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Centro di Referenza Nazionale

Studio sull'origine ed evoluzione di *Brucella melitensis* in Italia

Giuliano Garofolo

I RISULTATI DELLA RICERCA CORRENTE CONDOTTA DALL'ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE -
ANNO 2020 17 Giugno 2021 - Webinar





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Studi di filogenomica ed epigenomica per la caratterizzazione di Brucella

N° Progetto/anno	IZS AM 02/17 RC
Responsabile scientifico	Giuliano Garofolo
Data di scadenza del progetto	14/11/2020

24 mesi- Budget € 139.315,00

IZSAM-UO-1	Giuliano Garofolo
IZSAM-UO-2	Cesare Cammà
IZSAM-UO-3	Fabrizio De Massis

M2.1 Sviluppo dell'analisi SNP

M2.2 Sviluppo dello schema cgMLST

M2.3 Sviluppo di un sistema di riferimento per le attività di epidemiologia molecolare

M3.1 Studi di metilazione



BACTERIOLOGY
September 2018, Volume 56, Issue 9, e00517-18
<https://doi.org/10.1128/JCM.00517-18>

MICROBIAL GENOMICS

RESEARCH ARTICLE
Janowicz et al., *Microbial Genomics* 2020;6
DOI 10.1099/mgen.0.000446



Core Genome Multilocus Sequence Typing and Single Nucleotide Polymorphism Analysis in the Epidemiology of *Brucella melitensis* Infections

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Evolutionary history and current distribution of the West Mediterranean lineage of *Brucella melitensis* in Italy

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Materiale and metodi

Studio sull'origine ed evoluzione di Brucella melitensis in Italia

- Dataset: 441 Italian strains and 149 public strains
- WGS (NextSeq) and scaffold assembly (Spades 3.11)
- WGS typing and phylogenetic analysis:
 - * cgMLST followed by multiple spanning tree generation (Ridom Seqsphere+ task template with 2704 target core genes)
 - * SNP-based analysis (ISG tool) followed by Maximum-Likelihood tree generation (RAxML-NG)
- Temporal analysis performed using BEAST2 using a subset of 259 *B. melitensis* strains
- Population structure determined using BAPS6 in hierBAPS software





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Fig. 1. Restored laboratory of Bruce and others in Malta.

