IZSAM G.CAPORALE
TERAMO

Analisi filogenetica ed epidemiologia molecolare di ceppi di Brucella spp. isolati in Italia
IZSAM 02/12 RC

Giuliano Garofolo
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise - G. Caporale
I risultati della ricerca corrente condotta dall'Istituto Zooprofilattico Sperimentale dell'Abruzzo e Del Molise. Anno 2016 – 13 Giugno 2017

Brucellosi

- Una delle zoonosi più diffuse al mondo (500,000 casi l'anno)
- Infezioni nell'uomo non sono letali
- Zoonosi sottovalutata "negletta" che crea problemi di sanità pubblica
- Brucella è un patogeno poco studiato

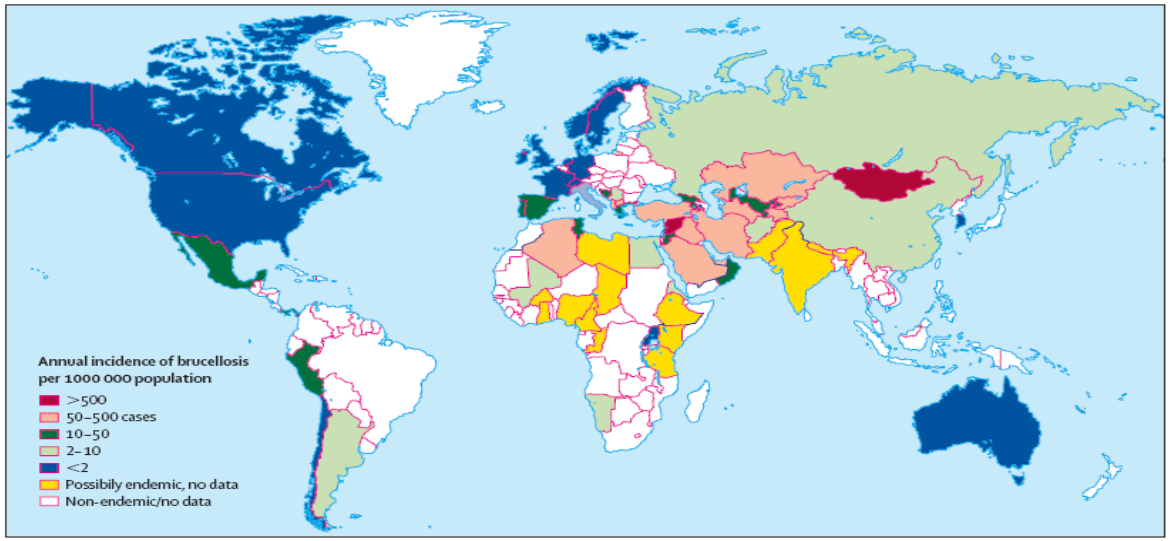
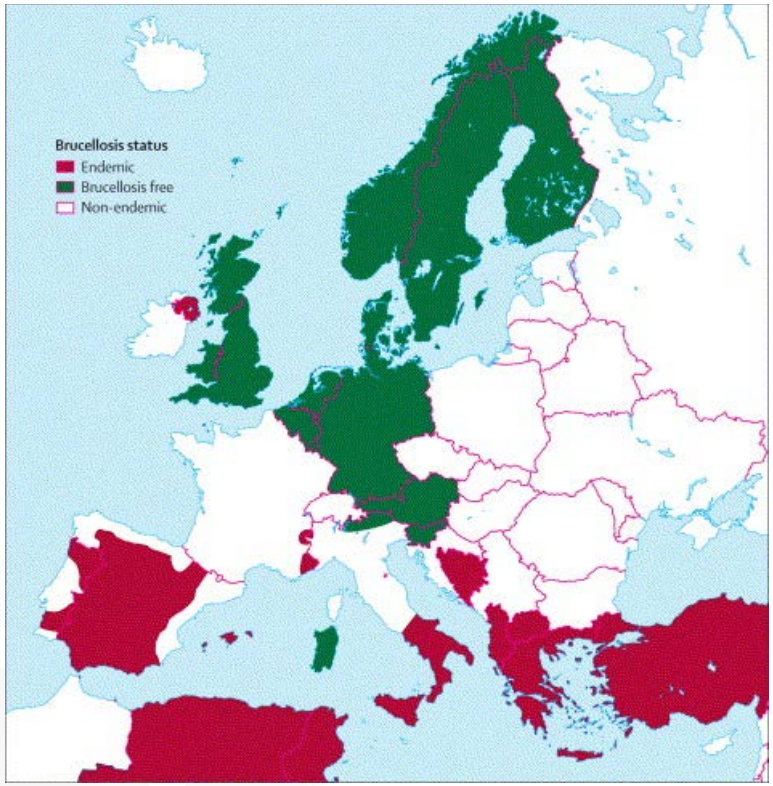


