



IZSAM G.CAPORALE
TERAMO



Brucellosi

Centro di Referenza Nazionale

Tipizzazione tramite SNPs – B. abortus dati preliminari

Giuliano Garofolo

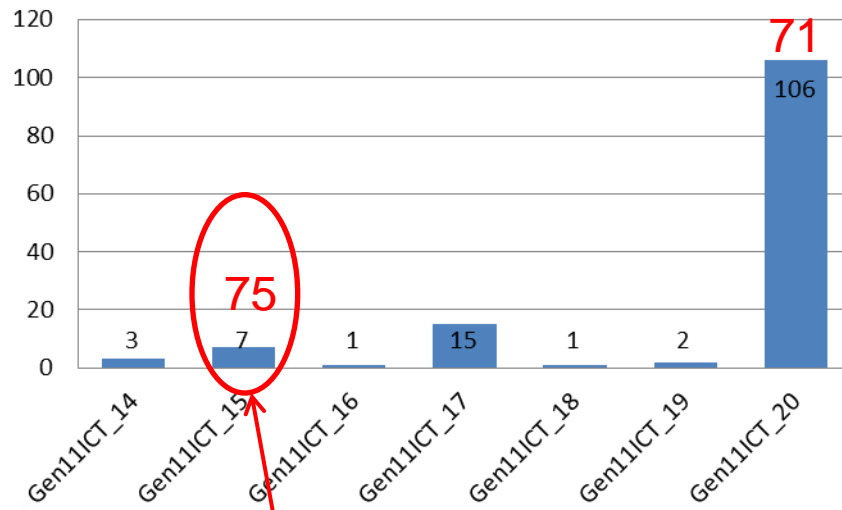
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'

Teramo 27 Febbraio 2015

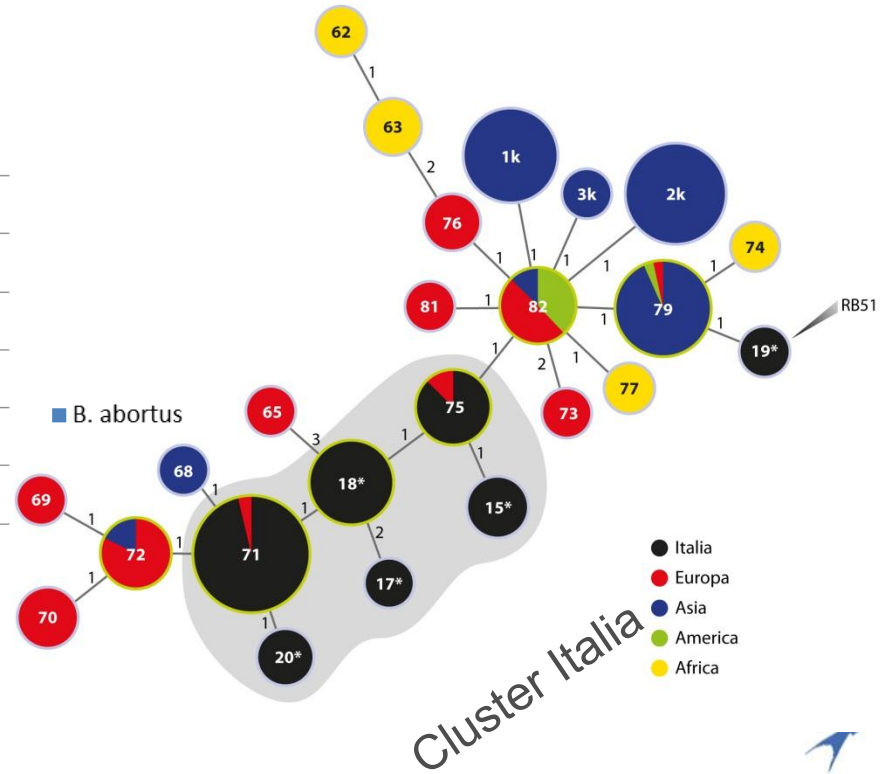
Centro Internazionale per la Formazione e l'Informazione Veterinaria "Francesco Gramenzi"