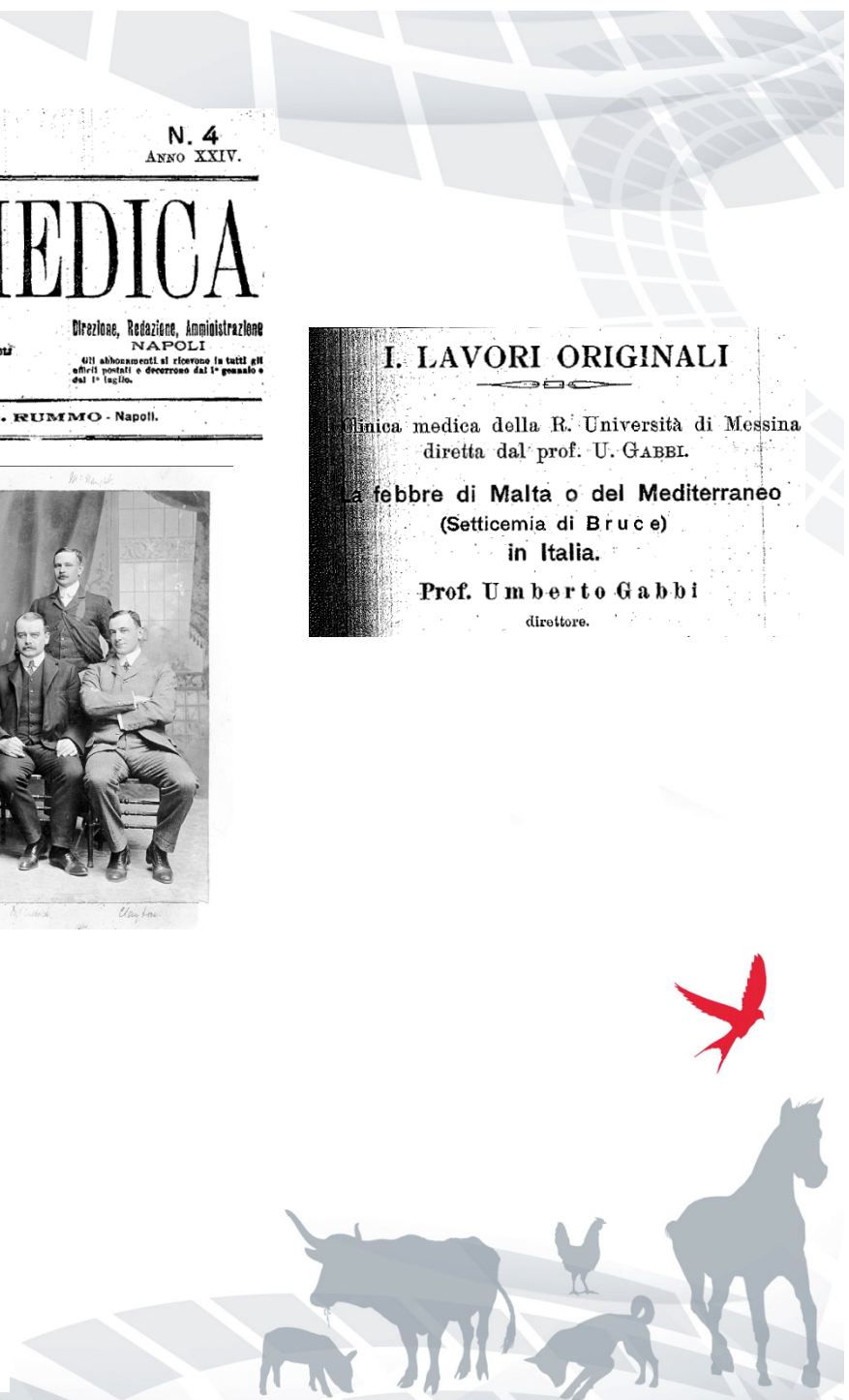


Si può affermare col dovuto riserbo che le prime sicure notizie scritte sulla esistenza di una infezione febbrile diversa dalla febbre tifoide per talune particolarità e stranezze del decorso clinico, per la sua lunga durata, e per la resistenza a qualsiasi medicazione si hanno a Napoli verso il 1872 sebbene già dal 1869 se ne constataessero i primi casi.

Il Galassi la descrisse a Roma, il Giura nell'Ariccia, il De Dominicis a Nola, il Cardarelli a Civitanova del Sannio, l'Orlando a Caserta, il D'Ortensio a Teramo il Testi a Fermo, il Cacciola e il D'ancona a Padova.....

I nomignoli

Febbricola tifoidea, tifoide intermittente, febbricola tifosa, febbre tifoidea atipica, febbre di Napoli, febbricola nostrana, febbre delle fogne, febbre urbana, febbre da anemia





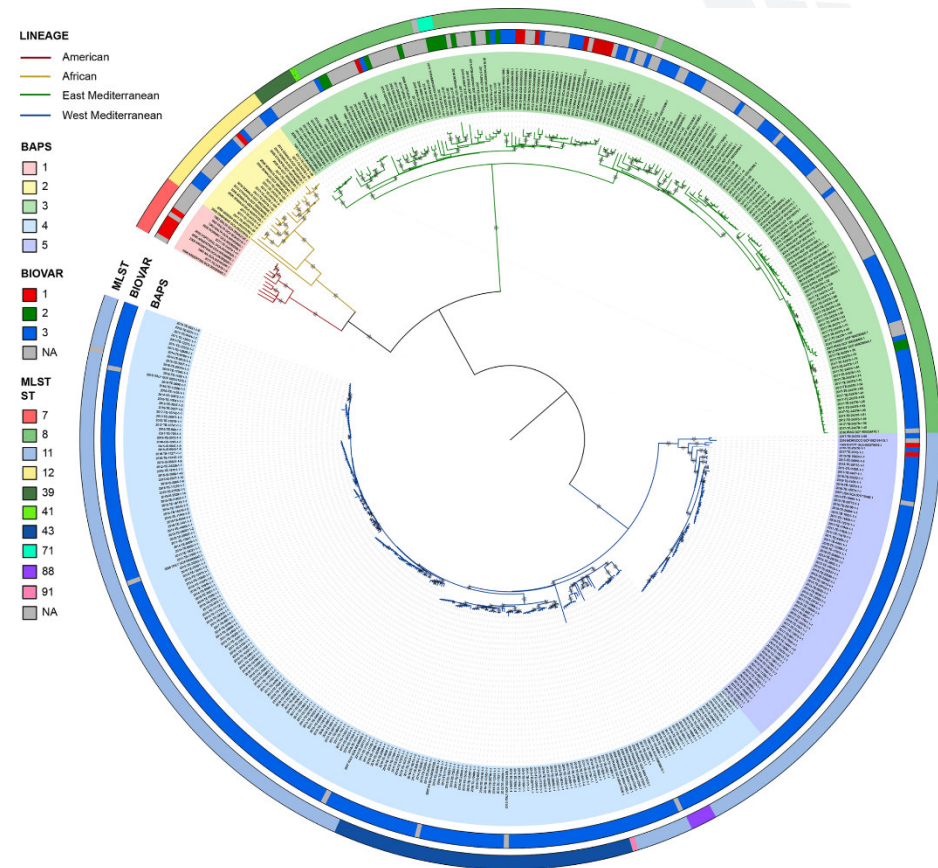
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Global phylogeny of *B. melitensis*

