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# EMIDA Brucmel - *Brucella melitensis*: biotyping and differential diagnostic

**Sacchini Flavio*, Domenica Travaglini, Manuela Tittarelli**
**f.sacchini@izs.it; m.tittarelli@izs.it**

**CIFIV, Teramo, 3 dicembre 2013**

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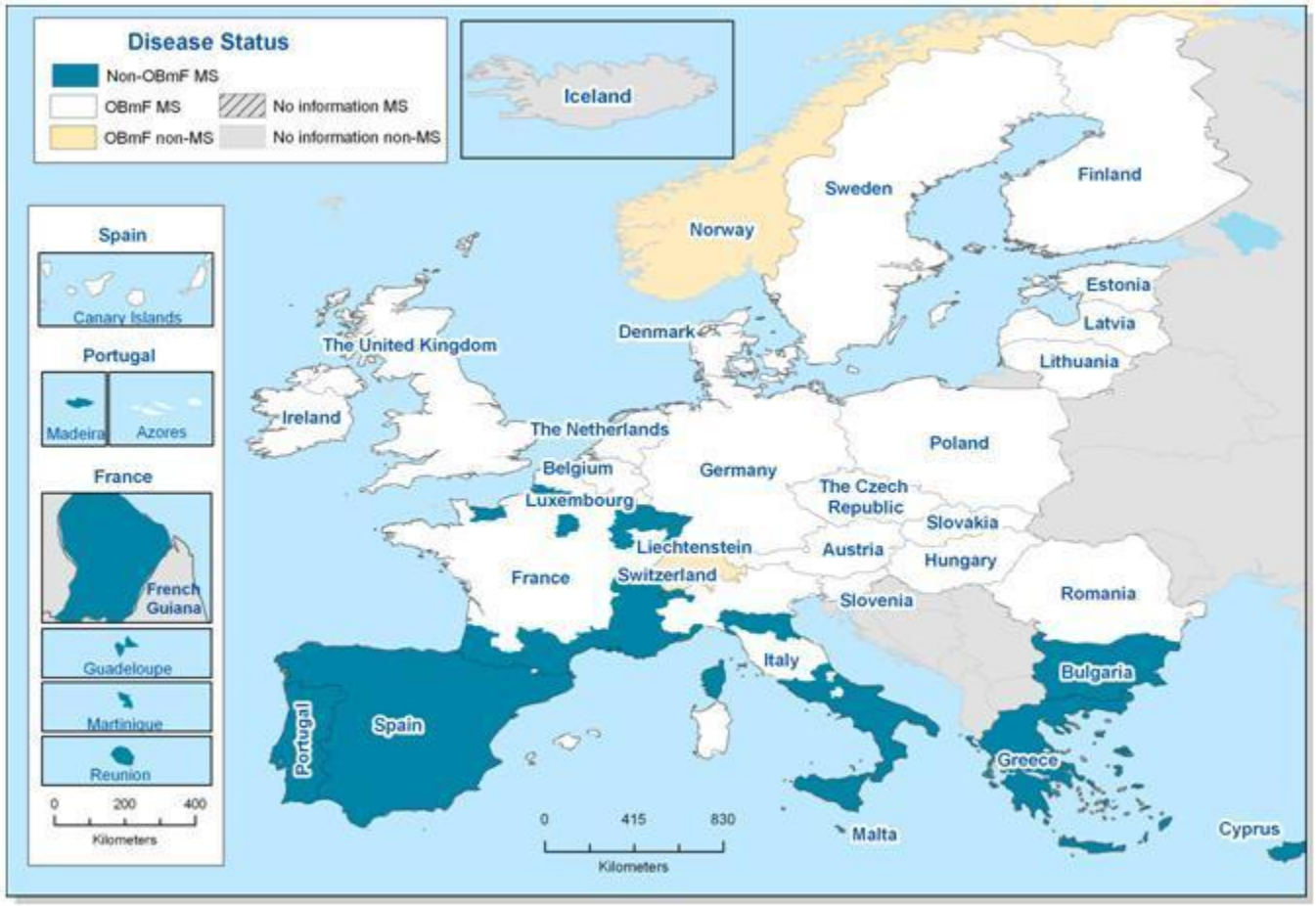
# EMIDA – ERANET

## Coordination of European research on emerging and major infectious diseases of livestock

- Il progetto EMIDA ERA-NET è finanziato da European Commission's Seventh Framework Programme (FP7), programma Cooperazione, tema "Knowledge based bio-economy – KBBE"
- Le ERA-Net (rete dello spazio europeo della ricerca) sono azioni di coordinamento e supporto, nell'ambito del Programma Quadro di ricerca dell'Unione Europea
- Obiettivo finale delle ERA-Net è favorire la cooperazione e il coordinamento di attività di ricerca gestite a livello nazionale e regionale dagli Stati Membri e Associati, attraverso lo sviluppo di attività congiunte (bandi e programmi di ricerca). **(Partner per l'Italia: Min. Salute e Min. Politiche Agricole Alimentari e Forestali)**
- EMIDA ERA-Net dal Decembre 2011 è stato sostituito dal progetto ERA-Net ANIHWA (http://www.anihwa.eu/) che include attività di ricerca relative sia al benessere animale che alla salute umana (outputs disponibili sul sito web del Collaborative Working Group for European Animal Health and Welfare Research (CWG) - http://www.scar-cwg-ahw.org/index.php/resources/emida)

# Brucellosi ovi-caprina in Europa, 2010

*Status of ovine and caprine brucellosis, 2010*




EFSA, ECDC: EU Summary Report 2010, EFSA Journal 2012;10(3):2597

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# *B. melitensis* nel Mediterraneo 2006 - 2011


http://www.oie.int/wahis_2/public/wahid.php/countrymapinteractive

# Partner coinvolti nel progetto Brucmel

## Italia

- Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'- Teramo (IZSAM) **(Project coordinator)**
- Istituto Zooprofilattico Sperimentale della Sicilia 'A. Mirri'- Palermo (IZS Sicilia)

## Grecia

- National Agricultural Research Foundation, Veterinary Research Institute of Thessaloniki (NAGREF)
- Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine of the University of Crete

## Israele

- Kimron Veterinary Institute, Bet Dagan (Kimron)

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# Obiettivi

**Migliorare le strategie di controllo della malattia**

**Migliorare il programma di profilassi nelle aree del Mediterraneo ed oltre**

**Potenziare gli strumenti epidemiologici necessari per le indagini di trace-back dei focolai**

**Sviluppare nuovi strumenti diagnostici in grado di discriminare animali infetti, animali vaccinati con Rev.1 e animali portatori di patogeni cross-reattivi**

