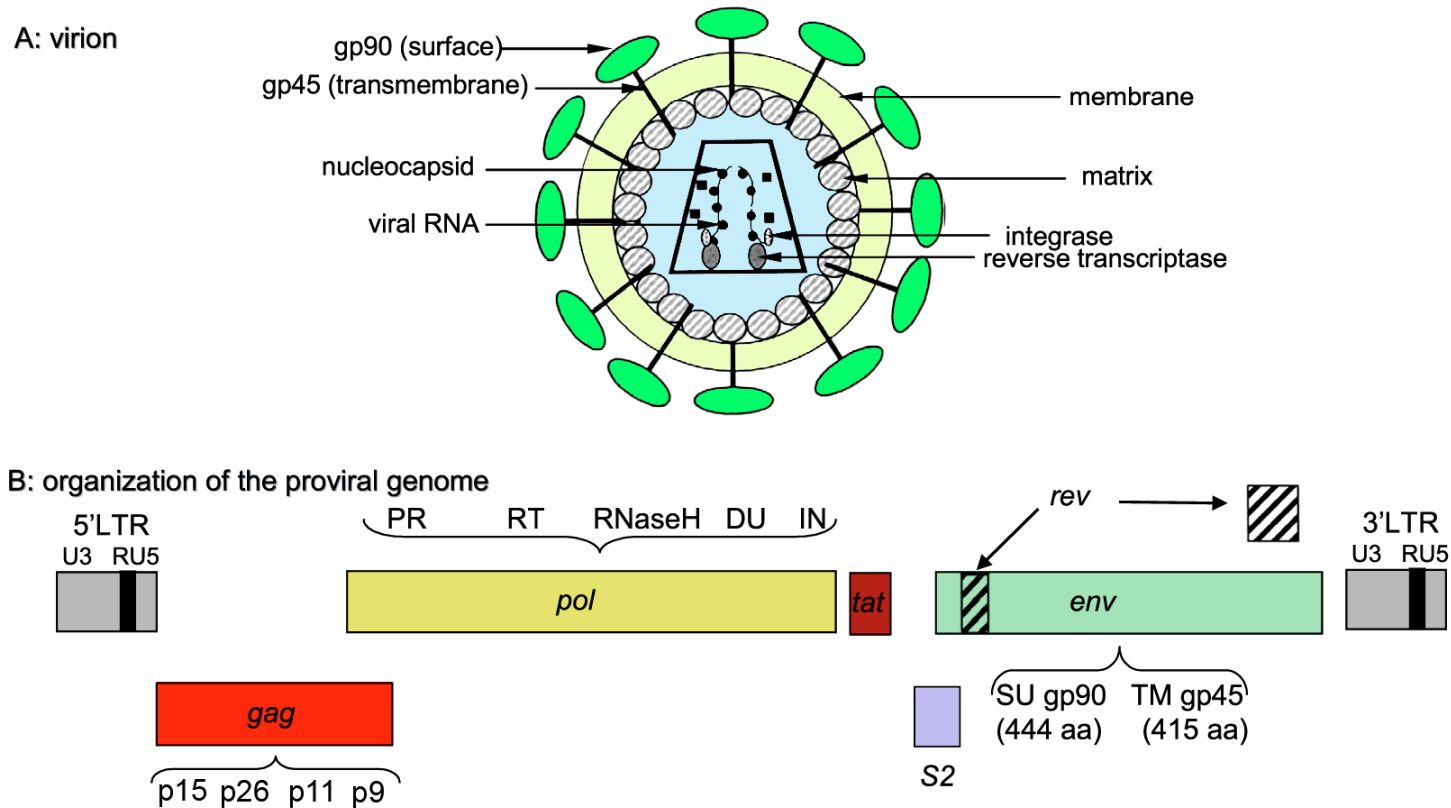
Classificazione OIE (Office International des
Epizooties):

"A **relevant disease** for the public health and



Virus dell'Anemia Infettiva Equina

Genoma piccolo: la più semplice organizzazione genomica di tutti i lentivirus
 8.2kb: solo 2/3 in lunghezza del genoma del virus HIV-1