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- 15,041 SNPs
- Ceppi Italiani appartengono al lignaggio genetico West Mediterranean
- 1,004 SNPs West Mediterranean clade
- West Mediterranean = bv3
- STs Italia **ST-11, ST-43, ST-88, ST-91**



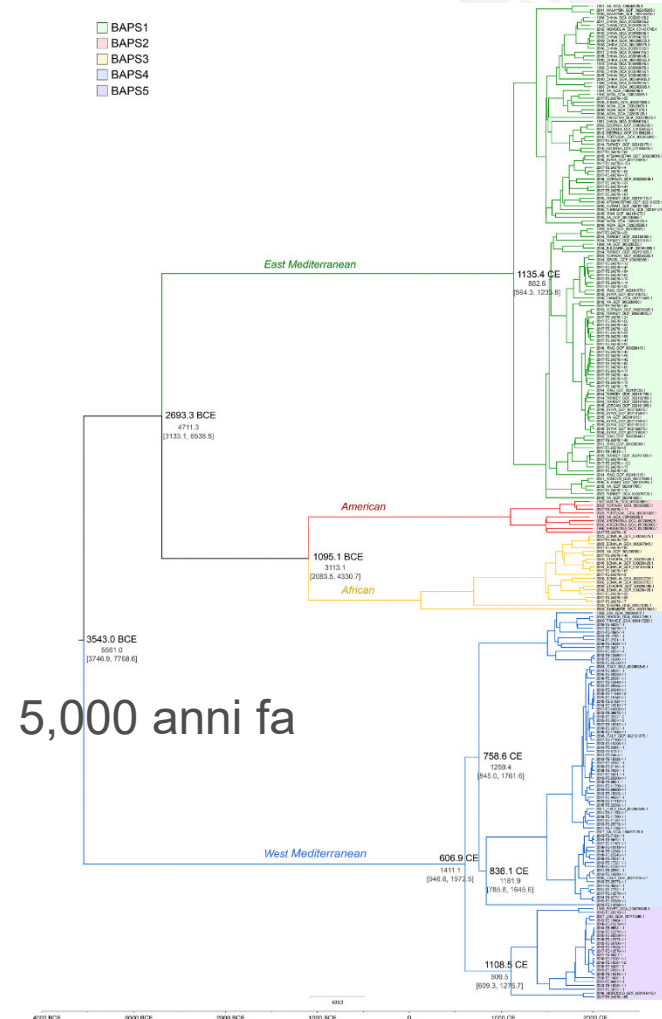
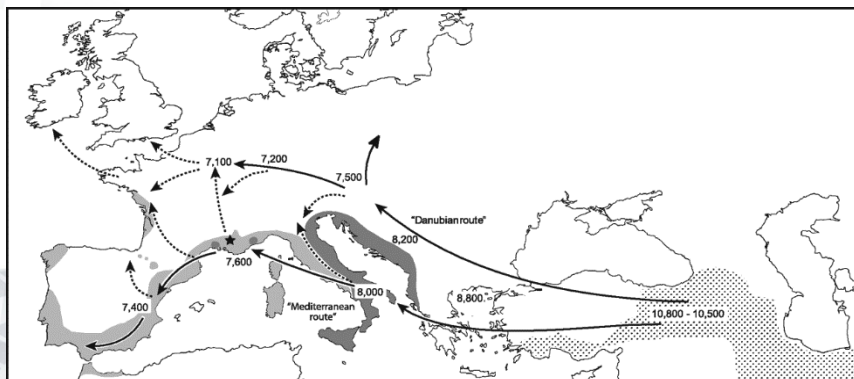
Tree scale: 0.0001



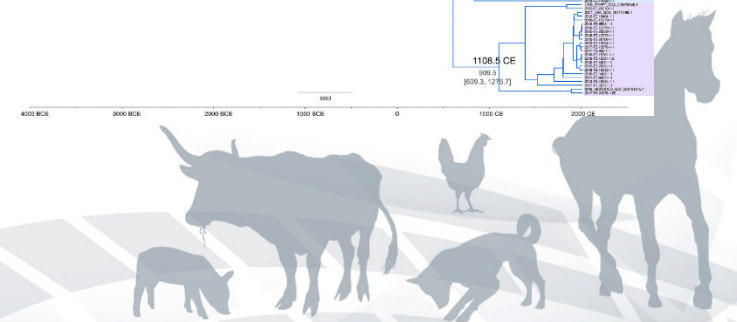
Time-structured phylogeny of *B. melitensis*

The West Mediterranean lineage diverged from the *B. melitensis* population in ~3500 BCE after the first introductions of domesticated sheep and goats occurred in the Central Mediterranean region.

West Mediterranean lineage is split into two branches corresponding to BAPS 4 and BAPS 5 subclades, with a most recent common ancestor (MRCA) around 1400 years ago.