**Screening di massa su latte di pecora mediante Milk test**

**Caratterizzare dal punto di vista molecolare il ceppo vaccinale Rev. 1 e i ceppi di campo di *B. melitensis***

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# Project strategy (Materiali e Metodi)

- 3 linee di ricerca: A, B, C
- 4 work packages: WP1, 2, 3 e 4

## A Identification of new protein antigens

**WP1**: Analysis of bacterial surface immunogenic proteins.

**WP2**: Immunogenic protein identification, synthesis of peptides and production of Monoclonal antibodies.

## B Molecular characterization

**WP3**: Analysis for different lineages of *B. melitensis* present in Israel, Greece and Italy

**WP4**: Genetic analysis of field and Rev.1 vaccine strains of *B. melitensis*

## C Validation of a *Brucella* 2.0 kit in sheep milk

Collection of positive milk samples

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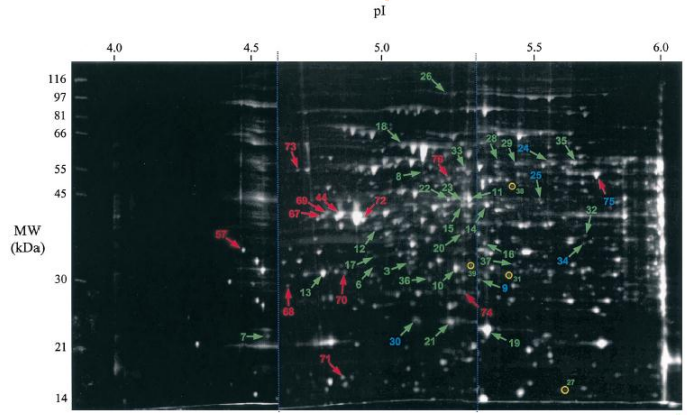
# Identificazione di nuovi antigeni proteici

## A Identification of new protein antigens

**WP1**: Analysis of bacterial surface immunogenic proteins.

**WP2**: Immunogenic protein identification, synthesis of peptides and production of Monoclonal antibodies.

## Proteomic analysis




## Bioinformatics analysis

**Target proteins identified:**

**D0B7Y2-Sugar ABC transporter; Q8YI0-periplasmic dipeptide transport protein:** not found in Rev. 1 strain. Possible use to discriminate Rev.1 vaccinated from infected or cross reactive animals.

**Q8YCE2-Sugar binding periplasmic protein BME11059O; Omp2a; DNA-dependent RNA polymerase beta chain–rpoB; Copper/Zinc superoxide dismutase IL gene–sodC; 50S ribomosal proteins L7/L12-rplL gene:** to distinguish infected from FPSRs.

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# Identificazione di nuovi antigeni proteici

## studi di proteomica

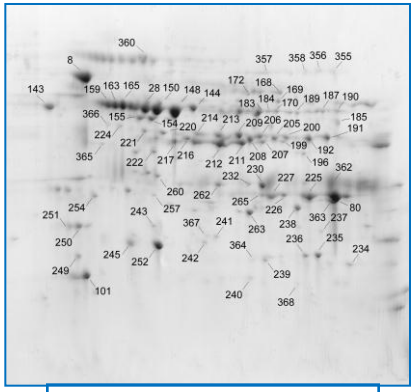
### 2-D elettroforesi + Immunoblotting

Sieri di animali vaccinati con Rev.1

Sieri di animali sani

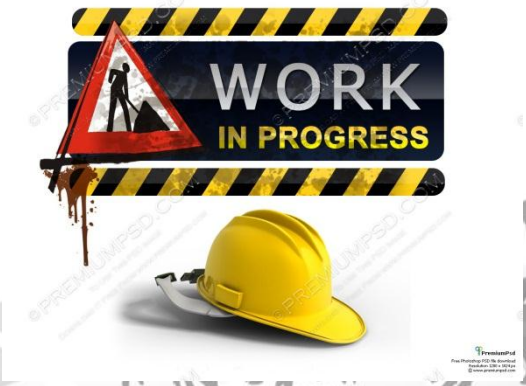
Sieri di animali infetti

Sieri di animali cross-reattivi



*B .melitensis Rev.1 Cross-reattivi*

Antigeni target proteici



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# *Identificazione di nuovi antigeni proteici studi di proteomica*

JOURNAL OF BACTERIOLOGY, Sept. 2002, p. 4962–4970 Vol. 184, No. 18
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.18.4962–4970.2002


## Comparative Proteome Analysis of *Brucella melitensis* Vaccine Strain Rev 1 and a Virulent Strain, 16M

Michel Eschenbrenner,[1] Mary Ann Wagner,[1] Troy A. Horn,[1] Jo Ann Kraycer,[1] Cesar V. Mujer,[1] Sue Hagius,[2] Philip Elzer,[2] and Vito G. DelVecchio[1]*

*Institute of Molecular Biology and Medicine, The University of Scranton, Scranton, Pennsylvania 18510,[1] and Department of Veterinary Science, Louisiana State University AgCenter, Baton Rouge, Louisiana 70803[2]*

Received 14 February 2002/Accepted 13 June 2002

**The genus *Brucella* consists of bacterial pathogens that cause brucellosis, a major zoonotic disease characterized by undulant fever and neurological disorders in humans. Among the different *Brucella* species, *Brucella melitensis* is considered the most virulent. Despite successful use in animals, the vaccine strains remain infectious for humans. To understand the mechanism of virulence in *B. melitensis*, the proteome of vaccine strain Rev 1 was analyzed by two-dimensional gel electrophoresis and compared to that of virulent strain 16M. The two strains were grown under identical laboratory conditions. Computer-assisted analysis of the two *B. melitensis* proteomes revealed proteins expressed in either 16M or Rev 1, as well as up- or down-regulation of proteins specific for each of these strains. These proteins were identified by peptide mass fingerprinting. It was found that certain metabolic pathways may be deregulated in Rev 1. Expression of an immunogenic 31-kDa outer membrane protein, proteins utilized for iron acquisition, and those that play a role in sugar binding, lipid degradation, and amino acid binding was altered in Rev 1.**

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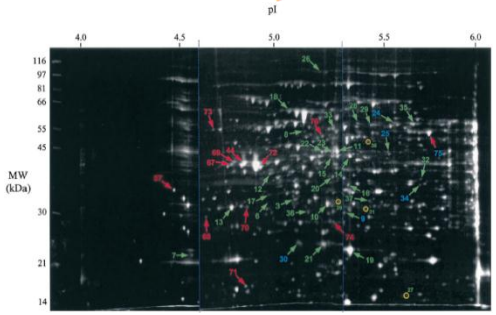
# *Identificazione di nuovi antigeni proteici*

## A Identification of new protein antigens

**WP1**: Analysis of bacterial surface immunogenic proteins.

**WP2**: Immunogenic protein identification, synthesis of peptides and production of Monoclonal antibodies.

→ **Proteomic analysis**




## Bioinformatics analysis

**Target proteins identified:**

**D0B7Y2-Sugar ABC transporter; Q8YI0-periplasmic dipeptide transport protein:** not found in Rev. 1 strain. Possible use to discriminate Rev.1 vaccinated from infected or cross reactive animals.