B. abortus



Biovar. 1

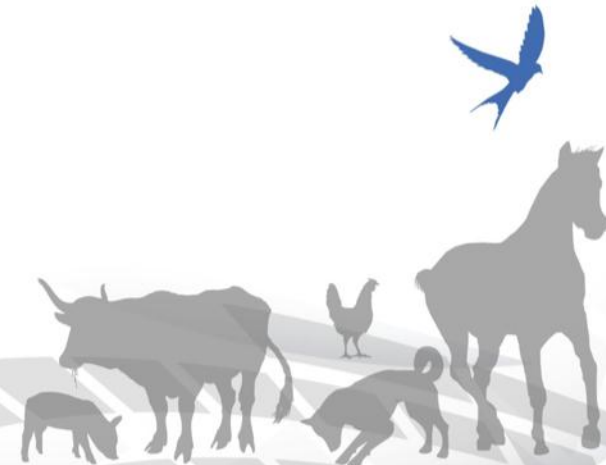


Distribuzione geografica degli isolati

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id	Year	Region	Province	Host	Species	Biovar	MLVA-11	MLVA-16
1046	2011	Abruzzo	CH	cattle	abortus	3	24	210
585*	2011	na	na	na	abortus	3		
5586	2011	Calabria	KR	cattle	abortus	3	9	72
8486	2011	Campania	SA	cattle	abortus	3	25	208
9060	2011	Calabria	CS	cattle	abortus	3	2	72
8979-3	2011	Campania	CE	water buffalo	abortus	1	30	82
11796	2011	Sicilia	ME	cattle	abortus	3	14	72
12183	2011	Campania	SA	cattle	abortus	3	19	72
15074/1	2011	Puglia	FG	cattle	abortus	3	7	72
1365/1	2011	Molise	IS	cattle	abortus	3	6	72
3272	2011	Puglia	FG	cattle	abortus	3	34	210
9261	2011	Calabria	CS	cattle	abortus	1	32	82