5,000 anni fa



Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact

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Edited by Jeremy A. Sabloff, University of Pennsylvania Museum of Archaeology and Anthropology, Philadelphia, PA, and approved May 27, 2008 (received for review March 20, 2008)

The past decade has witnessed a quantum leap in our understanding of the origins, diffusion, and impact of early agriculture in the Mediterranean Basin. In large measure these advances are attributable to new methods for documenting domestication in plants and animals. The initial steps toward plant and animal domestication in the Eastern Mediterranean can now be pushed back to the 12th millennium cal B.P. Evidence for herd management and crop cultivation appears at least 1,000 years earlier than the morphological changes traditionally used to document domestication. Different species seem to have been domesticated in different parts of the Fertile Crescent, with genetic analyses detecting multiple domestic lineages for each species. Recent evidence suggests that the expansion of domesticates and agricultural economies across the Mediterranean was accomplished by several waves of seafaring colonists who established coastal farming enclaves around the Mediterranean Basin. This process also involved the adoption of domesticates and domestic technologies by indigenous populations and the local domestication of some endemic species. Human environmental impacts are seen in the complete replacement of endemic island faunas by imported mainland fauna and in today's anthropogenic, but threatened, Mediterranean landscapes where sustainable agricultural practices have helped maintain high biodiversity since the Neolithic.

PERSPECTIVE

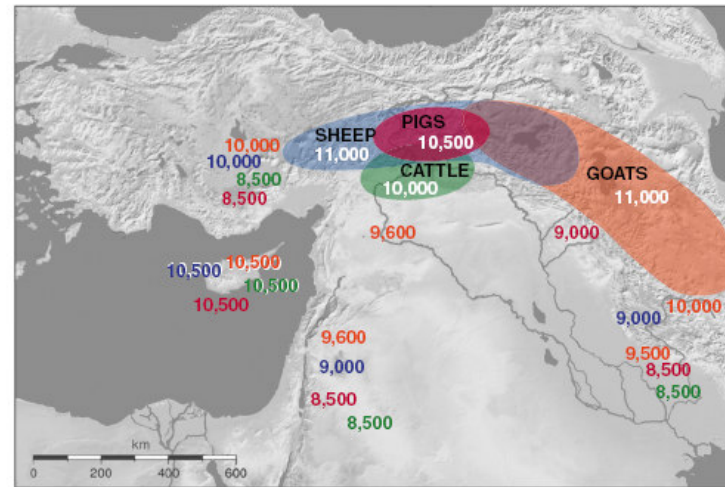


Fig. 1. The origin and dispersal of domestic livestock species in the Fertile Crescent. Shaded areas show the general region and the approximate dates in calibrated years B.P. in which initial domestication is thought to take place. Dates outside of the shaded areas show the approximate date when the domestic form first appears in a region. Orange, goats (*Capra hircus*); blue, sheep (*Ovis aries*); green, cattle (*Bos taurus*); fuscia, pigs (*Sus scrofa*).

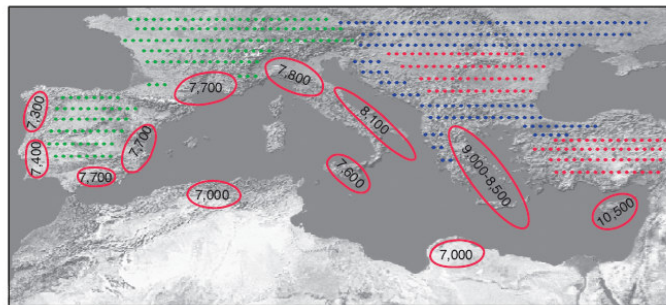
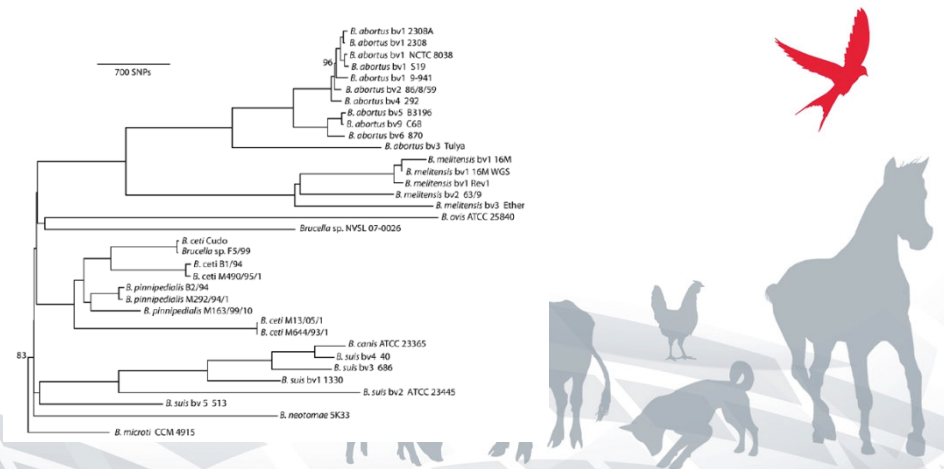


Fig. 2. An integrated model of the Neolithic expansion in the Mediterranean Basin. The location of colonist farming enclaves is shown in the red ellipses. Approximate dates of these enclaves are given inside the ellipses in calibrated years B.P. Red dots represent areas that are proposed to have been settled by colonist farmers; green dots indicate areas where indigenous foragers adopted elements of the Neolithic package; and blue dots indicate areas of proposed integration of colonist farmers with indigenous foraging groups. Data were compiled from refs. 52, 54, 56, 57, and 65 and figure 7.1 of ref. 74.



