Republic of Austria, Kingdom of Belgium, Czech Republic, French Republic, Federal Republic of Germany, Italian Republic, Kingdom of the Netherlands, Kingdom of Norway, Republic of Poland, Kingdom of Spain, Kingdom of Sweden and Republic of Finland

- forming

European Biodefence Laboratory Network (EBLN)

PROJECT ARRANGEMENT

No B-0060-EDAE-ESM04-GC
<table>
<thead>
<tr>
<th>Country</th>
<th>Participants - Principal Organisations</th>
</tr>
</thead>
</table>
| Austria      | Mag. Adelheid OBWALLER  
Bundesministerium fur Landesverteidigung, Wien                           |
| Belgium      | Prof. Jean-Luc GALA, Med Lt col  
Département des Laboratoires de la Défense, Brussels                       |
| Czech Republic | Prof. Ales MACELA  
National Institute for Nuclear, Chemical and Biological Safety, Milín |
| Finland      | Dr. Simo NIKKARI  
Centre for Military Medicine (BCYY/SOTLK), Helsinki                         |
| France       | Dr. Gilles VERGNAUD, ICA.  
DGA/DS/MRIS, Bagneux                                                         |
| Germany      | Dr. Wolf SPLETTSTÖESSER  
Bundeswehr Institute of Microbiology, München                               |
| Italy        | Dr. Florigio LISTA  
Army Medical and Veterinary Research Center, Roma                            |
| Netherlands  | Dr. Hugo-Jan JANSEN  
Ministry of Defence/TNO LOOSDRECHT/Delft                                     |
| Norway       | Dr. Jaran OLSEN  
FFI, Kjeller                                                                   |
| Poland       | Dr. Marcin NIEMCEWICZ  
Military Institute of Hygiene and Epidemiology, Pulowy                        |
| Spain        | Dr. Ricela SELLEK  
DGAM/SDGTECEN/ITM, Madrid                                                    |
| Sweden       | Dr. Mats FORSMAN, Chair  
FOI, Umea                                                                       |
Capability gaps*
*as defined by ECAP NBC project group, final report 2005.

no. 3
“Whole spectrum of biothreat detection not covered by individual nations”

no. 7
“No laboratory network in place for reach back and/or forensic purposes within Europe”
OBJECTIVE OF THE PROJECT

- establishment and management of a strategic European Biodefence Laboratory Network (EBLN)

- increase European preparedness for protection against Biological Warfare Agents (BWA)

- improve the European capability to verify the use of B-agents in context of the Biological and Toxin Weapon Convention (BTWC).

- design and construct a shared database with reference typing data – a common resource for unmistakable typing and identification of B-agents
Agents primarily covered within the project

- *Bacillus cer-thu-anthracis* group
- *Francisella* spp.
- *Burkholderia mallei/pseudomallei*
- *Vibrio cholerae* group
- *Brucella* spp.
- *Yersinia* spp.
- *Clostridium botulinum*
- *Coxiella burnetii*
### Example of distribution of coordination tasks for analysis and definition of reference strain panels

<table>
<thead>
<tr>
<th>Agent coordinator</th>
<th>Brucella</th>
<th>Francisella</th>
<th>Burkholderia</th>
<th>Y. pestis</th>
<th>Y. pseudotb + enterocolitica</th>
<th>B. anthracis</th>
<th>B. cereus</th>
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</thead>
<tbody>
<tr>
<td>MLVA</td>
<td>FR</td>
<td>SE</td>
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<td>FI</td>
<td>IT</td>
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<td>SE</td>
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<td>MLST</td>
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<td>GE</td>
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<td>GE</td>
<td>GE/AT</td>
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<td>FI</td>
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<td>MS-wholecell</td>
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<td>MS-extracts</td>
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<td>CZ/NL</td>
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<td>Classical phenotyping</td>
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<td>GE</td>
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<tr>
<td>Whole genome seq</td>
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</tbody>
</table>
Outline of workflow

- Coordination
- Inventory of available strains
- DNA/protein preparation & distribution among contributors
- MLVA-analysis
- MLST-analysis
- Large scale SNP-analysis
- Rapid methods for virulence target identification
- Toxin typing
- Phenotypic analysis (classical and Mass spectroscopy)
- Definition of nearest neighbours
- Definition of a representative strain panels
<table>
<thead>
<tr>
<th>No. of strains genotyped within agent</th>
<th>No. chosen to reflect diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>~ 350 strains of <em>Brucella</em> <em>spp.</em></td>
<td>15</td>
</tr>
<tr>
<td>~ 100 strains of <em>B. anthracis</em></td>
<td>13</td>
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<tr>
<td>~ 400 strains of <em>Francisella</em> <em>spp.</em></td>
<td>14</td>
</tr>
<tr>
<td>~ 350 strains of <em>Yersinia</em> <em>spp.</em></td>
<td>39</td>
</tr>
<tr>
<td>~ 300 strains of <em>B. cereus</em> <em>group</em></td>
<td>28</td>
</tr>
<tr>
<td>~ 50 strains of <em>Burkholderia</em></td>
<td>20</td>
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<tr>
<td>~ 140 strains of <em>Coxiella burnetii</em></td>
<td>20</td>
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</table>
Communications
- through a restricted web forum

<table>
<thead>
<tr>
<th>Forum Topics</th>
<th>User</th>
<th>Posts</th>
<th>Views</th>
<th>Last Post By</th>
<th>Last Post Date</th>
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<td>RCU Candidates Brucella</td>
<td>flonjio.lista</td>
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</tbody>
</table>
Typing methodology
Example of results – *Francisella* spp.