Figure 1: Worldwide incidence of human brucellosis

Pappas et al. 2006

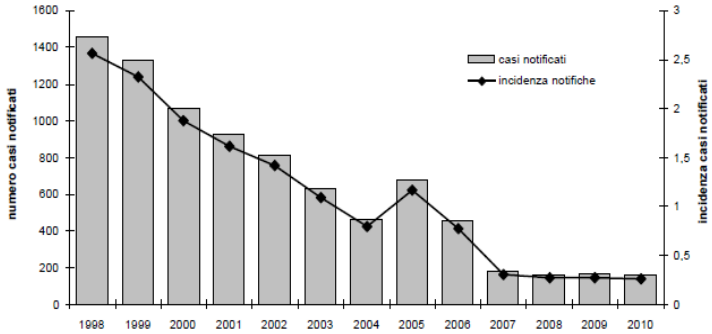
Mediterraneo

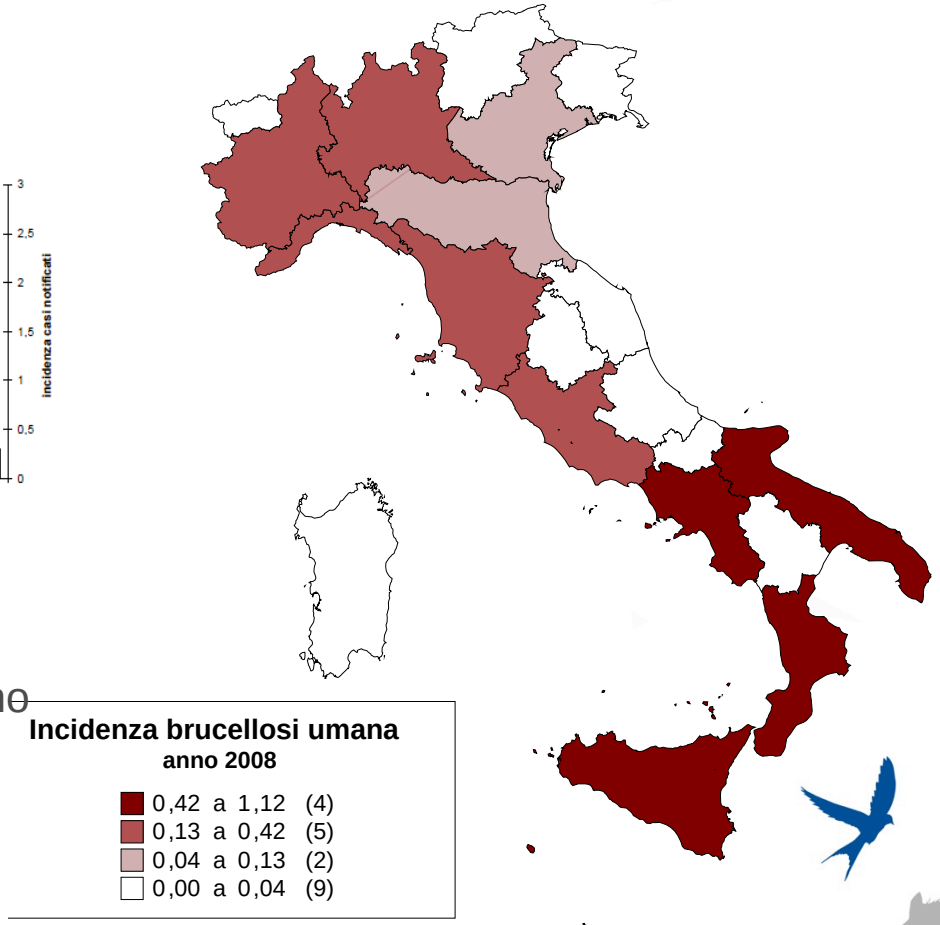


Pappas *et al.* 2006

Neglected disease in the developing countries "neglected disease of the poverty" in Europe (Hotez and Gurwith 2011)

Brucellosi in Italia



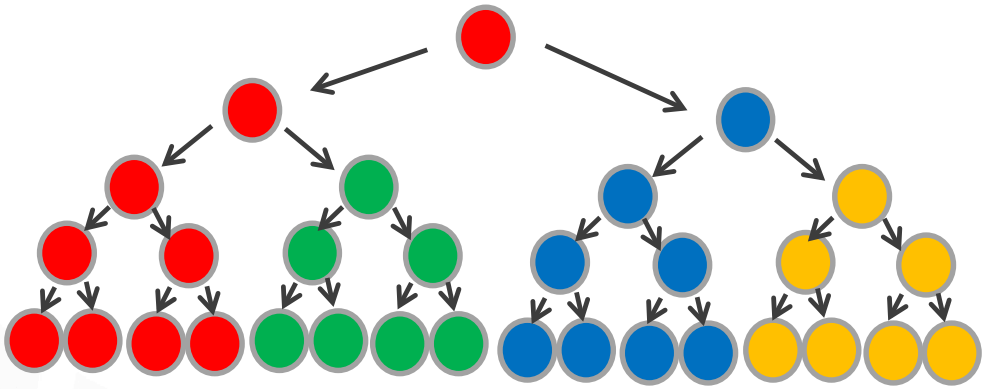


Negli anni ‘70 incidenza di 5 casi per 100000 mentre attualmente si registra incidenza 0,2
L’89% dei casi notificati tra il 1998 e il 2010 sono stati registrati in Campania, Puglia, Calabria e Sicilia.

Graziani, C., Mancini, F.R., Adone, R., Marianelli, C., Pasquali, P., Rizzo, C., Bella, A., De Massis, F., Danzetta, M.L., Calistri, P., Primavera, A., Ruocco, L., Busani, L., 2013. La brucellosi in Italia dal 1998 al 2011. Rapporti ISTISAN 13/45, 75 p.

Brucella evoluzione

- Brucella è geneticamente monomorfica
- Ricombinazione omologa è assente



Ancestrale

Mutati

Mutati

Mutati

Gli SNPs possono essere presenti sull'intero genoma quindi se esaminiamo e compariamo più genomi si possono identificare mutazioni anche in contesti temporali ristretti.

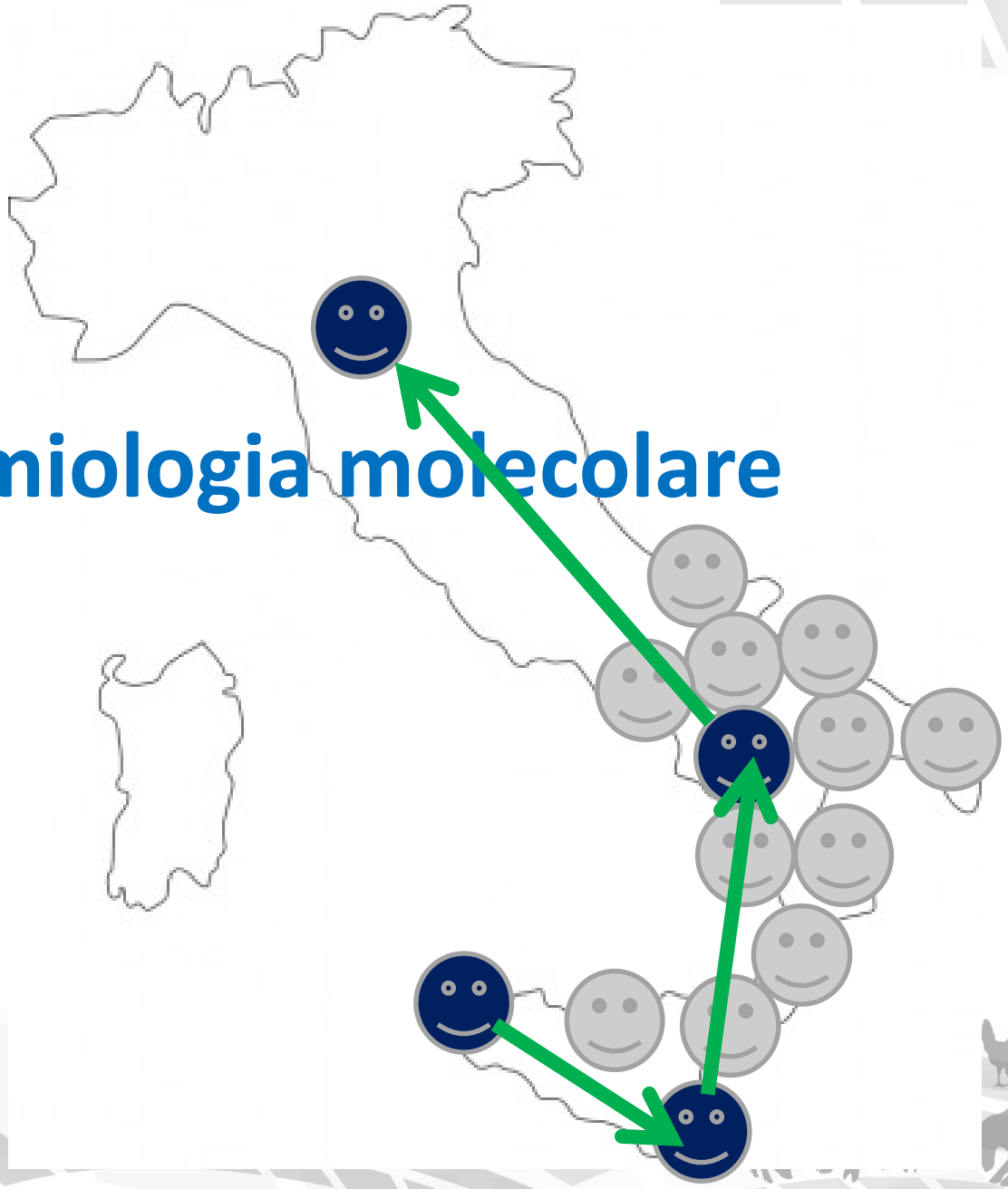
- **Bassa diversità genomica**
- **Selezione per bottlenecking**