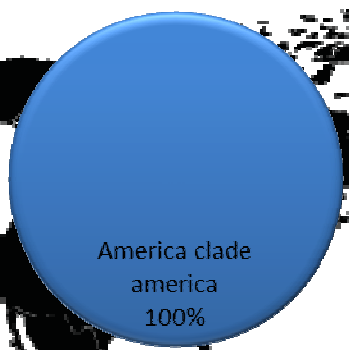


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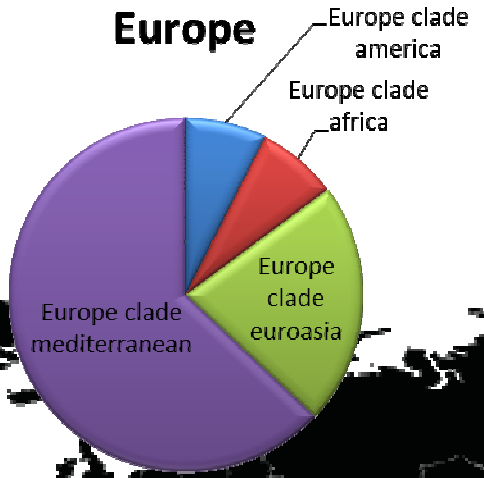
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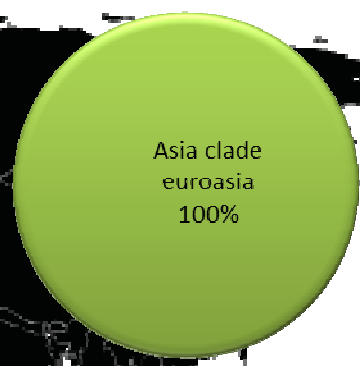
America



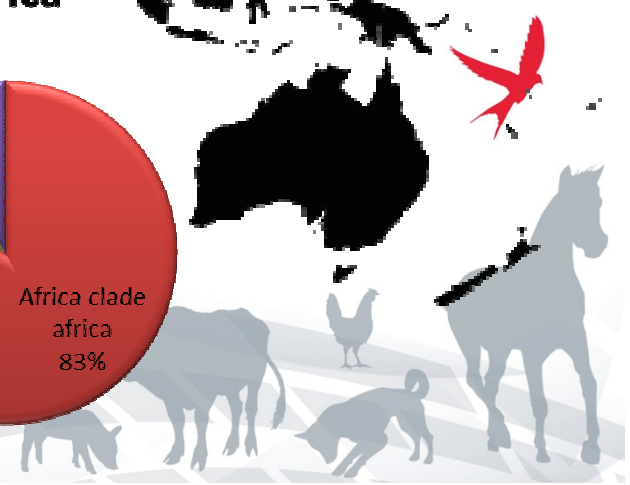
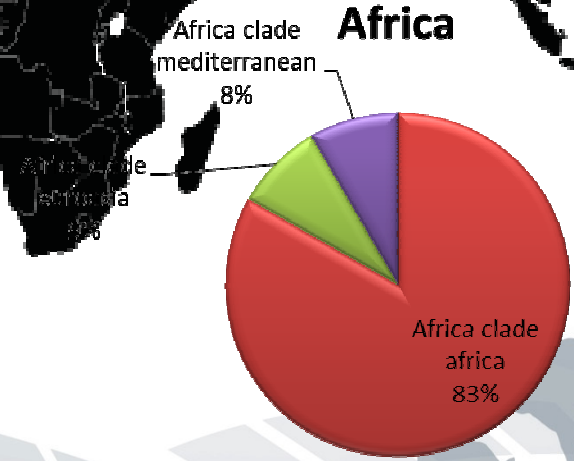
Europe



Euroasia



Africa





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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).