**Q8YCE2-Sugar binding periplasmic protein BME110590; Omp2a; DNA-dependent RNA polymerase beta chain–rpoB; Copper/Zinc superoxide dismutase IL gene–sodC; 50S ribomosal proteins L7/L12-rplL gene:** to distinguish infected from FPSRs.

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# *Identificazione di nuovi antigeni proteici*

## *Analisi bioinformatiche*

HCDCP
HELLENIC CENTER FOR DISEASE CONTROL & PREVENTION
MINISTRY OF HEALTH

NATIONAL SCHOOL OF PUBLIC HEALTH

PUBLIC HEALTH LABORATORIES NETWORK
REGIONAL PUBLIC HEALTH LABORATORY OF CRETE


University of Crete
School of Medicine

Laboratory of Clinical Bacteriology, Parasitology, Zoonoses, and Geographical Medicine

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# *Identificazione di nuovi antigeni proteici analisi bioinformatiche*

## Brucella Bioinformatics Portal

| Strains | Total Proteins |
|---|---|
| ***Brucella melitensis*** | **256** |
| ***Brucella melitensis*** **(strain M28)** | **3.351** |
| ***Brucella melitensis*** **(strain M5-90)** | **3.347** |
| ***Brucella melitensis*** **biotype 1 (strain 16M / ATCC 23456 / NCTC 10094)** | **4.966** |
| ***Brucella melitensis*** **biotype 2 (strain ATCC 23457)** | **3.125** |
| ***Brucella melitensis*** **bv. 1 str. Rev.1** | **3.220** |
| ***Brucella melitensis*** **bv. 2 str. 63/9** | **3.207** |
| ***Brucella melitensis*** **bv. 3 str. Ether** | **3.237** |
| ***Brucella melitensis*** **NI** | **3.221** |
| | **27.930** |

# Analisi bioinformatiche: *Criteri di selezione*

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## ➢ *localizzazione delle proteine*

| **Brucella melitensis** | |
|---|---|
| Cytoplasmic | 81 |
| Cytoplasmic Membrane | 102 |
| Extracellular | 1 |
| Outer Membrane | 43 |
| Periplasmic | 6 |
| Unknown | 23 |

| **Brucella melitensis (strain M28)** | |
|---|---|
| Cytoplasmic | 1428 |
| Cytoplasmic Membrane | 748 |
| Extracellular | 21 |
| Outer Membrane | 34 |
| Periplasmic | 105 |
| Unknown | 1015 |

| **Brucella melitensis (strain M5-90)** | |
|---|---|
| Cytoplasmic | 1423 |
| Cytoplasmic Membrane | 755 |
| Extracellular | 21 |
| Outer Membrane | 34 |
| Periplasmic | 105 |
| Unknown | 1009 |

| **Brucella melitensis biotype 1 (strain 16M / ATCC 23456 / NCTC 10094)** | |
|---|---|
| Cytoplasmic | 2248 |
| Cytoplasmic Membrane | 1130 |
| Extracellular | 32 |
| Outer Membrane | 60 |
| Periplasmic | 166 |
| Unknown | 1330 |

| **Brucella melitensis biotype 2 (strain ATCC 23457)** | |
|---|---|
| Cytoplasmic | 1379 |
| Cytoplasmic Membrane | 680 |
| Extracellular | 16 |
| Outer Membrane | 32 |
| Periplasmic | 93 |
| Unknown | 925 |

| **Brucella melitensis bv. 1 str. Rev.1** | |
|---|---|
| Cytoplasmic | 1427 |
| Cytoplasmic Membrane | 734 |
| Extracellular | 19 |
| Outer Membrane | 34 |
| Periplasmic | 102 |
| Unknown | 904 |

| **Brucella melitensis bv. 2 str. 63/9** | |
|---|---|
| Cytoplasmic | 1424 |
| Cytoplasmic Membrane | 738 |
| Extracellular | 19 |
| Outer Membrane | 34 |
| Periplasmic | 103 |
| Unknown | 889 |

| **Brucella melitensis bv. 3 str. Ether** | |
|---|---|
| Cytoplasmic | 1431 |
| Cytoplasmic Membrane | 738 |
| Extracellular | 21 |
| Outer Membrane | 32 |
| Periplasmic | 107 |
| Unknown | 908 |

| **Brucella melitensis NI** | |
|---|---|
| Cytoplasmic | 1442 |
| Cytoplasmic Membrane | 748 |
| Extracellular | 20 |
| Outer Membrane | 35 |
| Periplasmic | 102 |
| Unknown | 874 |

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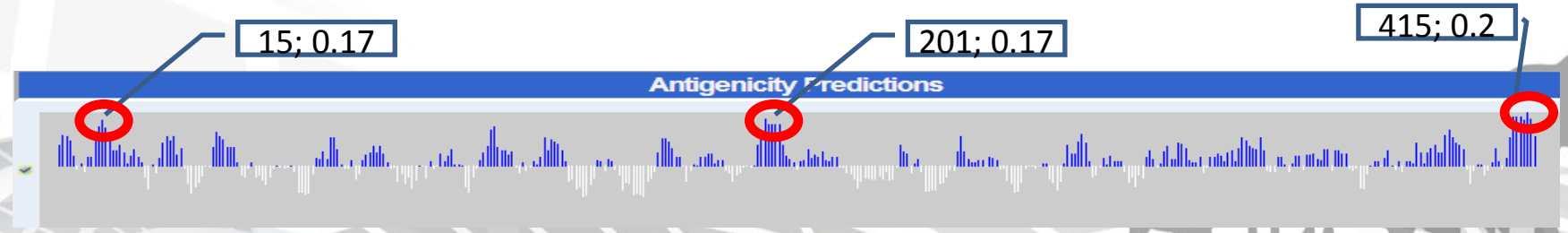
# Analisi bioinformatiche: *Criteri di selezione*