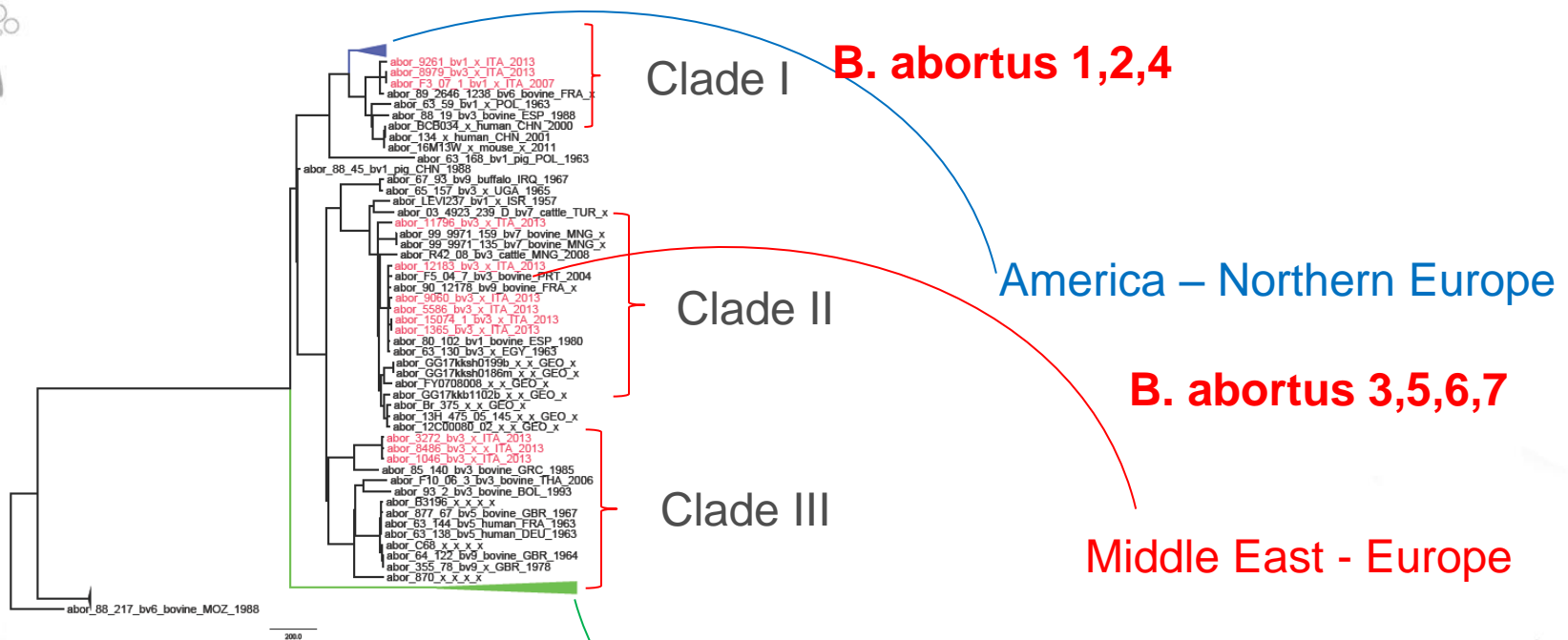


WGS *B. abortus*

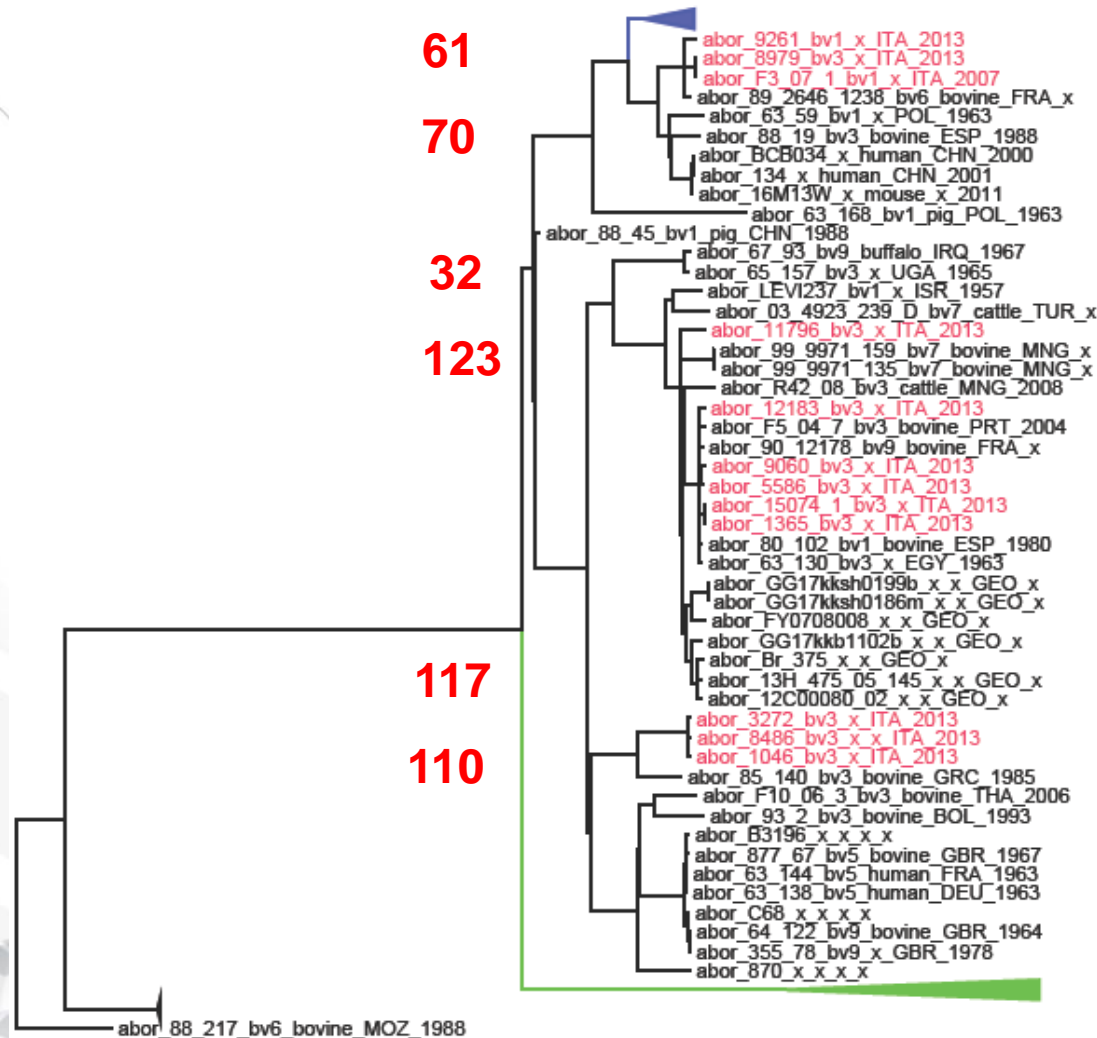
- Abbiamo sequenziato 12 genomi di *B. abortus*
- Abbiamo selezionato sequenze database Pathosystem Resource Integration Center (PATRIC)
- Abbiamo ricercato gli SNPs tra i nostri genomi e quelli disponibili usando la sequenza di riferimento della *B. abortus* 2308
- Per assegnare uno SNPs abbiamo tenuto un 10X di coverage
- Uno SNPs viene confermato solo se ha una proporzione di chiamata superiore al 90%
- Abbiamo usato la massima parsimonia per ricavare il nostro albero filogenetico