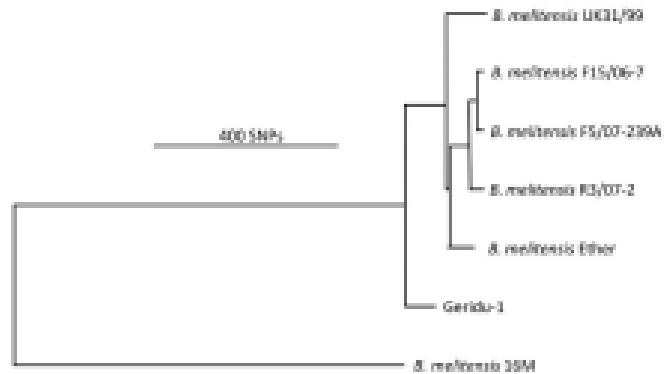


FIG 2. Phylogenetic tree showing the position of the medieval Geridu-1 strain within the Ether clade. Only those SNPs which correlated with sufficient coverage in the Geridu-1 alignment were included in the construction of the tree.

RESEARCH ARTICLE

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

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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

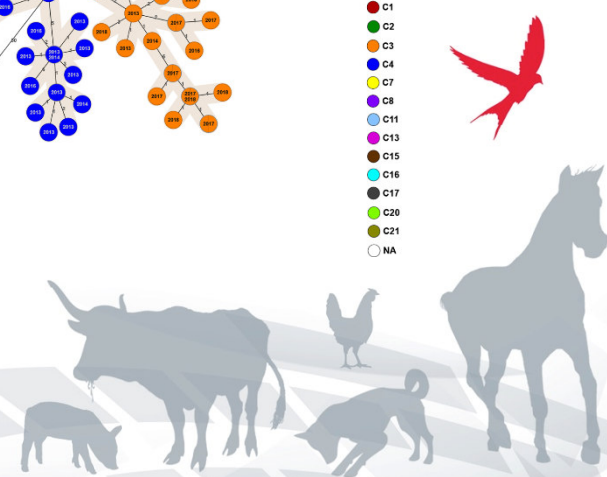
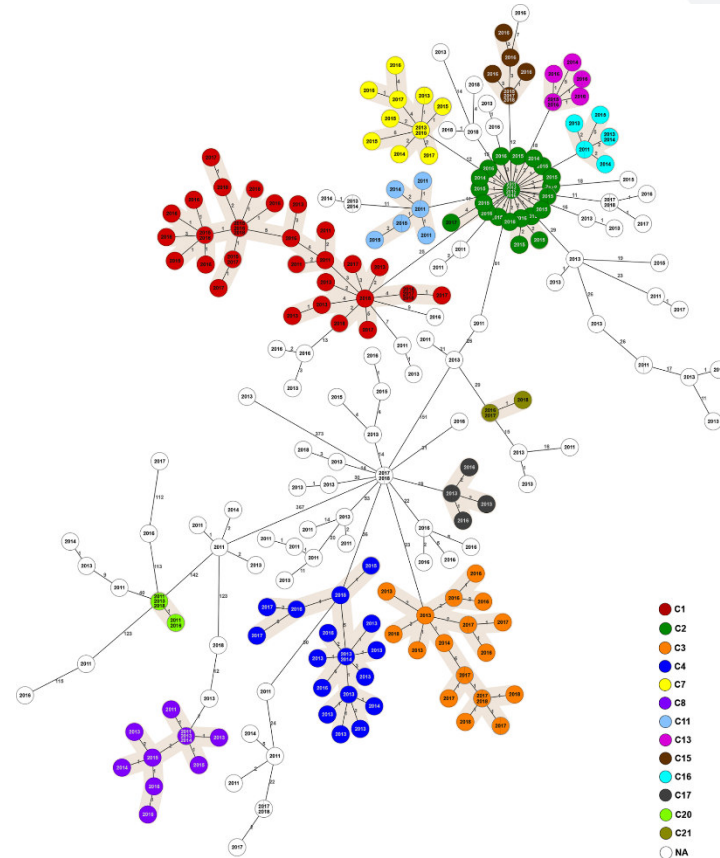
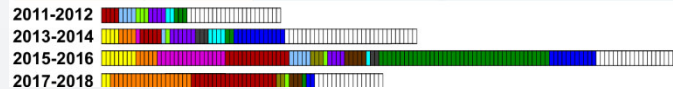
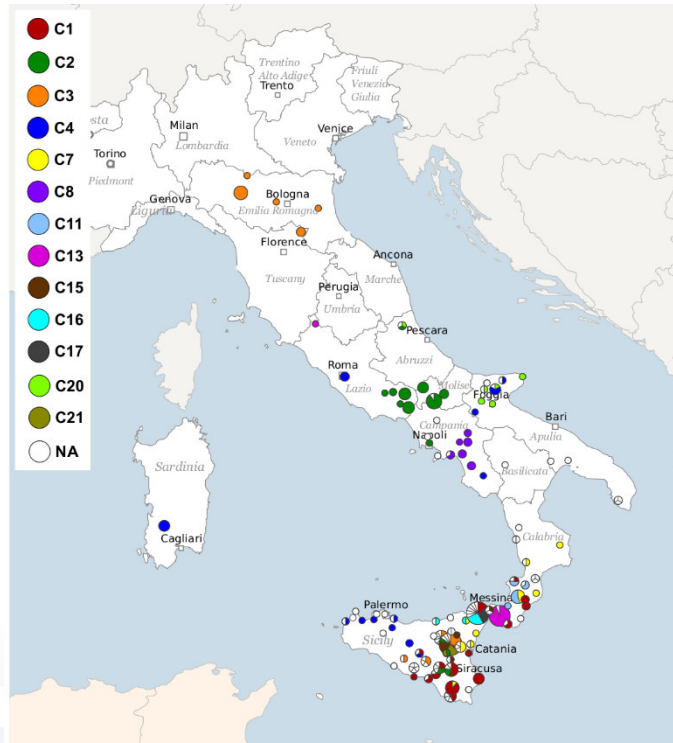




