Project Deliverable D-JRP17-WP7.Del6

Workpackage 7

Responsible Partner: ANSES

Contributing partners: APHA, FLI, IZSAM, INIAV, INSA, IZS, WBVR

GENERAL INFORMATION

European Joint Programme full title	**Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards**
European Joint Programme acronym	**One Health EJP**
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Start Date	**01/01/2018**
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DOCUMENT MANAGEMENT

Project deliverable	D-JRP17-WP7.Del6: Report of the last annual workshop including exchanges between partners and inputs from external stakeholders
Project Acronym	JRP17-ET2.1-IDEMBRU
Author	ANSES (Claire Ponsart, Luca Freddi, Vitomir Djokic)
Other contributors	APHA, FLI, IZSAM, INIAV, INSA, IZS, WBVR
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Dissemination level *PU: Public (default)* *CO: confidential, only for members of the consortium (including the Commission Services)*	PU **This is the default setting.** If this project deliverable should be confidential, please add justification here (may be assessed by PMT):
Dissemination *Author's suggestion to inform the following possible interested parties.*	OHEJP WP 1 ☐ OHEJP WP 2 ☐ OHEJP WP 3 ☐ OHEJP WP 4 ☐ OHEJP WP 5 ☐ OHEJP WP 6 ☐ OHEJP WP 7 ☐ Project Management Team ☐ Communication Team ☐ Scientific Steering Board ☐ National Stakeholders/Program Owners Committee ☐ EFSA ☐ ECDC ☐ EEA ☐ EMA ☐ FAO ☐ WHO ☐ OIE ☐ Other international stakeholder(s): ..... Social Media: ..... **Other recipient(s):** .....

DELIVERABLE REPORT D-JRP17-WP7.DEL6: REPORT OF THE LAST ANNUAL WORKSHOP INCLUDING EXCHANGES BETWEEN PARTNERS AND INPUTS FROM EXTERNAL STAKEHOLDERS

AGENDA –**November 9th and 10th** Hybrid meeting:

November 9th

(CET) **09:30 – 12:30**	**Project overview: Presentation of results for each partner institution**
09:30 – 09:50	ANSES
09:50 – 10:10	APHA
10:10 – 10:30	BfR
10:30 – 10:50	FLI
10:50 – 11:10	INIAV
11:10 – 11:30	INSA
11:30 – 11:50	IZSAM
11:50 – 12:10	NDRVMI
12:10 – 12:30	WbVr
12:30 – 14:00	Lunch break
14:00 – 17:30 15 minutes presentation per each WP leader/deputy	**WP6 - Toolkit.**
	Contribution of each WP to WP6 – Toolkit – presentations of WP leaders and deputies on: - Results - Deliverables that are supposed to be part of the toolkit(s) (1 for atypical Brucellae; 2. *B. canis* specific)
	Round table and merge of proposed flowcharts by each partner
DINNER	**SOCIAL EVENT**

November 10th

9:00 – 12:00	**TOOLKIT** Final flowcharts construction. Agreement on contents of Toolkit to underlay flowchart

	Agreement/allocation of tasks to be finished by the end of the project to deliver toolkit.
12:00 – 13:30	Lunch break
13:30 – 14:00	Padi-Web first results presentation – Vitomir Djokic
14:00 – 15:30	Final remarks: - financial closure of the project, - deadlines for reports - deadlines for publications

Presentations:

RÉPUBLIQUE FRANÇAISE
Liberté
Égalité
Fraternité

anses

World Organisation for Animal Health
Founded as OIE

Reference Laboratory for Brucellosis

ANSES UPDATE OF THE IDEMBRU PROJECT

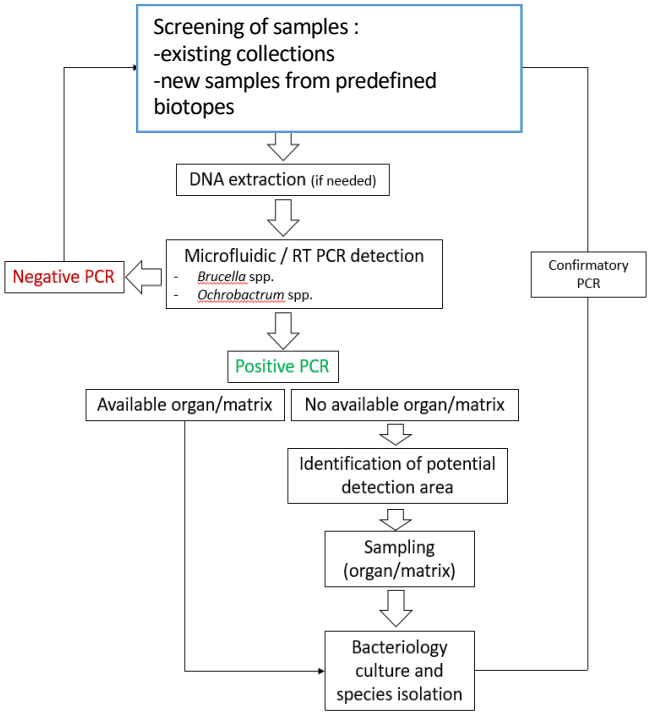
Vitomir Djokic, Claire Ponsart
French Agency for Food, Environmental and Occupational Health Safety