- ***Omologia*** **con altri batteri ritenuti responsabili di** ***cross-reattività*** **(falsi positivi) nei test sierologi per la diagnosi di Brucellosi (dati riportati in letteratura)**

| | |
|---|---|
| Other Brucellae | *Pseudomonas* spp |
| *Yersinia enterocolitica* O:9 | *Francisella tularensis* |
| *Escherichia coli* O:157 | *Pasteurella* spp |
| *Vibrio cholerae* 01 | *Moraxella phenylpyruvica* |
| *Salmonella* group N (0:30) | *Ochrobactrum anthropii* |

- ***Antigenicità presunta***



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# Analisi bioinformatiche: *Criteri di selezione*

➢ ***Studio 1:***

**508 proteine (170 Extracellular + 338 Outer Membrane)**

| **Brucella melitensis** | |
|---|---|
| Cytoplasmic | 81 |
| Cytoplasmic Membrane | 102 |
| Extracellular | 1 |
| Outer Membrane | 43 |
| Periplasmic | 6 |
| Unknown | 23 |

| **Brucella melitensis (strain M28)** | |
|---|---|
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| Cytoplasmic Membrane | 748 |
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| Outer Membrane | 34 |
| Periplasmic | 105 |
| Unknown | 1015 |

| **Brucella melitensis (strain M5-90)** | |
|---|---|
| Cytoplasmic | 1423 |
| Cytoplasmic Membrane | 755 |
| Extracellular | 21 |
| Outer Membrane | 34 |
| Periplasmic | 105 |
| Unknown | 1009 |

| **Brucella melitensis biotype 1 (strain 16M / ATCC 23456 / NCTC 10094)** | |
|---|---|
| Cytoplasmic | 2248 |
| Cytoplasmic Membrane | 1130 |
| Extracellular | 32 |
| Outer Membrane | 60 |
| Periplasmic | 166 |
| Unknown | 1330 |

| **Brucella melitensis biotype 2 (strain ATCC 23457)** | |
|---|---|
| Cytoplasmic | 1379 |
| CytoplasmicMembrane | 680 |
| Extracellular | 16 |
| OuterMembrane | 32 |
| Periplasmic | 93 |
| Unknown | 925 |

| **Brucella melitensis bv. 1 str. Rev.1** | |
|---|---|
| Cytoplasmic | 1427 |
| Cytoplasmic Membrane | 734 |
| Extracellular | 19 |
| Outer Membrane | 34 |
| Periplasmic | 102 |
| Unknown | 904 |

| **Brucella melitensis bv. 2 str. 63/9** | |
|---|---|
| Cytoplasmic | 1424 |
| Cytoplasmic Membrane | 738 |
| Extracellular | 19 |
| Outer Membrane | 34 |
| Periplasmic | 103 |
| Unknown | 889 |

| **Brucella melitensis bv. 3 str. Ether** | |
|---|---|
| Cytoplasmic | 1431 |
| Cytoplasmic Membrane | 738 |
| Extracellular | 21 |
| Outer Membrane | 32 |
| Periplasmic | 107 |
| Unknown | 908 |

| **Brucella melitensis NI** | |
|---|---|
| Cytoplasmic | 1442 |
| Cytoplasmic Membrane | 748 |
| Extracellular | 20 |
| Outer Membrane | 35 |
| Periplasmic | 102 |
| Unknown | 874 |

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# Analisi bioinformatiche: *Criteri di selezione*

➢ ***Studio 2:***

## ulteriori 889 proteine (Periplasmic)

| **Brucella melitensis** | |
|---|---|
| Cytoplasmic | 81 |
| Cytoplasmic Membrane | 102 |
| Extracellular | 1 |
| Outer Membrane | 43 |
| Periplasmic | 6 |
| Unknown | 23 |

| **Brucella melitensis (strain M28)** | |
|---|---|
| Cytoplasmic | 1428 |
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| Cytoplasmic Membrane | 755 |
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|---|---|
| Cytoplasmic | 2248 |
| Cytoplasmic Membrane | 1130 |
| Extracellular | 32 |
| Outer Membrane | 60 |
| Periplasmic | 166 |
| Unknown | 1330 |

| **Brucella melitensis biotype 2 (strain ATCC 23457)** | |
|---|---|
| Cytoplasmic | 1379 |
| CytoplasmicMembrane | 680 |
| Extracellular | 16 |
| OuterMembrane | 32 |
| Periplasmic | 93 |
| Unknown | 925 |

| **Brucella melitensis bv. 1 str. Rev.1** | |
|---|---|
| Cytoplasmic | 1427 |
| Cytoplasmic Membrane | 734 |
| Extracellular | 19 |
| Outer Membrane | 34 |
| Periplasmic | 102 |
| Unknown | 904 |

| **Brucella melitensis bv. 2 str. 63/9** | |
|---|---|
| Cytoplasmic | 1424 |
| Cytoplasmic Membrane | 738 |
| Extracellular | 19 |
| Outer Membrane | 34 |
| Periplasmic | 103 |
| Unknown | 889 |

| **Brucella melitensis bv. 3 str. Ether** | |
|---|---|
| Cytoplasmic | 1431 |
| Cytoplasmic Membrane | 738 |
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| Periplasmic | 107 |
| Unknown | 908 |

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| Cytoplasmic | 1442 |
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| Extracellular | 20 |
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| Unknown | 874 |

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# *Identificazione di nuovi antigeni proteici analisi bioinformatiche - risultati*

## ➢ 7 proteine target identificate:

- D0B7Y2 - Sugar ABC transporter
- Q8YIL0 - Periplasmic dipeptide transport protein

→ **Animali vaccinati con Rev.1 vs animali infetti o cross-reattivi**

- Q8YCE2 - Probable sugar-binding periplasmic protein (P39)
- Omp2a
- DNA-dependent RNA polymerase beta chain – rpoB
- Copper/Zinc superoxide dismutase IL gene – sodC
- 50S ribomosal proteins L7/L12 - rplL gene