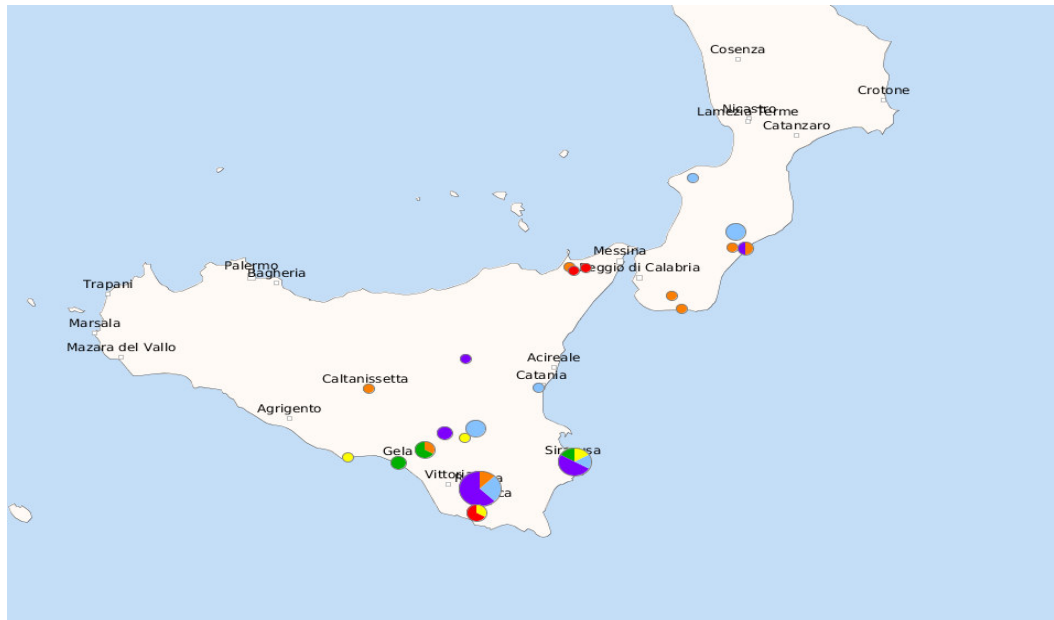
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Distribuzione geografica *B. melitensis* BAPS4