Global *B. abortus*



Global B. abortus



Tree length 9689 SNPs
Parsimony informative sites 5885
Consistency index 0.9707
Homoplasy (1-C) = 0,029

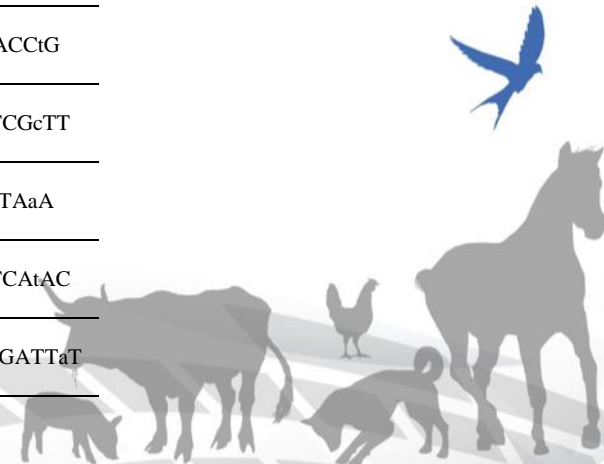


Saggi PCR real time

13 saggi di PCR disegnati per la detection degli SNPs

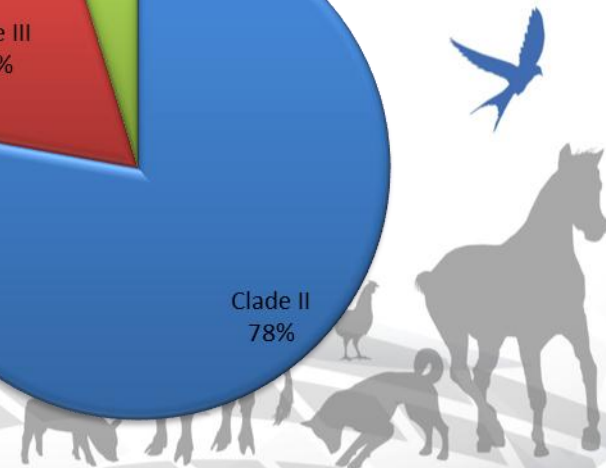
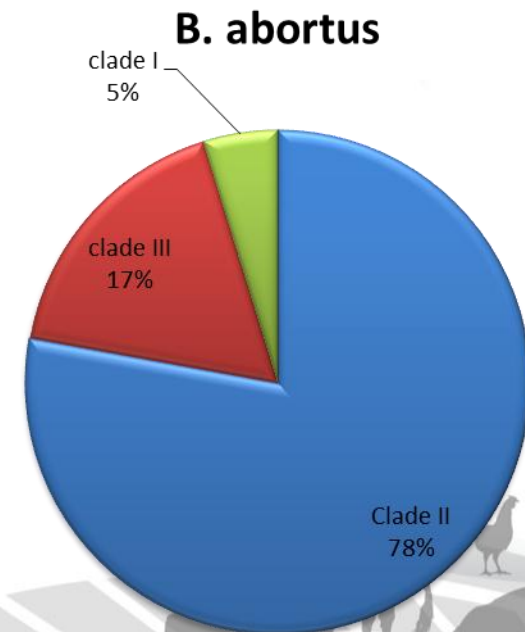
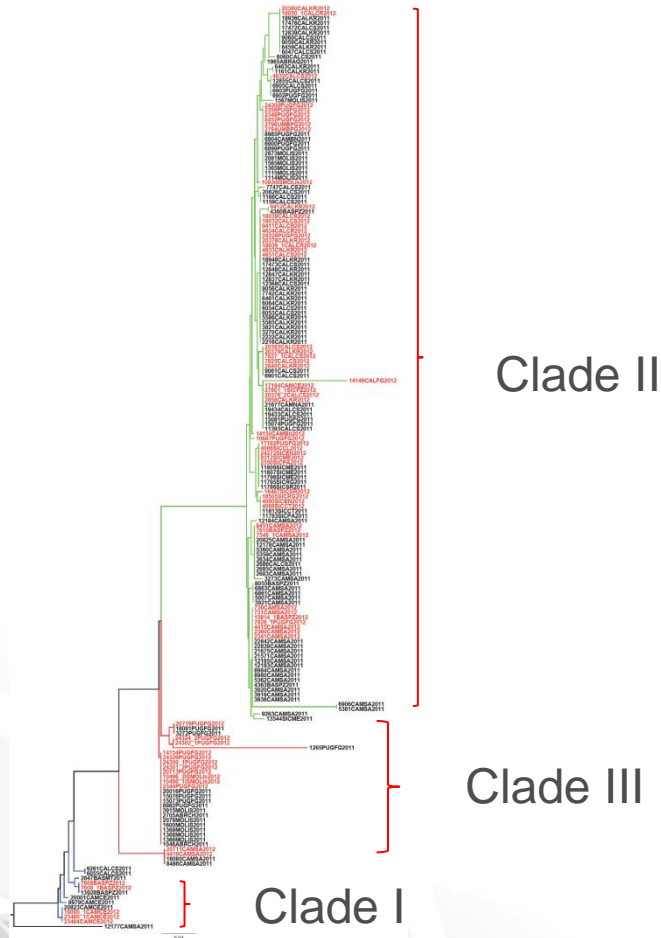
- 7 saggi sono stati scartati
- Percentuale di successo 46%