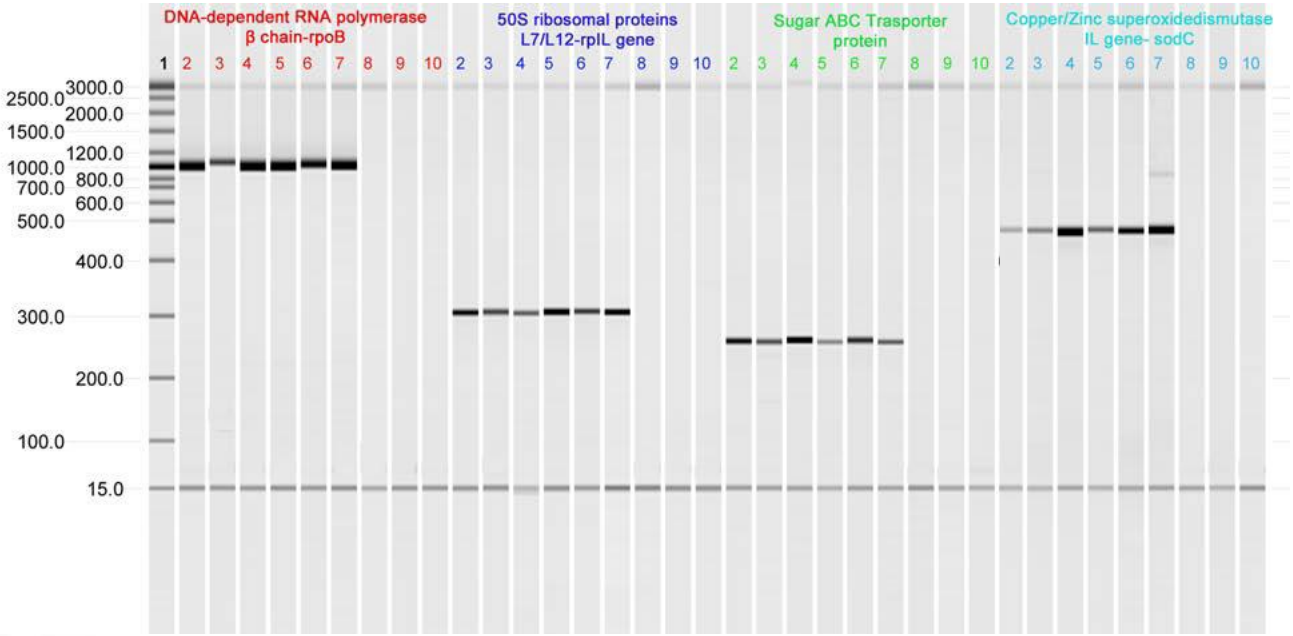
↓

**Animali infetti (non vaccinati con Rev.1) vs animali cross-reattivi**

# *Identificazione di nuovi antigeni proteici*

## Analisi di verifica delle proteine target identificate con metodi bioinformatici

### a. PCR




1. Marker dei PM; 2. *B. melitensis* 3; 3. *B. abortus* 1: 4. B. *suis* 1330; 5. B. *ovis*; 6. B. *ceti*; 7. B. *melitensis Rev-1*; 8. *E. coli* O:157; 9. *Salmonella* O:30; 10. *Y. Enterocolitica* O:9.

### b. Sequenziamento

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# Identificazione di nuovi antigeni proteici

## Antigeni ricombinanti

**A** Identification of new protein antigens

**WP1:** Analysis of bacterial surface immunogenic proteins.

**WP2:** Immunogenic protein identification, synthesis of peptides and production of Monoclonal antibodies.

→ Proteomic analysis (in progress)

↓

**Bioinformatics analysis**

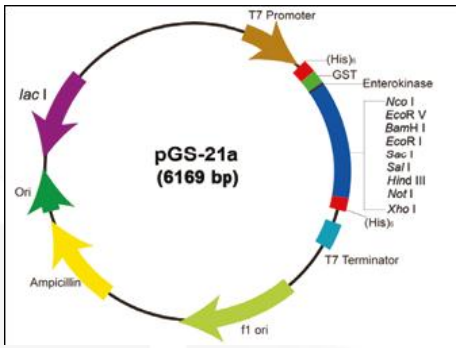
**Target proteins identified:**

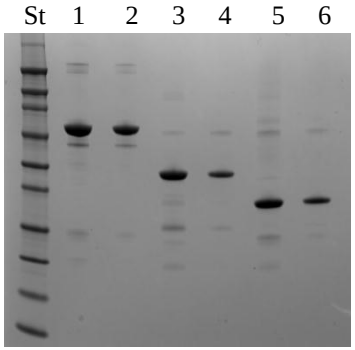
**D0B7Y2-Sugar ABC transporter; Q8YI0-periplasmic dipeptide transport protein:** not found in Rev. 1 strain. Possible use to discriminate Rev.1 vaccinated from infected or cross reactive animals.

**Q8YCE2-Sugar binding periplasmic protein BMEII0590; Omp2a; DNA-dependent RNA polymerase beta chain–rpoB; Copper/Zinc superoxide dismutase IL gene–sodC; 50S ribomosal proteins L7/L12-rplL gene:** to distinguish infected from FPSRs.

↓

**Recombinant Antigens**




St: Novex Sharp pre-Stained Protein Standards

1: Probable sugar binding periplasmic protein (3.5 µl)

2: Probable sugar binding periplasmic protein (2 µl)

3: Periplasmic dipeptide transport protein (5 µl)

4:Periplasmic dipeptide transport protein (2 µl)

5: Sugar ABC transporter (6 µl)

6: Sugar ABC transporter (2 µl)

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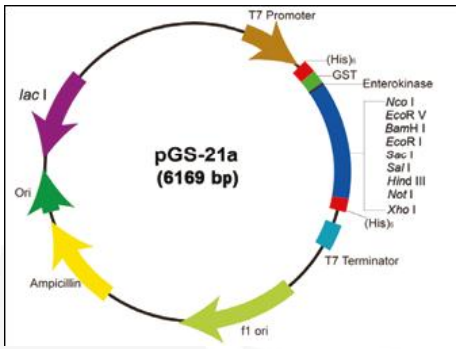
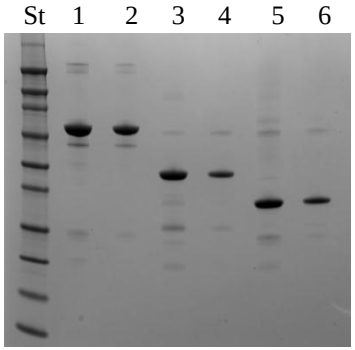
# *Identificazione di nuovi antigeni proteici*
# *Antigeni ricombinanti*

**Produzione di antigeni ricombinanti**:

- D0B7Y2 - Sugar ABC transporter
- Q8YIL0 - Periplasmic dipeptide transport protein
- Q8YCE2 - Probable sugar-binding periplasmic protein (P39)




***E. coli***




St: Novex Sharp pre-Stained Protein Standards

1: Probable sugar binding periplasmic protein (3.5 µl)

2: Probable sugar binding periplasmic protein (2 µl)

3: Periplasmic dipeptide transport protein (5 µl)