IZSAM G.CAPORALE
TERAMO

Sorveglianza epidemiologica

IZSAM G.CAPORALE TERAMO

Epidemiologia molecolare



Epidemiologia molecolare

- **Epidemiologia molecolare** cerca relazioni **fenotipiche e genetiche** degli agenti responsabili di malattie infettive per suggerire la loro sorgente, le loro relazioni biologiche, le vie di trasmissione

Brucella

B. abortus (biovar 1,2,3,4,5,6,9)
B. melitensis (biovar 1,2,3)
B. suis (biovar 1-5)
B. ovis
B. canis
B. neotomae
B. ceti
B. pinnipedialis
B. microti

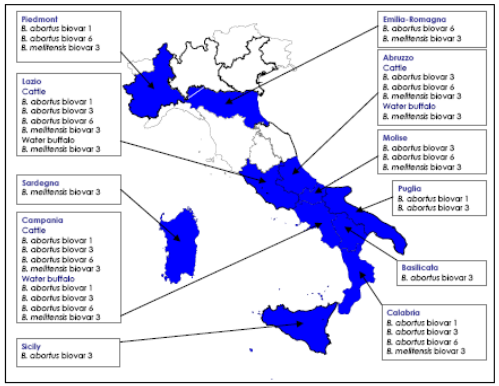


Figure 1
Geographic distribution of *Brucella* strains isolated from cattle and water buffalo in Italy between 2001

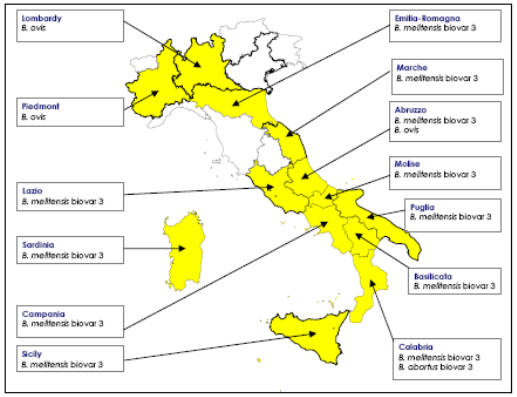


Figure 2
Geographic distribution of *Brucella* strains isolated from sheep and goats in Italy between 2001 and 2006

Typing of *Brucella* field strains isolated from livestock populations in Italy between 2001 and 2006

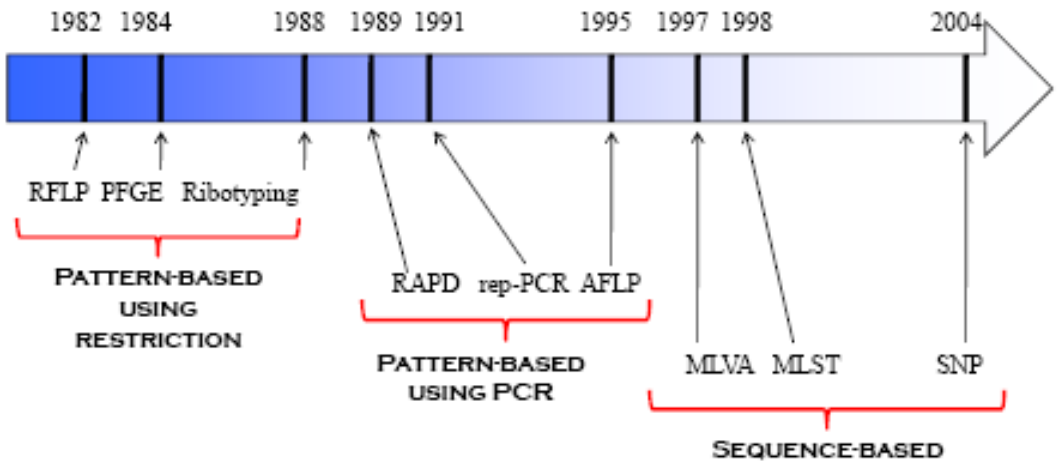
Elisabetta Di Giannatale[1], Fabrizio De Massis[1, 2], Massimo Ancora[1], Katiuscia Zilli[1] & Alessandra Alessiani[1]

B. abortus biovar 1, 3, 6
B. melitensis biovar 3

Obiettivi del progetto di ricerca

- Tipizzazione molecolare degli isolati di *Brucella* provenienti dal territorio italiano
- Analisi del genoma completo dei ceppi rappresentativi di cluster circolanti in Italia
- Sviluppo di un sistema di tipizzazione per la sorveglianza epidemiologica

Brucella typing



Metodi su base genomica:
Diventati disponibili dal momento che sono state disponibili le prime Sequenze complete di Brucella

1. dati generati da tali approcci sono facilmente conservabili e comparabili laboratori
2. Approccio multi locus mitiga gli errori che potrebbero generarsi con le metodiche a locus singolo
3. Vantaggiosi perché utilizzano marker a diversa velocità di mutazione