SNPs	Position	Genome SNPs state	Melt-MAMA primer	Melt-MAMA Primer sequences (5' to 3')
Br.w.l.1	951620	C/T	A	GCGACCGCATCCTCTAaTC
			D	cggggcggggcggggGCGACCGCATCCTCTAcTT
			C	CATGGTAGGCAGGCTGGTT
Br.w.l.2	1336380	A/G	A	CGAAGTTTCCCACACcA
			D	cggggcggggcggggCGAAGTTTCCCACACcTg
			C	GATGAAGAAATCGATGAGCG
Br.e.l.1	104052	G/T	A	TGGAGGACGAAGTCTTCGaTG
			D	cggggcggggcggggGGAGGACGAAGTCTTCGcTT
			C	ATCCTCGACAGTTGCGGC
Br.e.l.2	360724	C/A	A	TACCGCGGCCGTCTAaC
			D	cggggcggggcggggTACCGCGGCCGTCTAaA
			C	AATCAAAGCGGCTTGCCTC
Br.t.l.1	740090	T/C	A	CAAAAGCGGACGGTCAgAT
			D	cggggcggggcggggTCAAAAGCGGACGGTCAaC
			C	CCCTCAACCCAGACGTTCCG
Br.t.l.2	864308	C/T	A	AGGCATTACCGTTTCAGATTc
			D	cggggcggggcggggAGGCATTACCGTTTCAGATTaT
			C	TCTTCCC GGCTCCGAAT



B. abortus Italy

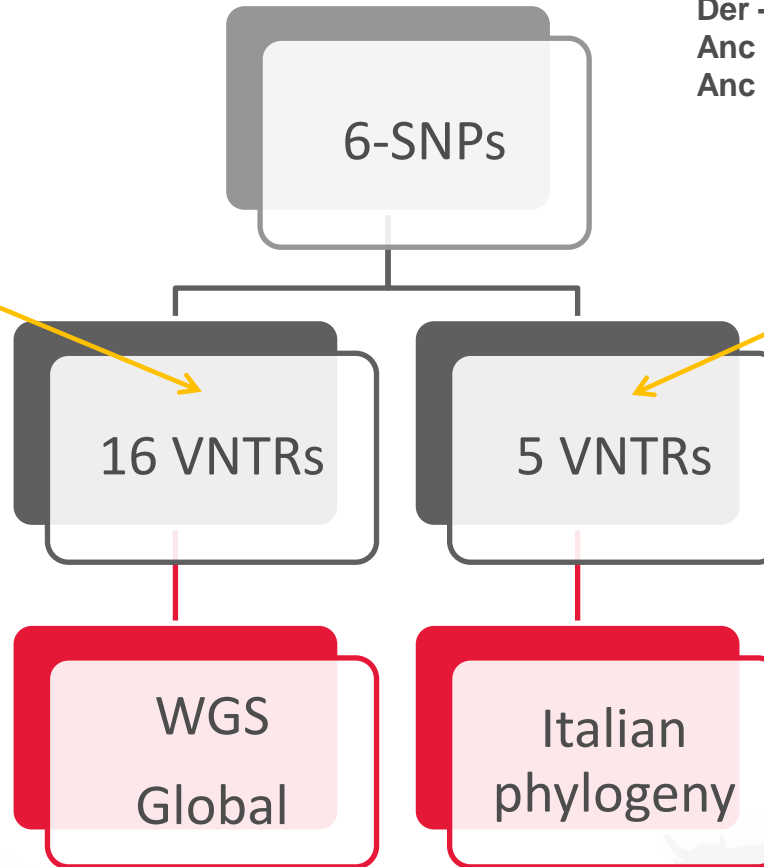
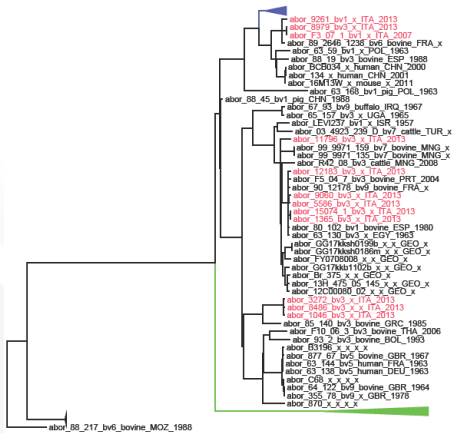
- 200 isolati testati
- 160 clade 2
- 25 clade 3
- 15 clade 1