4:Periplasmic dipeptide transport protein (2 µl)

5: Sugar ABC transporter (6 µl)

6: Sugar ABC transporter (2 µl)

# Q8YCE2 - Probable sugar-binding periplasmic protein (P39)

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>sp|O06875|SP39_BRUAB **Probable sugar-binding periplasmic protein** OS=***Brucella abortus*** biovar 1 (strain 9-941) GN=BruAb2_0537 PE=1 SV=3

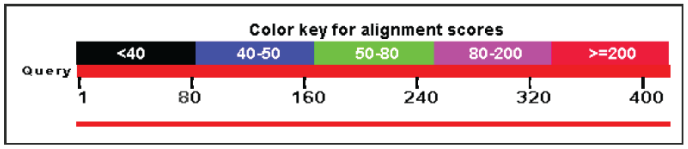
>sp|Q8YCE2|SP39_BRUME **Probable sugar-binding periplasmic protein** OS=***Brucella melitensis*** biotype 1 (strain 16M / ATCC 23456 / NCTC 10094) GN=BMEII0590 PE=3 SV=2

NCBI Blast:Protein Sequence (421 letters)

**BLAST ®**

**Basic Local Alignment Search Tool**

**Distribution of 1 Blast Hits on the Query Sequence**




| Identities | Positives | Gaps |
|---|---|---|
| 420/421(99%) | 420/421(99%) | 0/421(0%) |

| Score | Expect | Method | Identities | Positives | Gaps | Frame |
|---|---|---|---|---|---|---|
| 858 bits(2216) | 0.0() | Compositional matrix adjust. | 420/421(99%) | 420/421(99%) | 0/421(0%) | |

Features:

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Query  421  K  421
           K
Sbjct  421  K  421
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# Q8YCE2 - Probable sugar-binding periplasmic protein (P39)

INFECTION AND IMMUNITY, Feb. 1997, p. 495–502
0019-9567/97/$04.00+0

Vol. 65, No. 2

## Characterization, Occurrence, and Molecular Cloning of a 39-Kilodalton *Brucella abortus* Cytoplasmic Protein Immunodominant in Cattle

PHILIPPE A. DENOEL,[1]* THI KIM-OANH VO,[1] ANNE TIBOR,[1] VINCENT E. WEYNANTS,[1] JEAN-MARC TRUNDE,[2] GERARD DUBRAY,[2] JOSEPH N. LIMET,[1] AND JEAN-JACQUES LETESSON[1]

*Laboratoire de Microbiologie et d'Immunologie, U. R. Biologie Moléculaire, Facultés Universitaires Notre-Dame de la Paix, B-5000 Namur, Belgium,[1] and Laboratoire de Pathologie Infectieuse et Immunologie, Institut National de la Recherche Agronomique, 37380 Nouzilly, France[2]*

Received 6 May 1996/Returned for modification 10 July 1996/Accepted 22 November 1996

**Monoclonal antibodies and polyclonal antisera recognizing a 39-kDa protein (P39) of brucellin, a cytoplasmic extract from *Brucella melitensis* rough strain B115, were produced. The P39 was purified by anion-exchange chromatography. Eleven of fourteen *Brucella*-infected cows whose infections had been detected by the delayed-type hypersensitivity (DTH) test with brucellergen also developed a DTH reaction when purified P39 was used as the trigger. The T-cell proliferative responses to P39 of peripheral blood lymphocytes from *Brucella*-infected cows were also positive. None of the animals infected with other bacterial species that are presumed to induce immunological cross-reactions with *Brucella* spp. reacted to P39, either in DTH tests or in lymphocyte proliferation assays. A λgt11 genomic library of *Brucella abortus* was screened with a monoclonal antibody specific for P39, and the gene coding for this protein was subsequently isolated. The nucleotide sequence of the P39 gene was determined, and the deduced amino acid sequence is in accordance with the sequence of an internal peptide isolated from P39.**

IZSAM G.CAPORALE TERAMO

Brucellosi
Centro di Referen

INFECTION AND IMMUNITY, Dec. 1994, p. 5361–5366
0019-9567/94/$04.00+0

Vol. 62, No. 12

# Brucella Ribosomal Protein L7/L12 Is a Major Component in the Antigenicity of Brucellin INRA for Delayed-Type Hypersensitivity in Brucella-Sensitized Guinea Pigs

G. BACHRACH,[1] M. BANAI,[2]* S. BARDENSTEIN,[2] G. HOIDA,[3] A. GENIZI,[4] AND H. BERCOVIER[1]

*Department of Clinical Microbiology, The Hebrew University-Hadassah Medical School, Jerusalem,[1] and Department of Bacteriology, The Kimron Veterinary Institute,[2] The Israeli Veterinary Services,[3] and Department of Statistics, Agricultural Research Organization,[4] Beit Dagan 50250, Israel*

Received 13 May 1994/Returned for modification 16 July 1994/Accepted 2 September 1994

## DISCUSSION

Ribosomal vaccines against different diseases have been proposed, but their rationale has been controversial (16, 20, 25, 31–34). It has been shown that brucella (11) and mycobacterium (3, 4, 23) ribosomes can elicit a DTH response when injected into sensitized animals. However, what exactly in the ribosomes induced the DTH was not definitively identified. The results reported in our present work clearly identify brucella ribosomal protein L7/L12 as an antigen capable of eliciting DTH in brucella-sensitized guinea pigs. We showed that this protein is present in Brucellergen (Fig. 2). The questions about the nature of the antigenic motif that is recognized by the immune response of the infected host.

Ribosomal proteins L7/L12 are encoded by the same gene and differ only by acetylation of the $NH_3$ terminus of the L12 protein, which then becomes the L7 protein (21, 30). Which one of these two proteins, if not both, is responsible for the DTH reaction remains to be determined.

The reason(s) why the recombinant L7/L12 protein was not antigenic in the DTH reaction is not yet understood. The lack of brucella-specific posttranslational modification in *E. coli* could be one explanation. It seems more reasonable to suggest that the six histidines added to the protein N terminus in the pQE3012 construct might have interfered with protein antigenicity. We are now building new recombinant proteins lacking these histidines and trying specific polypeptides for DTH-inducing activity.