MLST

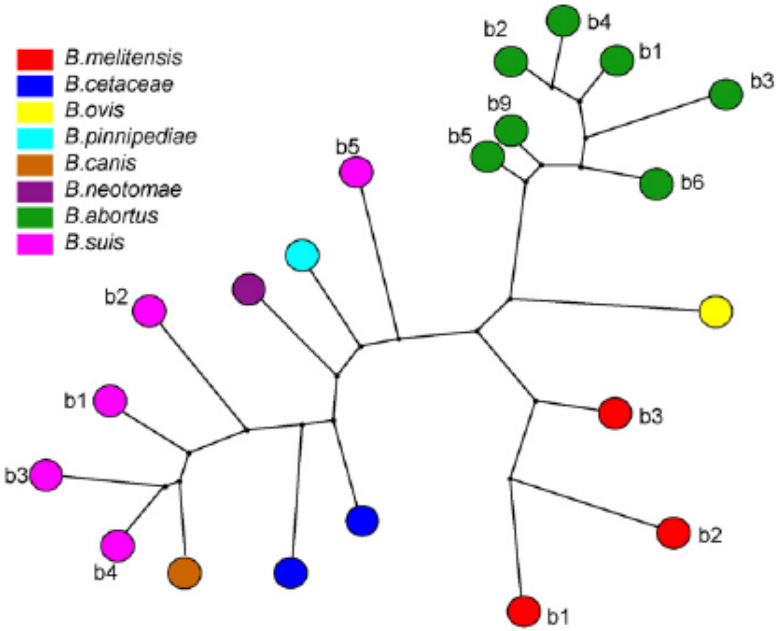
MLVA

WGS

MLST

B. Melitensis	B. Abortus
6 Gt	6 Gt
B. Suis	B. Marinae
6 Gt	5 Gt

MLST



Whatmore *et al.* 2007

MLST

DATI

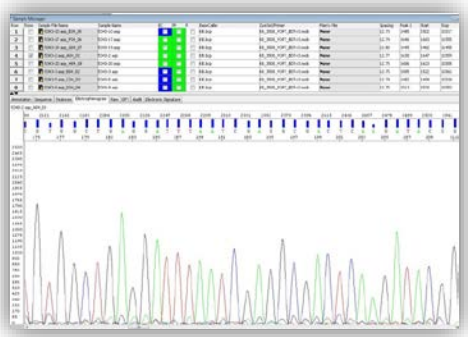
Isolato

DNA

PCR

sequenza

Ricerca nel database



MLST analisi

Indagini epidemiologche per singoli focolai

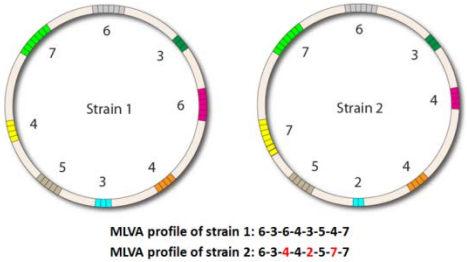
Andamento locale della malattia

Dinamiche di popolazione

Analisi evolutive

MLVA Brucella

Variable Number Tandem Repeats
sono sequenze di DNA che possono variare da 2 fino a 100 nucleotidi organizzate in domini ripetuti



ATTCGATTCGATTCG

ATTCG è ripetuta 3 volte

Indagini epidemiologche per singoli focolai

Andamento locale della malattia

Dinamiche di popolazione

Analisi evolutive

MLVA – Metodi

HOOF-Prints
MLVA8-MLVA10-MLVA11-MLVA15-MLVA16 Orsay
MLVA21 Weybridge
MLVA15 Flagstaff
MLVA10 Ames
Bricker et al. 2003, Le Fleche et al. 2006 Whatmore et al (2006), Tiller et al (2010)

MLVA-16

Esistenza di un database online

MLVAbank for Microbes Genotyping

Public Databases

Brucella v4

Owned by public_databases

Journal of Microbiological Methods 92 (2013) 103–107

Contents lists available at SciVerse ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

MLVA-16 loci panel on *Brucella spp.* using multiplex PCR and multicolor capillary electrophoresis

Giuliano Garofolo *, Massimo Ancora, Elisabetta Di Giannatale

Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" — National and OIE Reference Laboratory for Brucellosis, Italy

ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form 8 November 2012
Accepted 8 November 2012
Available online 19 November 2012

Keywords:
MLVA
Capillary electrophoresis
Brucella spp.
Genotyping

ABSTRACT

The Multi Locus Variable Number Tandem Repeat Analysis 16 loci panel (MLVA-16), involving singleplex PCRs and agarose gel electrophoresis, is the standard genotyping method for *Brucella spp.*, used also for the *Brucella* international online database. We describe an alternative, reliable, high-throughput MLVA-16 protocol using multiplex PCRs and multicolor capillary electrophoresis.

© 2012 Elsevier B.V. All rights reserved.

Infection, Genetics and Evolution 19 (2013) 59–70

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Investigating genetic diversity of *Brucella abortus* and *Brucella melitensis* in Italy with MLVA-16

Giuliano Garofolo [a,*], Elisabetta Di Giannatale [a], Fabrizio De Massis [a], Katiuscia Zilli [a], Massimo Ancora [a], Cesare Cammà [a], Paolo Calistri [a], Jeffrey T. Foster [b]

[a] *Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale", National and OIE Reference Laboratory for Brucellosis, Via Campo Boario, 64100 Teramo, Italy*
[b] *Center for Microbial Genetics & Genomics, Northern Arizona University, Flagstaff, AZ 86011-4073, USA*