**Tularemia**

**Causative agent:** *Francisella tularensis*
- Gram-negative, facultative intracellular
- 3 subspecies; Only type A and B clinically relevant

**Reservoirs and transmission vectors**
- Reservoir unknown: small rodents / free living protozoa?
- Infects >250 species
- Arthropod vectors: ticks, biting flies, mosquitoes
- Contact with infected animals, water, food, dust, aerosols

**Variable clinical manifestation**
- Enlarged lymph nodes, fever
- Ulceroglandular, oropharyngal, pneumonic, oculoglandular
Whole genome phylogeny of *Francisella tularensis* and *novicida*.

Modified from Larsson et al., PLoS Pathogens, 2009
Choice of typing method
- dependent on population structure of the biological agent

Bacterial population structures

**Clonal structure**
(tree structure)

Mutations (X) are passed on to daughter clones, i.e. they are “canonical"

Ex: *Francisella tularensis*

**Panmitic structure**
(web structure)

Mosaic genomes - high degree of recombination

Mutations (X) and relationships are difficult to trace,

Ex: *Clostridium botulinum*
Depending on purpose of typing - strain panel and need of taxonomic and geographical resolution may differ

Clinical samples, Environmental samples, Endemic areas, Non-endemic areas

**Bioterrorism preparedness**

![Diagram showing taxonomic and geographic resolution with Genus, Species, Subspecies, and Isolates branches, and East, West North America, and Europe regions.]
Whole genome phylogeny of *Francisella* spp.

Modified from Svensson et al., PLoS One 2009
Francisella typing based on 16S rRNA

Data from EBLN database
Francisella typing based on 23S rRNA

Data from EBLN database
Francisella typing based on MLST

Data from EBLN database
Francisella typing based on MLVA

MLVA_1-25_repeats

Francisella tularensis tularensis  FSC237
Francisella tularensis tularensis  FSC041
Francisella tularensis mediasiatica  FSC147
Francisella tularensis mediasiatica  FSC148
Francisella tularensis novicida  FSC595
Francisella tularensis novicida  FSC040
Francisella tularensis tularensis  FSC604
Francisella tularensis tularensis  FSC054
Francisella tularensis holarctica  FSC022
Francisella tularensis holarctica  FSC021
Francisella tularensis holarctica  FSC012
Francisella tularensis holarctica  FSC035
Francisella tularensis holarctica  FSC257
Francisella tularensis holarctica  FSC200

Data from EBLN database
Francisella typing based BIOLOG

Data from EBLN database
Francisella typing using MALDI-TOF

Distance Level
Canonical SNP’s Typing

Modified from Svensson et al., PLoS One 2009
<table>
<thead>
<tr>
<th><strong>Method</strong></th>
<th><strong>Resolution within <em>F. tularensis</em></strong></th>
<th><strong>Type A subpopulations identified</strong>&lt;sup&gt;1&lt;/sup&gt;</th>
<th><strong>Type B subpopulations identified</strong></th>
<th><strong>Individual isolates</strong></th>
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<tbody>
<tr>
<td>Ribosomal gene sequencing</td>
<td>Subspecies</td>
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<tr>
<td>Multi-locus sequence typing</td>
<td>Subspecies, major subpopulations</td>
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<td>Japanese, United States</td>
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</tr>
<tr>
<td>Signature based PCR identification</td>
<td>Subspecies, major subpopulations</td>
<td>A1 A2</td>
<td>Central vs. Western Europe</td>
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<td>Biochemical phenotypic methods</td>
<td>Subspecies, major subpopulations</td>
<td>A1 A2</td>
<td>B1, B2, B3, B4, B5</td>
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<td>Mass-spectroscopy phenotypic methods</td>
<td>Subspecies, major subpopulations</td>
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<td>B1, B2, B3, B4, B5</td>
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<tr>
<td>Multi-locus variable-number tandem repeat analysis</td>
<td>Subspecies, subpopulations, strains</td>
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<td>B1, B2, B3, B4, B5</td>
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<tr>
<td>Single nucleotide polymorphisms</td>
<td>Subspecies, subpopulations, strains</td>
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<td>InDel polymorphism</td>
<td>Subspecies, subpopulations, strains</td>
<td>A.I, A1a, A1b, A2</td>
<td>B1, B2, B3, B4, B5</td>
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<td>Combinatorial approach</td>
<td>Subspecies, subpopulations, strains</td>
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<td>Whole genome sequencing</td>
<td>Subspecies, subpopulations, strains</td>
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<td>B1, B2, B3, B4, B5</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<sup>1</sup> Identification of Type A subpopulations: A1, A2, A3, A4, A5

*Summary: Resolution of different typing methods within *F. tularensis*


Output of EBLN

• Integrated EU biodefence laboratory expertise

• Increased capability in MS reach-back laboratories, support to BioEDEP

• Shared database and reference strain panels for validation of diagnostic tests and identification methods and instruments

• Improved tools for B-DIM and epidemiological investigations

• Microbial forensic capability
What next?

• Exchange version 2 collections within a new project
• Invent a way to be able to organise forensic microbiology ring trials across Europe without the need to exchange biological material for each exercise
• Explore the potential of Next Generation Sequencing technologies
• Help biotech derive products from expertise (such as typing kits)
• This will require a way to work more closely with academic laboratories and small biotech industry : funding ?
Recommendations EDA IBDSA study 2008

Integrated Biological Defence System Architecture

R16: set up of an European genotype database with its associated genotyping kit reagents, regrouping the fingerprint of the representative strains of each of the pathogenic species considered as the military threat, for unambiguous identification.