Canonical SNPs plus VNTRs

SNPs state Italian Clade
 Der – Der– Anc – Anc -Anc – Anc
 Anc – Anc– Der – Der- Anc – Anc
 Anc – Anc -Anc – Anc - Der – Der-

All the SNPs show ancestral state
 Anc – Anc – Anc – Anc - Anc– Anc



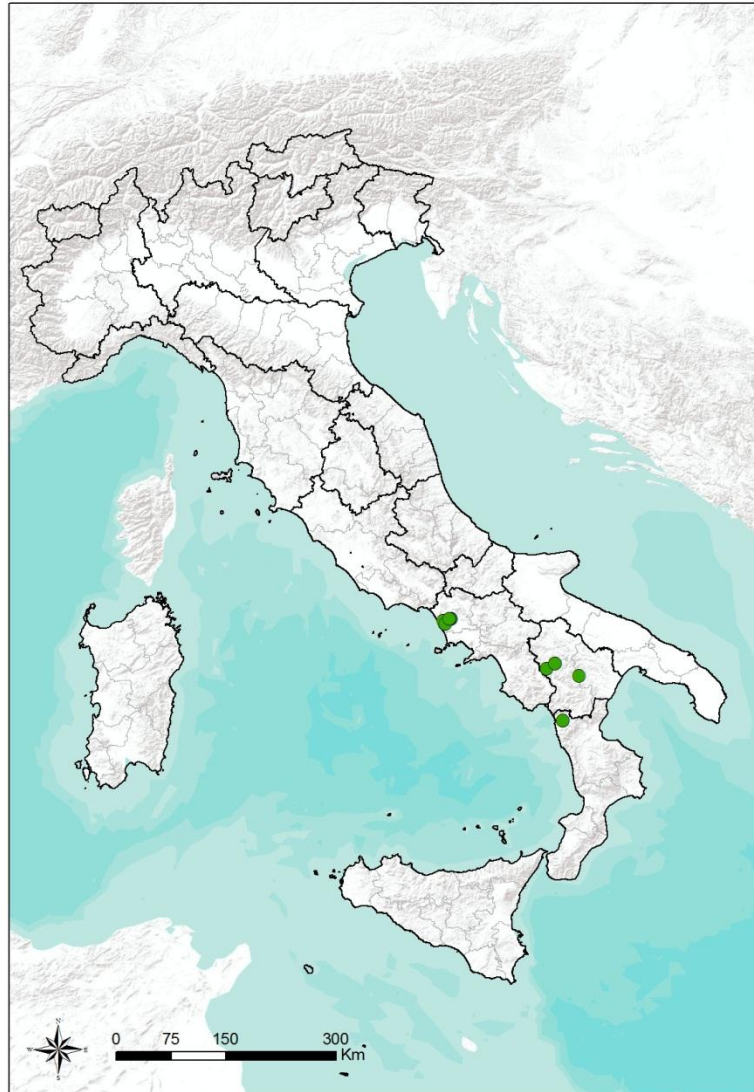
BRUCE 55
BRUCE 04
BRUCE 07
BRUCE 09
BRUCE 16