WGS
Reference assisted – SNPs

Sequenze con Illumina NextSeq™ 500

Mappare contro RefSeq genome Brucella melitensis bv. 1 str. 16M

Variant calling di alta qualità

AGT AGG TGT TGT

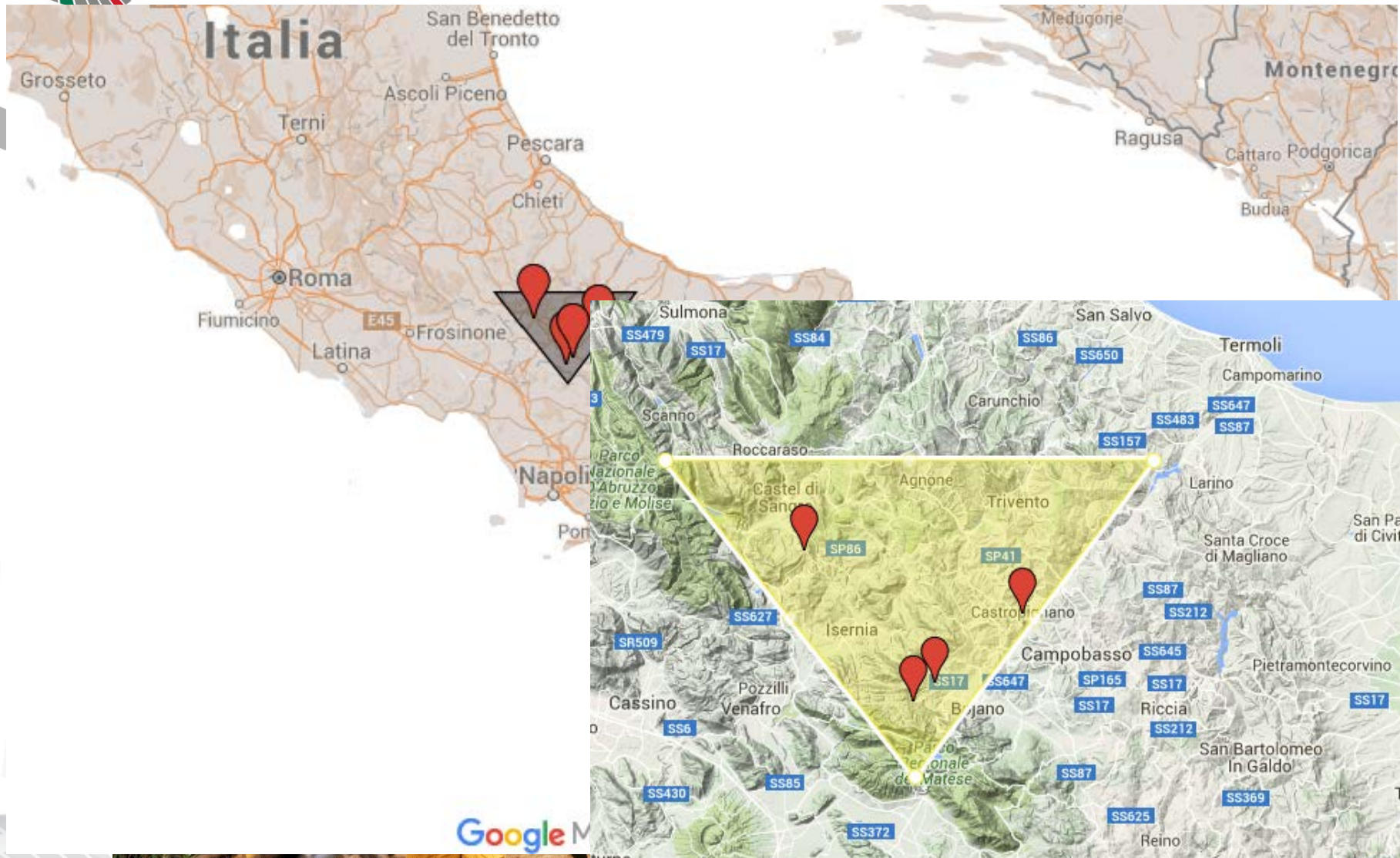
Metodo di analisi

- FASTQC per controllo qualità
- BWAmem per mappare le sequenze
- SNVs usando GATK con metodo UnifiedGenotyper
- ISG per estrarre gli SNVs da tutti i genomi
- Ispezione dei SNV usando un BAM viewer
- SNPs coverage > 30X
- SNPs proporzione > 90%
- SNPs da regioni duplicate vengono rimossi come quelli definiti ambigui e mancanti in tutti gli altri genomi

Costruire albero filogenetico

- SI può costruire un albero filogentico usando il metodo Neighbour-Joining (NJ) methods
- Ma si preferisce il metodi di massima verosimiglianza: RaxML e FastTree
- Verifica della filogenesi con metodo di massima parsimonia
- Bootstrap viene utilizzato per verificare la filogenesi

IZSAM G.CAPORALE
TERAMO

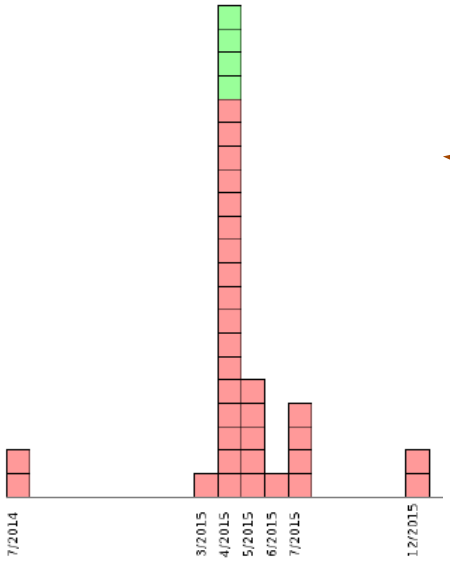


Focolaio

Territori UI

2014-2016

19 aziende
41 isolati



Epi Curve
Grouped by column "Epi Info"
FOCOLAIO_FROSINONE_2014_2016
FOCOLAIO_ROMA_2014_2015
ROUTINE