Possibili limiti delle proteine ricombinanti

IZSAM G.CAPORALE TERAMO

Brucellosi
Centro di Referenza Nazionale

# *Identificazione di nuovi antigeni proteici*
# *selezione target immunogenici*

**A** Identification of new protein antigens

**WP1**: Analysis of bacterial surface immunogenic proteins.

**WP2**: Immunogenic protein identification, synthesis of peptides and production of Monoclonal antibodies.

→ Proteomic analysis (in progress)

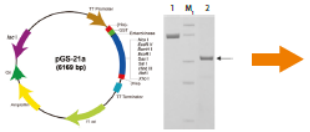
Bioinformatics analysis

**Target proteins identified:**

**D0B7Y2-Sugar ABC transporter; Q8YI0-periplasmic dipeptide transport protein:** not found in Rev. 1 strain. Possible use to discriminate Rev.1 vaccinated from infected or cross reactive animals.

**Q8YCE2-Sugar binding periplasmic protein BMEII0590; Omp2a; DNA-dependent RNA polymerase beta chain-rpoB; Copper/Zinc superoxide dismutase IL gene-sodC; 50S ribomosal proteins L7/L12-rplL gene:** to distinguish infected from FPSRs.

Recombinant Antigens




**SDS-PAGE analysis**
Lane 1: BSA (2.00 µg)
Lane 2: Sugar ABC transporter (2.00 µg)

**Selection of immunogenic protein targets**

**MAbs**

**Project Serum Bank**
(infected and REV.1 vaccinated animals)

**Cross reactive sera**
(experimentally infected sheep with *Salmonella* group N, *E. coli* O:157, *Y. enterocolitica* O:9)

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Brucellosi
Centro di Referenza Nazionale

# *Identificazione di nuovi antigeni proteici*

## *Banca sieri*

- ***Banca sieri***
  - **Raccolta di sieri (bovini e ovi/caprini) provenienti da animali vaccinati con Rev.1 e animali infettati da *B. melitensis:***
  - **ad oggi oltre 300 sieri**

- ***Produzione sieri cross-reattivi***
  - **Infezione sperimentale su pecore, volta alla produzione di sieri contro *Yersinia enterocolitica* O:9, *E. Coli* O:157 e *Salmonella* group N**

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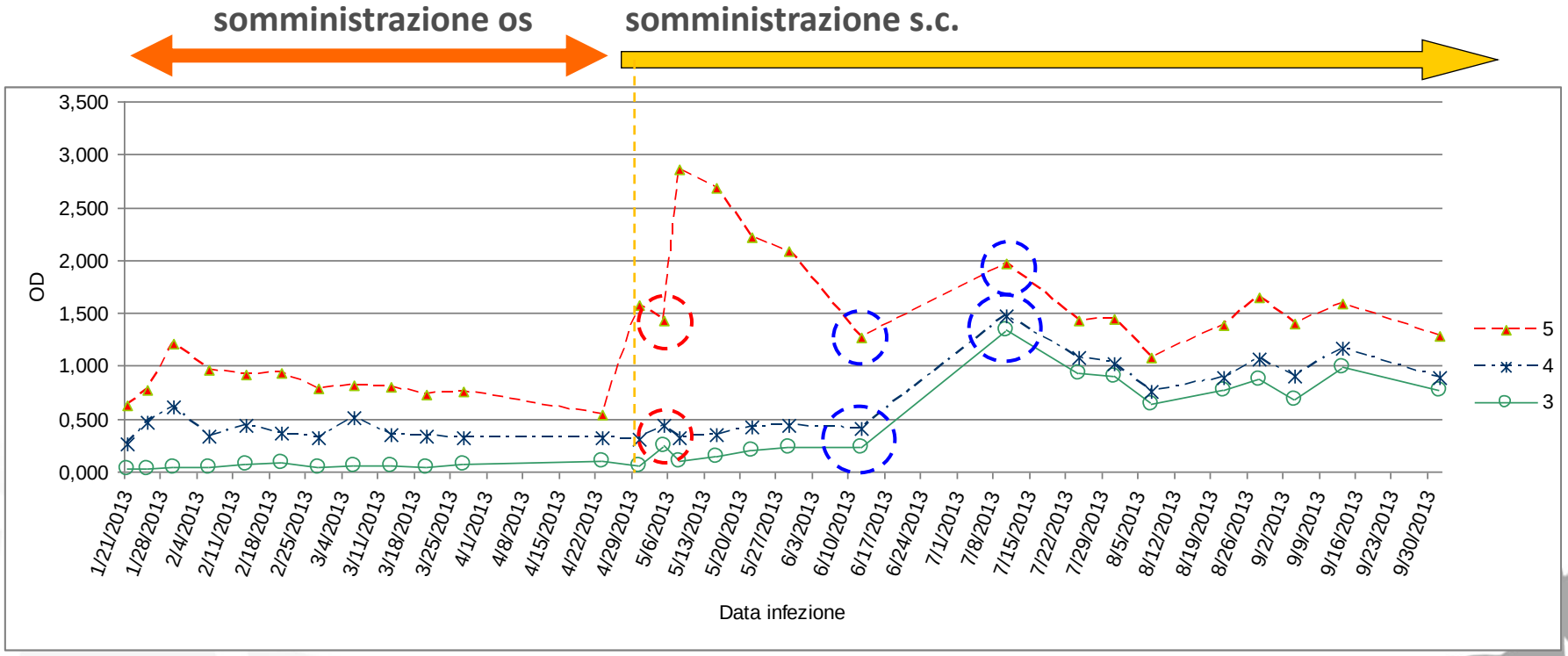
Brucellosi
Centro di Referenza Nazionale

# *Identificazione di nuovi antigeni proteici*
# *Banca sieri – infezione sperimentale*

## Risultati test Elisa - *E. Coli* O:157



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Centro di Referenza Nazionale

# *Identificazione di nuovi antigeni proteici*

## *outputs and Impact*

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**WP1**: Analysis of bacterial surface immunogenic proteins.