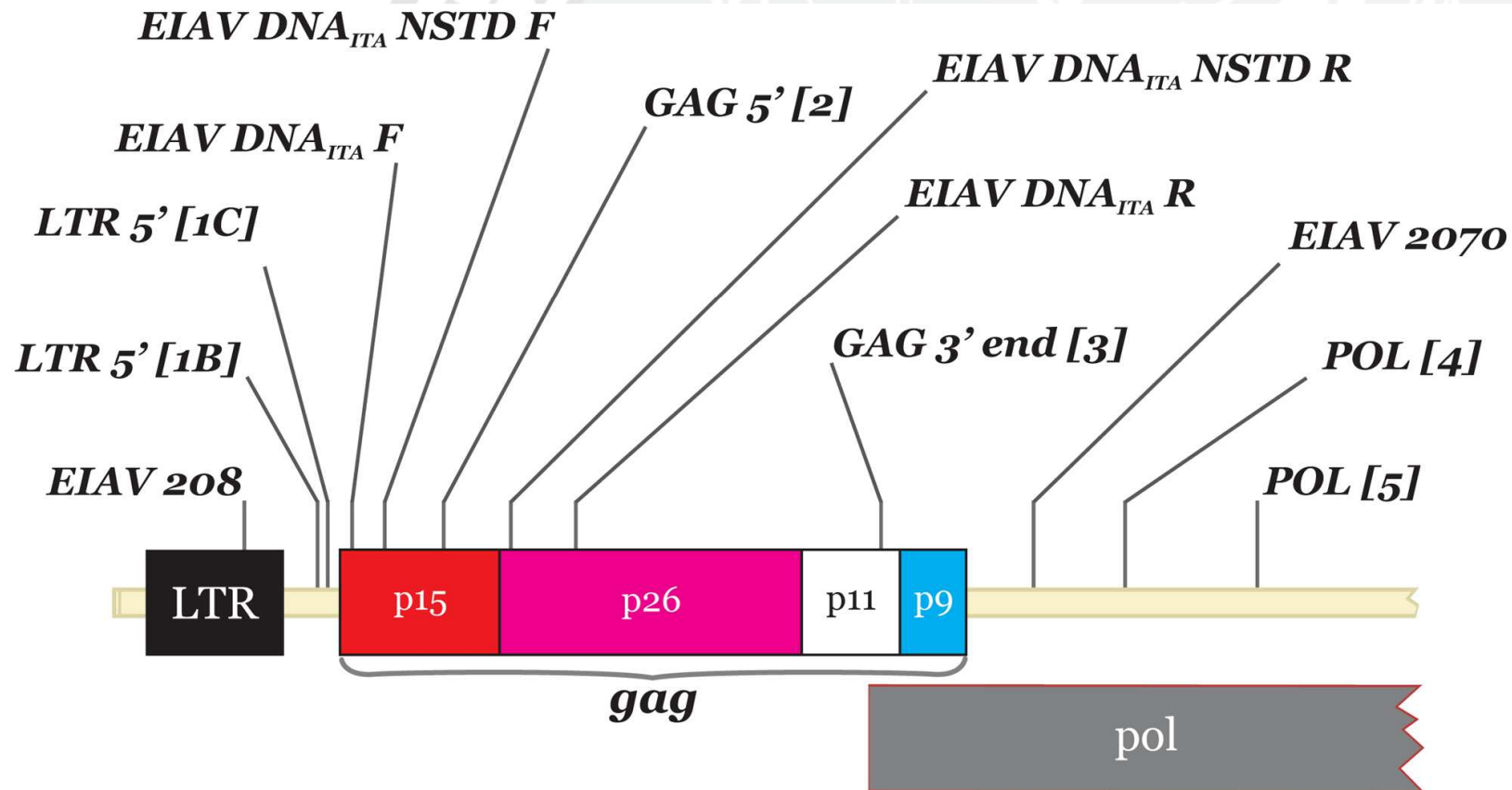
Leroux et al., Vet. Res. 35 (2004) 485-512

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



Isolamento del gene GAG del

- Estrazione RNA and DNA **virus EIAV**
- Amplificazione (RT-PCR, PCR)
- Sequenziamento (39 sequenze ottenute)



Protocolli di amplificazione

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] GTCTTCTGGAGGTTCCTGGCCA	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 TGTGGGCGCTAAGTTTGGTG	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 GGTCATCTTCTCGCGTTGG			