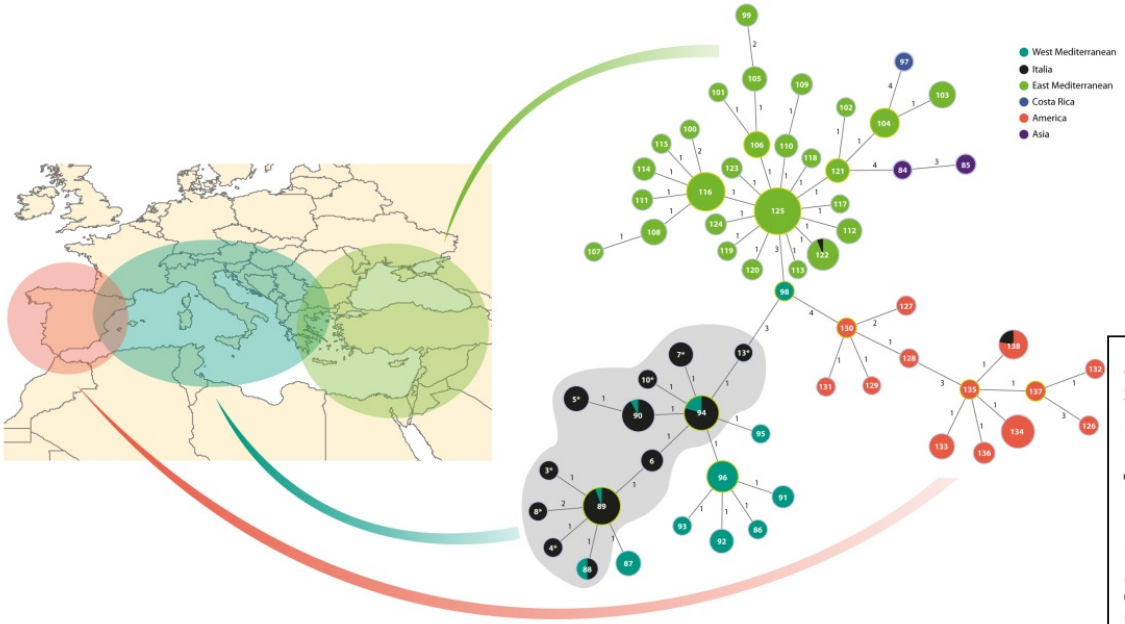
MLVA11

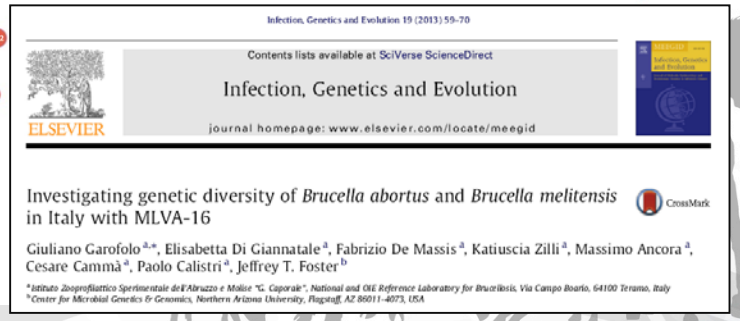
MLVA

Genotipo 87
West Mediterranean

=

Isolato Italiano





Infection, Genetics and Evolution 19 (2013) 59–70

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

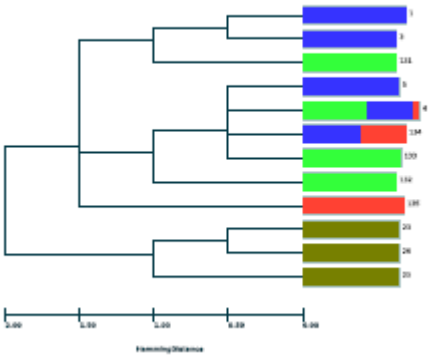
journal homepage: www.elsevier.com/locate/meegid

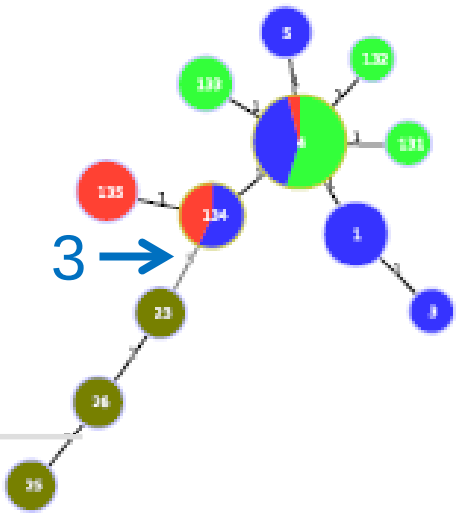
Investigating genetic diversity of *Brucella abortus* and *Brucella melitensis* in Italy with MLVA-16

Giuliano Garofolo [a,*], Elisabetta Di Giannatale [a], Fabrizio De Massis [a], Katiuscia Zilli [a], Massimo Ancora [a], Cesare Cammà [a], Paolo Calistri [a], Jeffrey T. Foster [b]

[a] Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale", National and OIE Reference Laboratory for Brucellosis, Via Campo Boario, 64100 Teramo, Italy
[b] Center for Microbial Genetics & Genomics, Northern Arizona University, Flagstaff, AZ 86011-4073, USA

Outbreak investigation - MLVA16



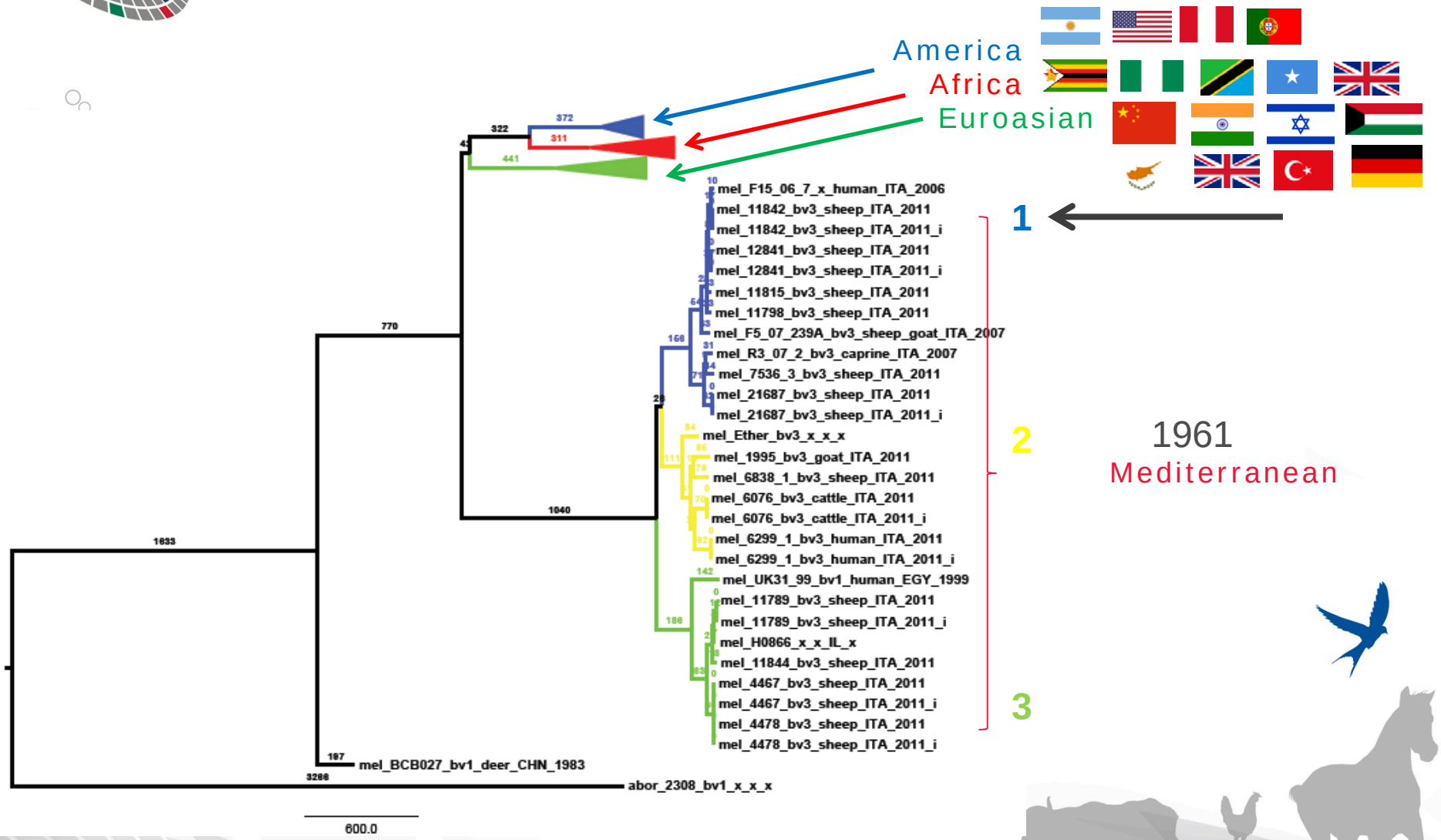


Genotipo MLVA11 identico

Mutazioni per 3 dei cinque loci ipervariabili!!!!