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↓

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↓

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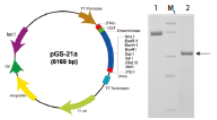


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↓

MAbs

## Outputs

**A** **New diagnostic tests**

to discriminate infected from Rev.1 vaccinated animals as well infected from cross-reactive animals

## Impact

**Improvement of disease control strategies**

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Brucellosi
Centro di Referenza Nazionale

# Caratterizzazione molecolare

**B** **Molecular characterization**

**WP3**: Analysis for different lineages of *B. melitensis* present in Israel, Greece and Italy

**WP4**: Genetic analysis of field and Rev.1 vaccine strains of *B. melitensis*

**Omp2 genetic analyses and MLVA 16**

**Genetic Finger print identification of strain specific molecular patthern**

IZSAM G.CAPORALE TERAMO

Brucellosi
Centro di Referenza Nazionale

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## Outputs

**Molecular tools and database of regional *B. melitensis* strains**

**B**

for epidemiological investigations and trace-back analyses to identify the outbreak origin

**Improvement of disease control strategies**

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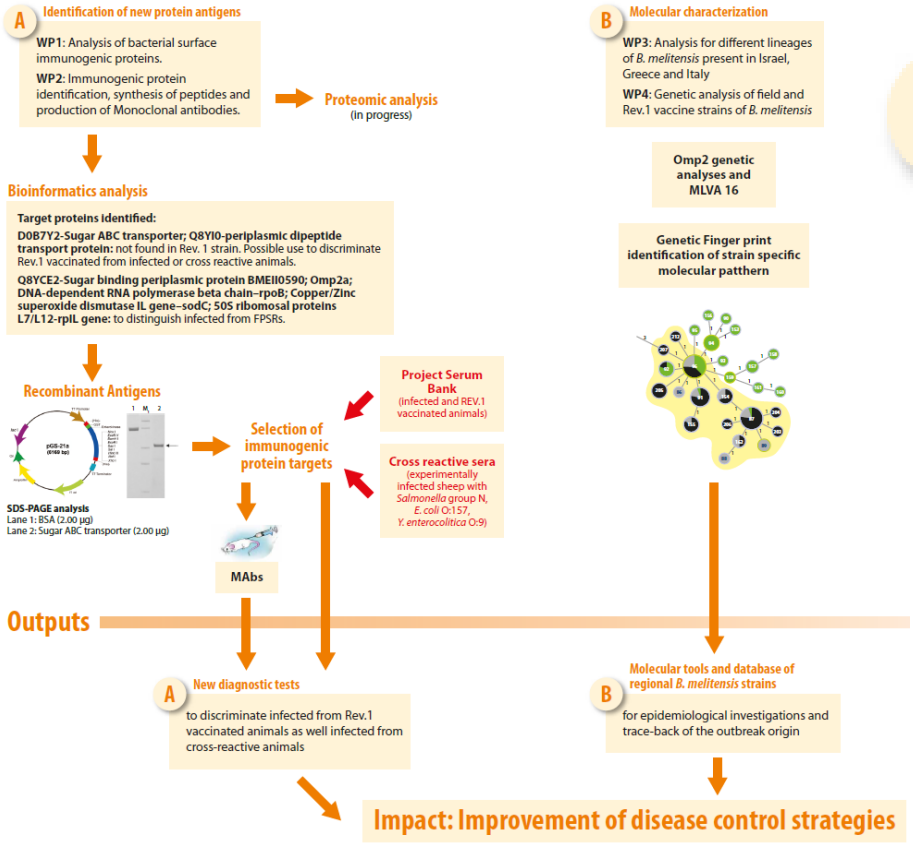
Brucellosi
Centro di Referenza Nazionale

# Project strategy (Materials and Methods)




## Validation of a *Brucella* 2.0 kit in sheep milk

C

Collection of positive milk samples

# Valutazione del Brucella 2.0 test kit su latte di pecora (i ELISA)

Brucellosi
Centro di Referenza Nazionale

**Raccolta di campioni di latte ovino positivi e negativi**

↓

**Ottimizzazione del protocollo di separazione della parte grassa**

↓

**Brucella 2.0 test kit (iELISA)**

↓

**Analisi ed interpretazione dei risultati**

**Diagnosi microbiologica di Brucellosi mediante separazione immunomagnetica nel latte (DynaBeads)**

**I risultati ottenuti da prove su campioni di latte di campo provenienti da allevamenti ovini e bovini mostrano una maggiore sensibilità del metodo Dyna-Bru**

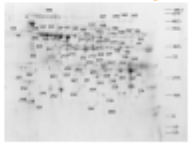
**Batteriologia e Igiene delle produzioni lattiero casearie/Laboratorio Nazionale di Riferimento per Campylobacter – IZSA&M**

## A Identification of new protein antigens

**WP1:** Analysis of bacterial surface immunogenic proteins

**WP2:** Immunogenic protein identification, synthesis of peptides and production of Monoclonal Antibodies (MAbs)

### Proteomic analysis



### Bioinformatics analysis

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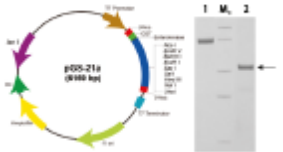
### Recombinant Antigens





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**MAbs**

## B Molecular characterization

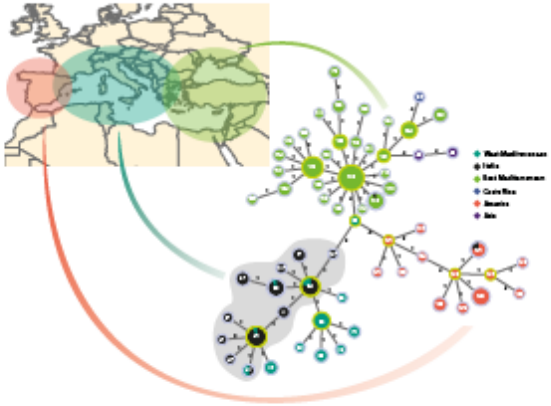
**WP3:** Analysis for different lineages of *B. melitensis* present in Israel, Greece and Italy

**WP4:** Genetic analysis of field and Rev-1 vaccine strains of *B. melitensis*

**RFLP and DGGE Omp2**

**MLVA-16 typing**

***Brucella* DNA fingerprints to asses genetic diversity of vaccine and field strains**



## C Assessment of Brucella 2.0 test kit on sheep milk (i ELISA)

Collection of positive and negative milk samples

Optimisation of protocol for fat separation

Test analyses

Result analyses and interpretation

# Outputs

### A New diagnostic tests

to discriminate infected from Rev-1 vaccinated animals as well infected from cross-reactive animals

### B Molecular tools and database of regional *B. melitensis* strains

for epidemiological investigations and trace-back analyses to identify the outbreak origin

### C Milk test for mass screening of sheep milk

# Impact

## Improvement of disease control strategies

***IZS AM***

Manuela Tittarelli
Domenica Travaglini
Ivanka Krasteva
Cesare Cammà
Katiuscia Zilli
Giuliano Garofalo
Federica Di Giacinto
Mirella Luciani

***IZS Sicilia***

Aldo Vesco
Sara Villari
Valeria Gargano

***Nagref e Università di Creta***

Loukia Ekateriniadou
Vicky Boukouvala
Vassilis Sandalakis

***Kimron Veterinary Institute***

Menachem Banai

***Ministero della Salute***

Marina Bagni

***Ministero delle Politiche Agricole Alimentari e Forestali***

# *Grazie per l'attenzione!*