Campioni Italiani 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

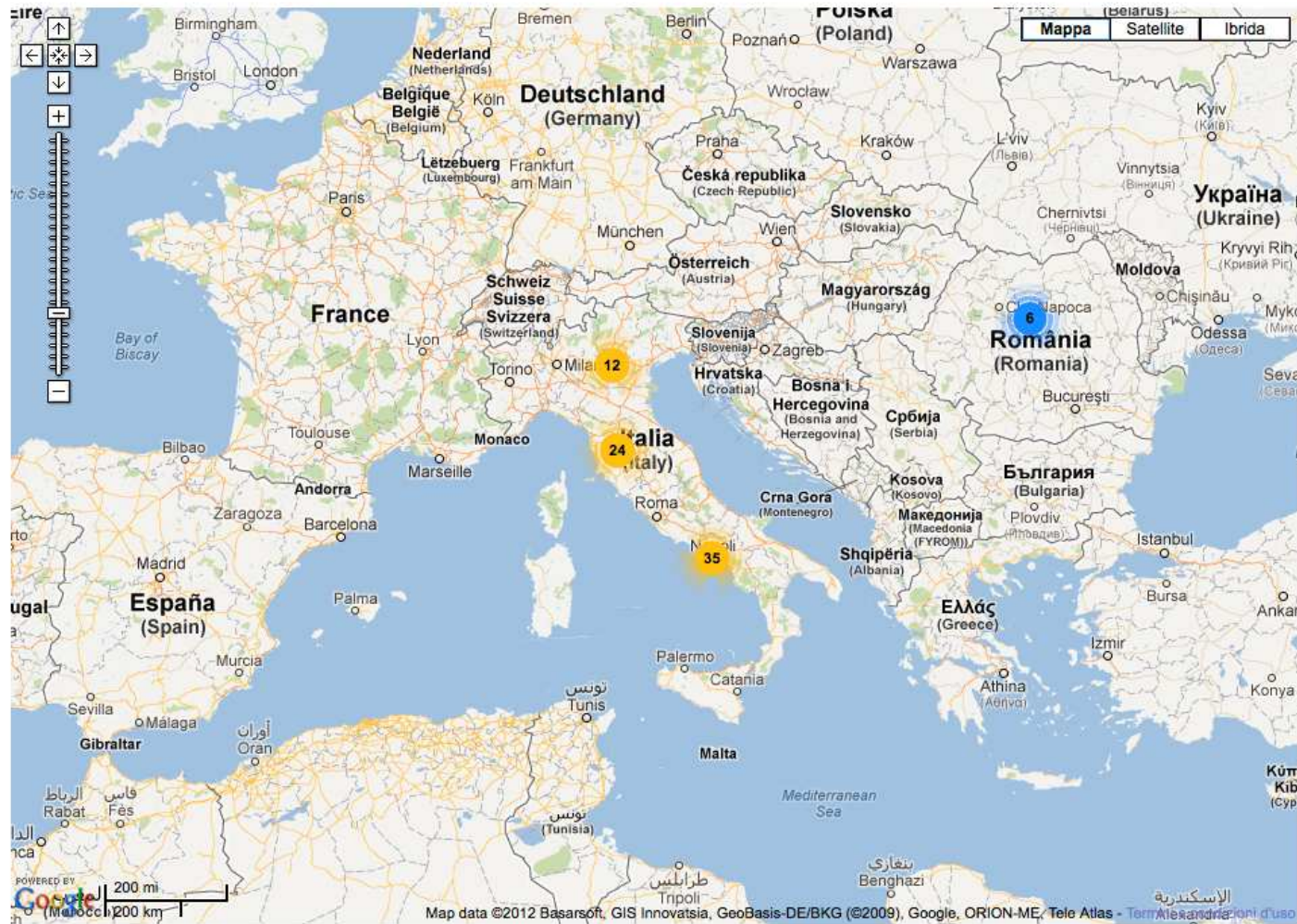
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Country Selection
 ITALY

- ← HOME
- New Sample
- Geo Localization

All samples / Symptomatics / Asymptomatics



Center Latitude: Center Longitude:

Reset View



Geo-localizzazione dei campioni



Dati anamnestici e molecolari

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



Allineamenti aminoacidici di alcune

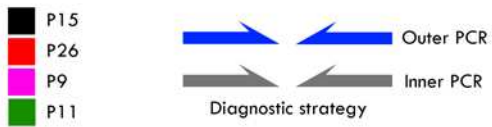
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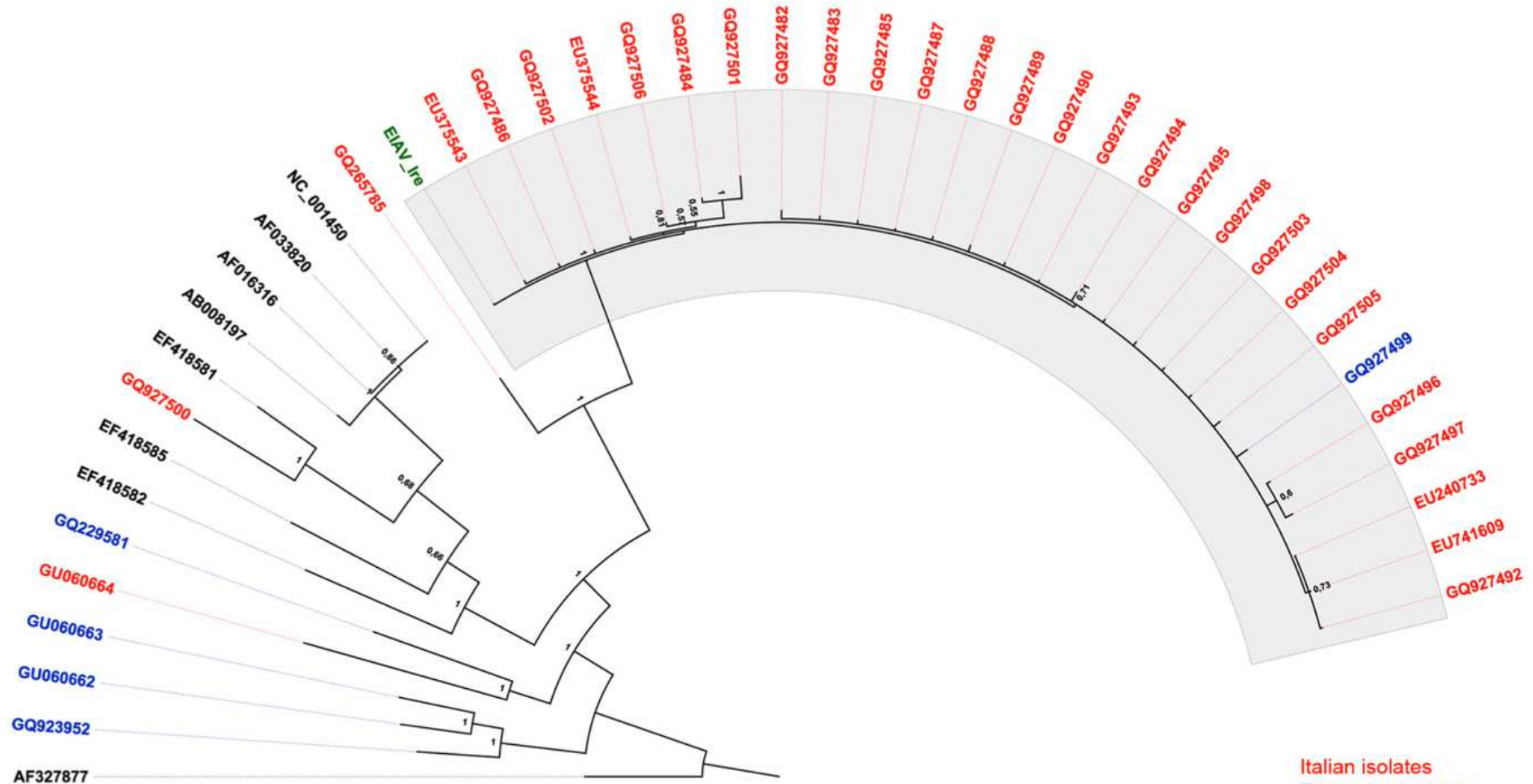
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Filogenesi del virus



Italian isolates
 Romanian isolates
 Irish isolate
 International isolates



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.

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).

Stipiti circolanti in Italia



Conclusioni

- Ottenimento di un saggio per l'identificazione del pro-virus
- Individuazione di almeno 7 stipiti circolanti in Italia
- Ipotesi sull'origine del virus EIAV: come il cavallo ha un'origine Euroasiatica e si è differenziato in seguito alle migrazioni umane
- Ipotizzata l'origine comune dei ceppi Americani durante la reintroduzione del cavallo da parte dei coloni Europei



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