BRUCE_06	BRUCE_08	BRUCE_11	BRUCE_12	BRUCE_42	BRUCE_43	BRUCE_45	BRUCE_55	BRUCE_18	BRUCE_19	BRUCE_21	BRUCE_04	BRUCE_07	BRUCE_09	BRUCE_16	BRUCE_30
3	6	3	14	1	1	3	3	7	42	8	6	7	8	6	3
3	6	3	14	1	1	3	3	7	42	8	7	7	6	8	3

WGS *B. melitensis* approccio globale



B. melitensis from Italy

***Brucella melitensis* bv. 3 str. Ether**
Strain also known as NCTC10509; ATCC 23458; BCCN R3. This is the *Brucella melitensis* biovar 3 reference strain originally isolated by Prof. G. Gargani (Univ. di Firenze, Italy) from a goat in Italy in 1961 (Meyer and Morgan 1973).

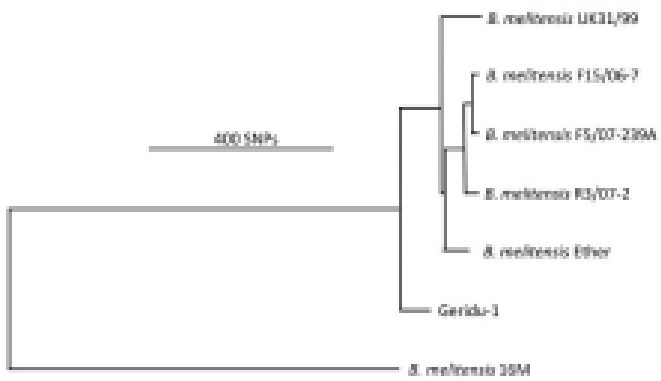


FIG 2 Phylogenetic tree showing the position of the medieval Geridu-1 strain within the Ether clade. Only those SNPs which correlated with sufficient coverage in the Geridu-1 alignment were included in the construction of the tree.

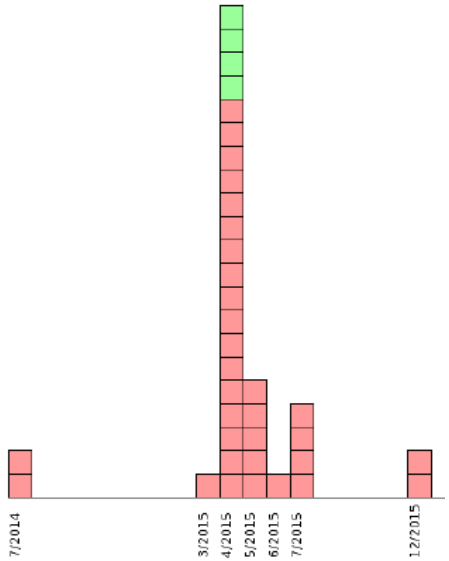
RESEARCH ARTICLE

Recovery of a Medieval *Brucella melitensis* Genome Using Shotgun Metagenomics

Gemma L. Kay,[a] Martin J. Sergeant,[a] Valentina Giuffra,[b,c] Pasquale Bandiera,[c] Marco Milanese,[d] Barbara Bramanti,[e] Raffaella Bianucci,[e,f,g] Mark J. Pallen[a]

Division of Microbiology and Infection, Warwick Medical School, University of Warwick, Coventry, United Kingdom[a]; Division of Paleopathology, Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy[b]; Department of Biomedical Sciences[c] and Department of History,[d] University of Sassari, Sassari, Italy; Department of Biosciences, Centre for Ecological and Evolutionary Synthesis, University of Oslo, Oslo, Norway[e]; Department of Public Health and Pediatric Sciences, Laboratory of Physical Anthropology, University of Turin, Turin, Italy[f]; Anthropologie Bioculturelle, Droit, Ethique et Santé, Aix Marseille Université, Marseille, France[g]