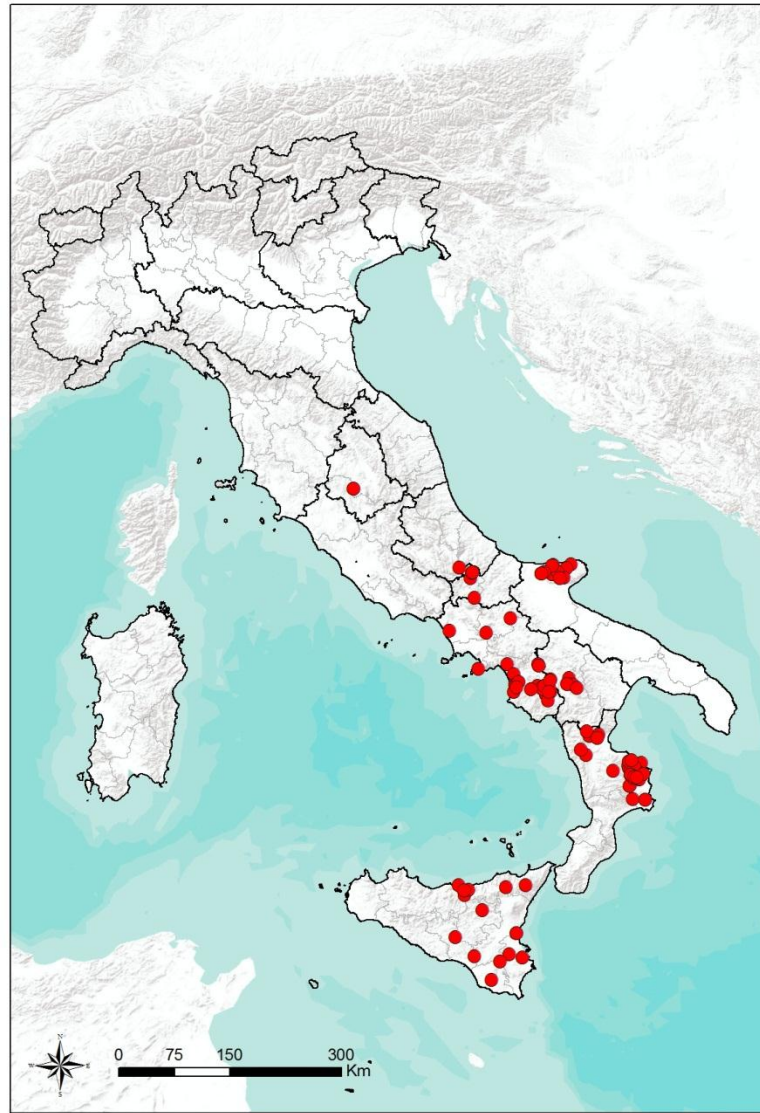


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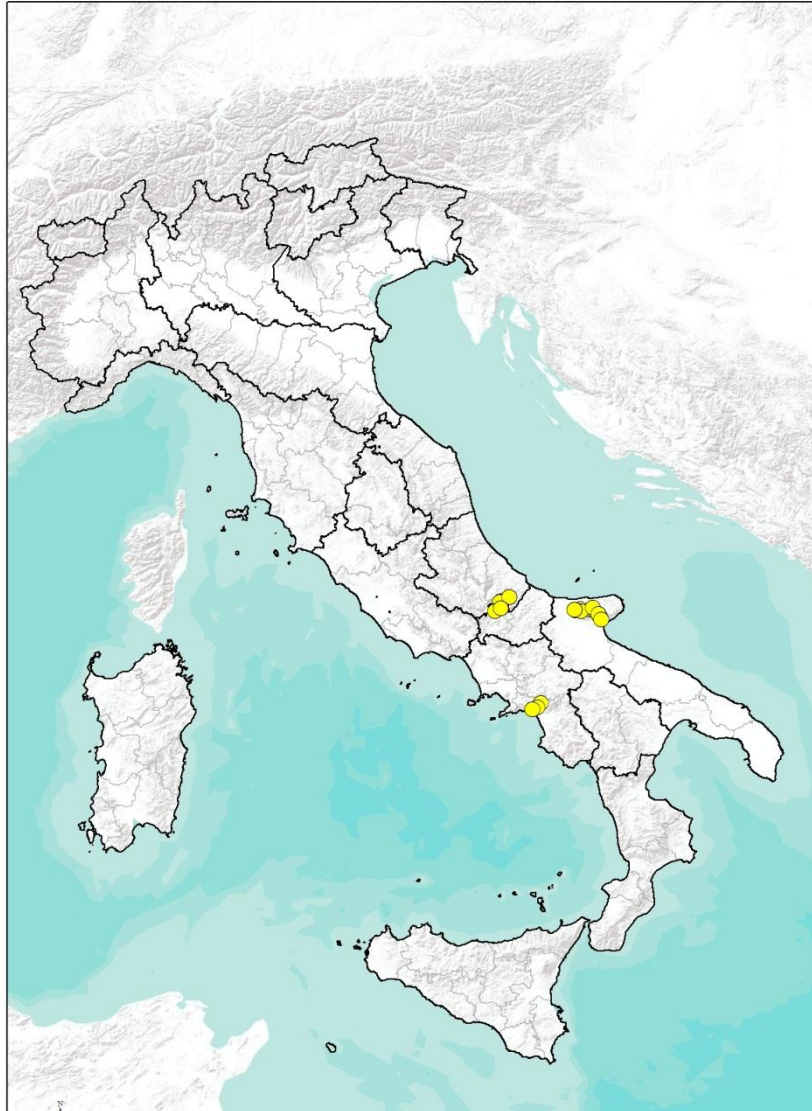
Clade 1