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Distribuzione del Complex 1



C1 presente in un periodo di 8 anni

Ragusa and Siracusa maggiore prevalenza

- 2011
- 2013
- 2015
- 2016
- 2017
- 2018

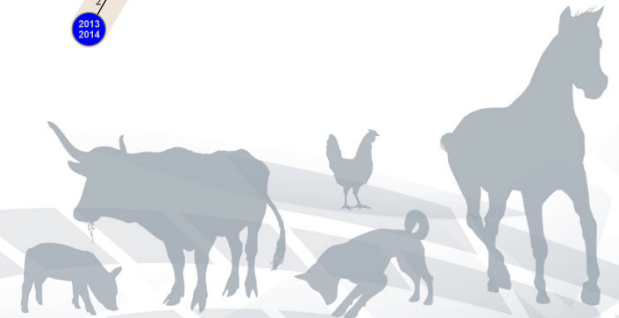
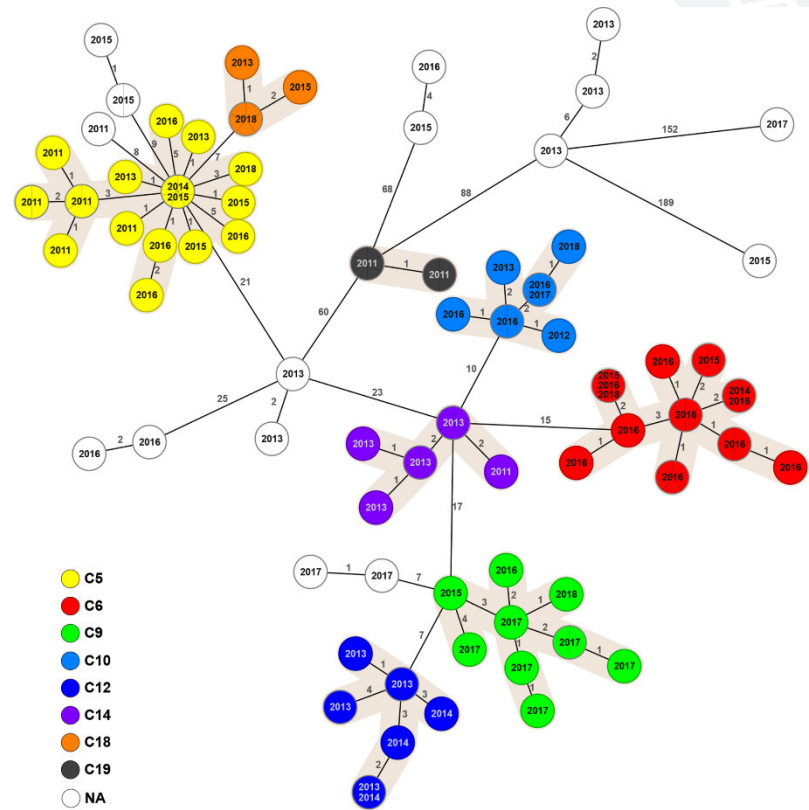
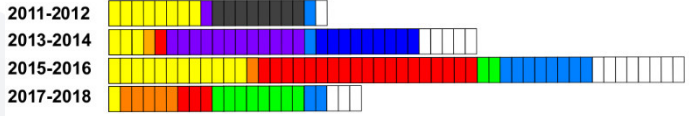
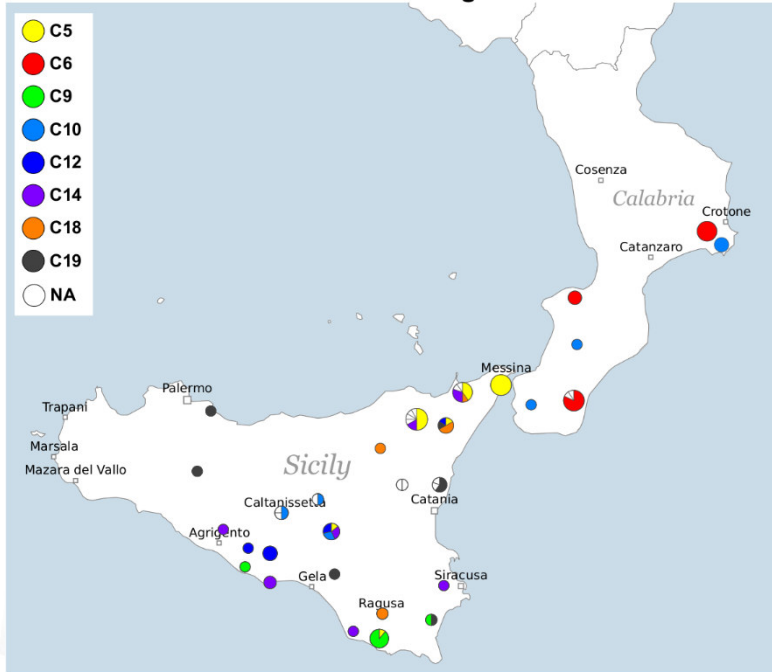




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Distribuzione geografica *B. melitensis* BAPS5 population in Italy

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- Nel nostro studio, abbiamo utilizzato strumenti e metodi WGS per analizzare un ampio set di isolati fornendo un quadro completo dell'epidemiologia, della diversità genetica e della storia evolutiva di *B. melitensis* in Italia.
- Nel nostro lavoro, abbiamo effettuato un'analisi completa del lignaggio West Mediterranean che risulta essere la popolazione di *Bmel* presente in Italia. Dove in abbiamo trovato la presenza di due sottopopolazioni con una delle quali presente solo in Sicilia e Calabria.
- All'interno della sotto popolazione BAPS 5, abbiamo identificato due rami corrispondenti a ceppi provenienti da Egitto, Marocco ed Eritrea che si sono discostati dai ceppi italiani circa 800-1000 anni fa. La storia della brucellosi diffusa nel bacino del Mediterraneo potrebbe essere spiegata dai collegamenti commerciali tra le regioni dell'Italia meridionale, in particolare la Sicilia, e gli arabi e berberi dell'Africa.
- I nostri risultati sui tempi di genesi di *B. melitensis* coincidono con i tempi dell'espansione neolitica. Quindi, il lignaggio del west mediterranean si è evoluto nella regione (Italia) circa 5000 anni fa.

Conclusioni





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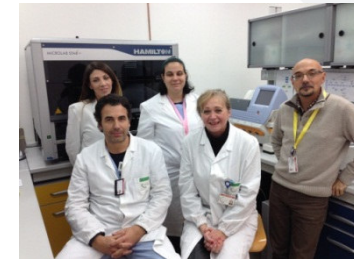


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Ringraziamenti



Ministero della Salute