SNPs analysis

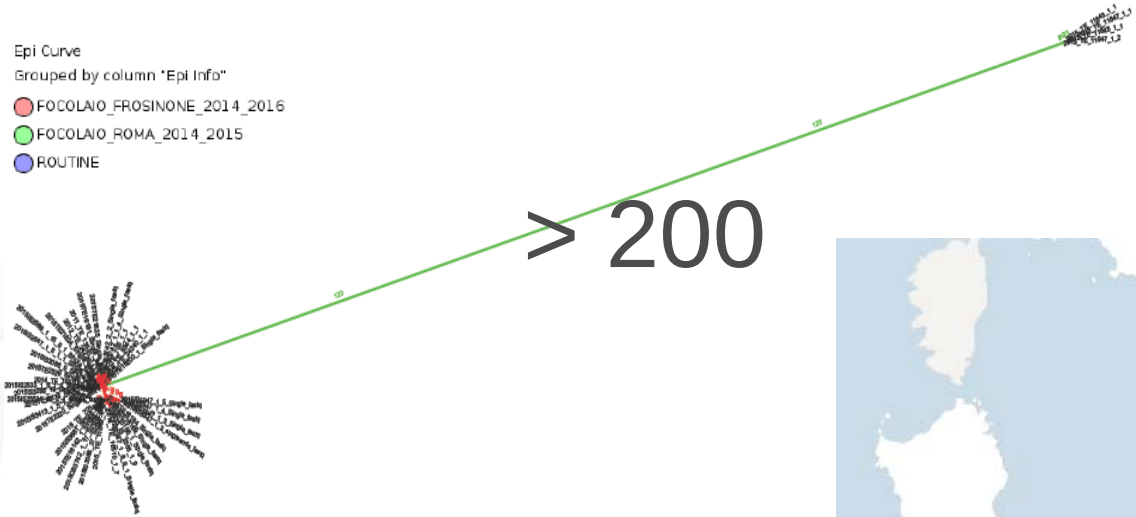


41 isolati

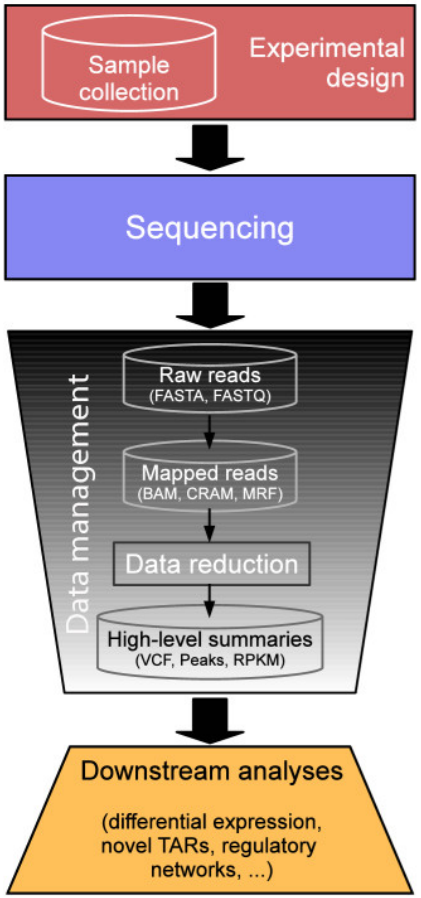
2 clusters separati da > 200 SNPs

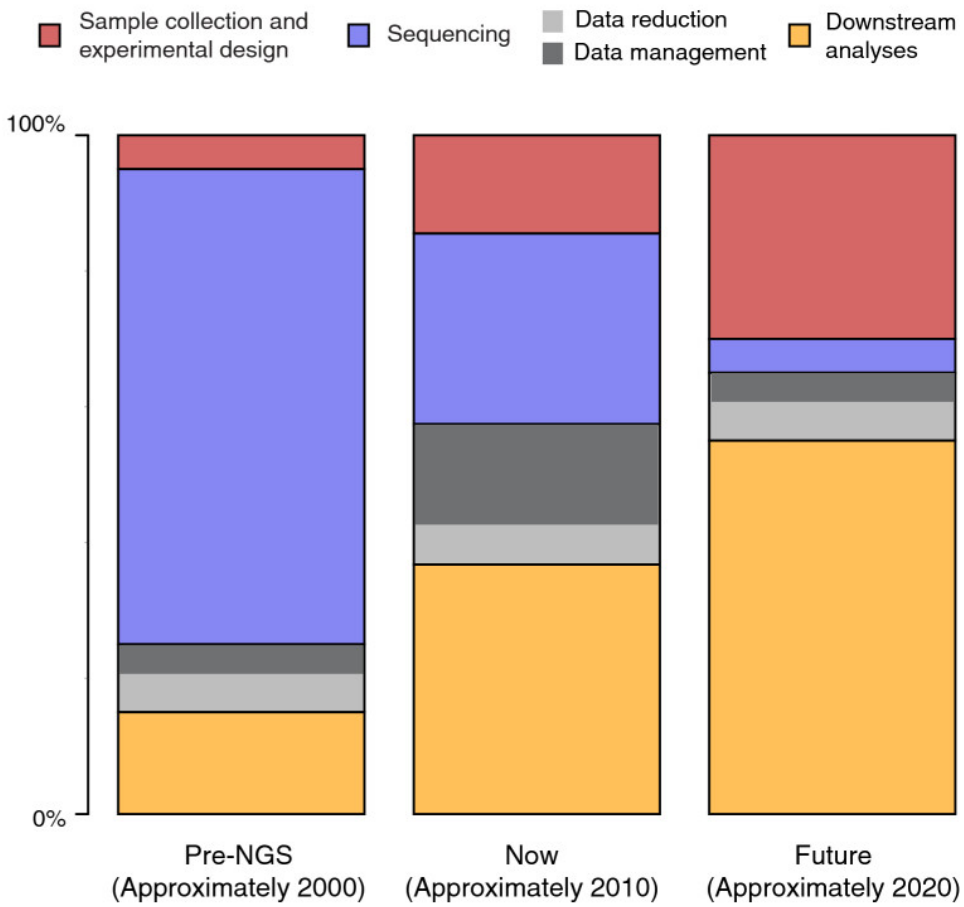
Epi Curve
Grouped by column "Epi Info"
FOCOLAIO_FROSINONE_2014_2016
FOCOLAIO_ROMA_2014_2015
ROUTINE

> 200



Whole Genome Sequencing

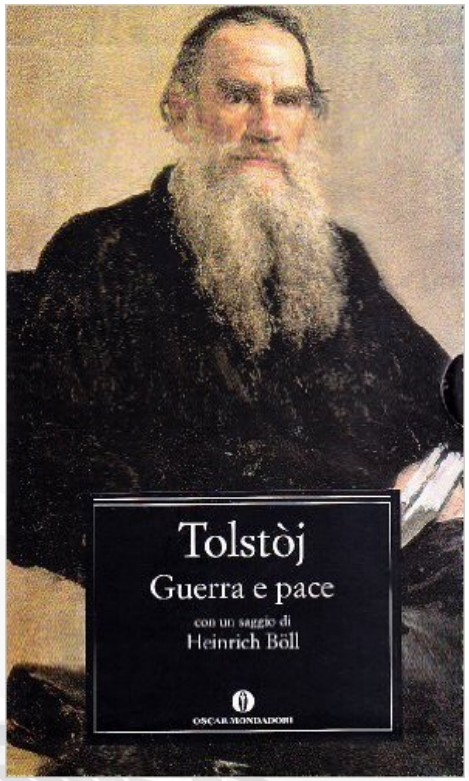




Sboner, Andrea, et al. "The real cost of sequencing: higher than you think!." *Genome biology* 12.8 (2011): 125.

WGS vs MLVA

Guerra e Pace in 140 caratteri
Finiranno nel 2138 (non abbiamo mica fretta)



Conclusioni

- MLVA è un’alternativa valida ed economica
- WGS tramite NGS non può essere utilizzato per ottenere MLVA
- SNP analysis è estremamente risolutiva ma male interpreta i fenomeni di riombinazione
- Nessun metodo genomico può rimpiazzare l’epidemiologia tradizionale

Next

- Who infected whom…….
- Verificare la data di introduzione della Brucellosi
- Brucellosi e metagenomica
- Genomica comparativa per testare l’evoluzione della virulenza di Brucella

Ringraziamenti

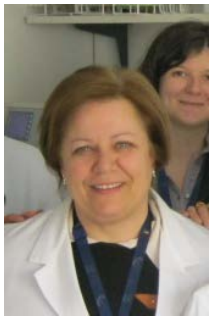
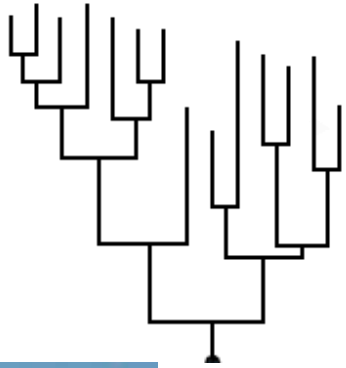
Ministero della Salute

BATTERIOLOGIA
Elisabetta Di Giannatale
Katiuscia Zilli
Lorena Sacchini
Alessandra Alessiani
Tiziana Persiani

EPIDEMIOLOGIA
Fabrizio De Massis
Paolo Calistri

DIAGNOSTICA
Massimo Scacchia
Andrea Di Provvido
Daniela Averaimo
Giovanni Foschi

RICERCA E SVILUPPO
Cesare Cammà
Massimo Ancora
Marco Di Domenico
Maurilia Marcacci

BIOINFORMATICA
Adriano Di Pasquale
Massimiliano Orsini
Claudio Patavino
Iolanda Mangone

SIEROLOGIA
Manuela Tittarelli
Barbara Bonfini
Flavio Sacchini