Clade 2

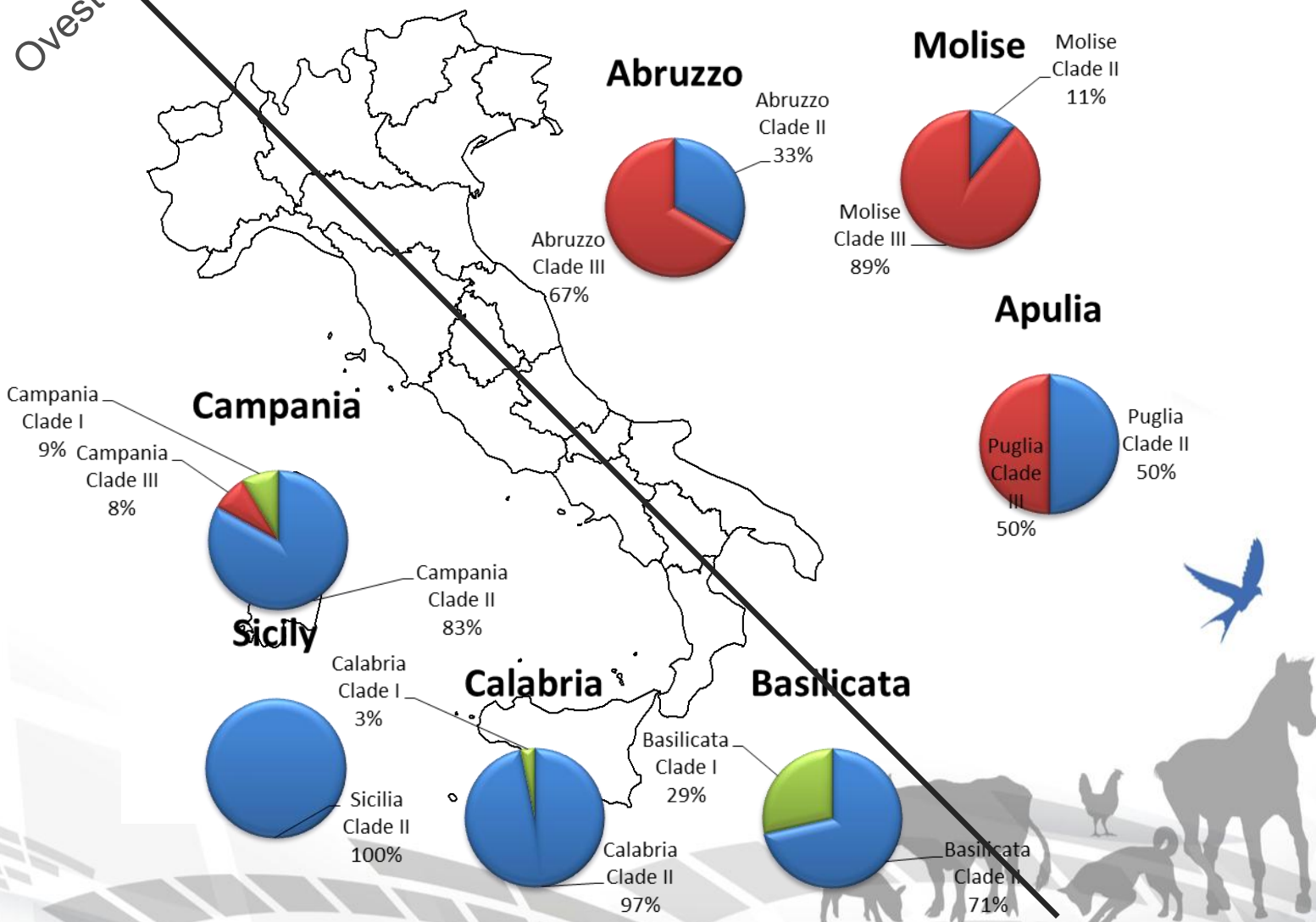


Clade 3



B. abortus - Italy

Ovest
Est





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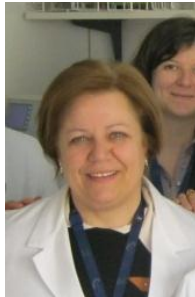
Ricerca e sviluppo

Batteriologia

Epidemiologia

Diagnostica

Sierologia



Grazie per l'attenzione