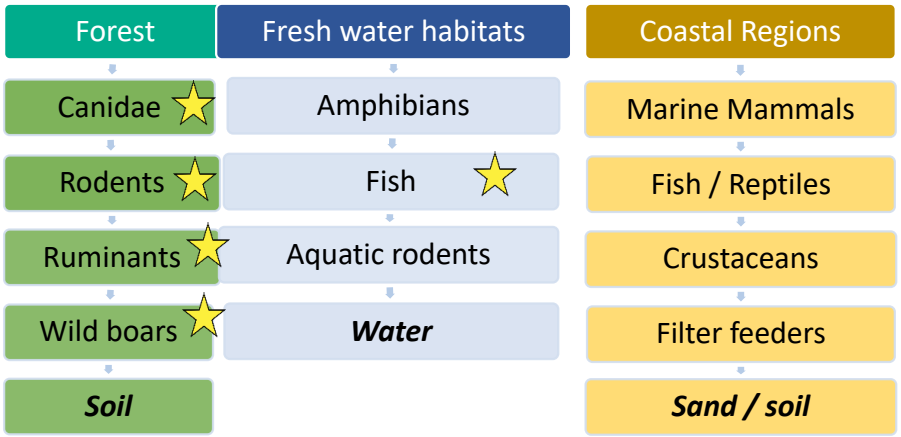
WP-1 - Recording the situation of brucellosis in emergent wild and environmental reservoirs, as well as canine brucellosis

Pipeline strategy: to develop diagnostics criteria from domestic and wild animals as well as complex environmental matrices

Complex environmental matrices:

1. Soil
2. Faeces
3. Boot swabs
4. Water
5. Sand





In total 986 samples obtained and counting…
Out of which:
364 from Bulgarian wildlife
300 from French forests
176 from French coastal regions
146 from French fresh water aquatic rodents

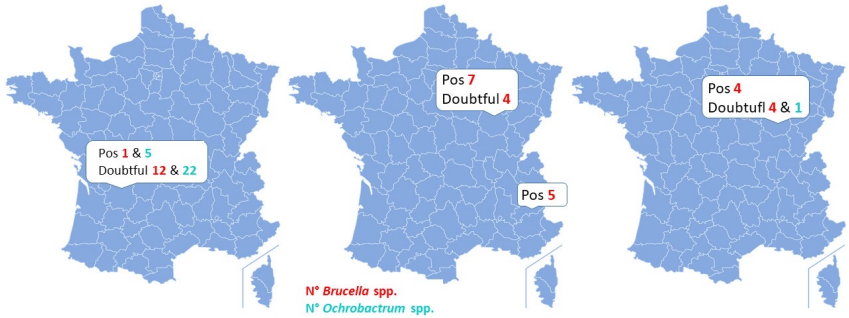
+ samples of 830 dogs

WP-1 - Recording the situation of brucellosis in emergent wild and environmental reservoirs, as well as canine brucellosis

Tested on animal samples from forest biotops, new multiplex PCR identified several hotspots and species of interest

Both *Brucella* and *Ochrobactrum* are circulating in forest ecosystems

Species (n)	*Brucella* IS711 detected			*Ochrobactrum* Och3* detected		
	Pos (%)	Doubtful (%)	Neg (%)	Pos (%)	Doubtful (%)	Neg (%)
Fox (481)	0,2	2,5	97,3	**1,0**	**4,6**	94,4
Wild boar (60)	21,7	6,7	71,7	0,0	0,0	100,0
Deer (36)	**11,1**	**11,1**	77,8	0,0	**2,8**	97,2



All qPCR positive and doubtful results are inoculated onto non specific media and further characterization was performed

WP-1 - Recording the situation of brucellosis in emergent wild and environmental reservoirs, as well as canine brucellosis

Marine mammals:

- Dolphins, dauphins, seals and porpoises
- Geolocalisation : Atlantic and Mediterenian coast
- Sample types: Spleens, livers, lymph nodes.
- Total number of samples: 76

N°BM	Host	Sample type	Ct on IS711 April 2021	IS711 (Ct) June 2021
I024	Stenella coeruleoalba	Liver	32,02	33,18
I026	Halichoerus grypus	Spleen	32,31	32,70
I031	Phoca vitulina	Liver	34,59	32,35
I032	Grampus griseus	Spleen	12,42	16,54
I033	Phocoena phocoena	Liver	33,06	31,28
I069	Stenella coeruleoalba	Lnn mesenterial	15,58	32,71

= 6 positives out of 76

- After first positive PCR results culturing in non specific and Brucella specific media tried without success.

- Second time tried enriched cultures also without success.

Bacteria are dead?

Not enough to isolate?

Amplified the similar sequence of some other bacterial species?

One *B. microti* strain isolated from fresh water wild frog in the south of France. Further characterisation needed = by the end of the year.

One Ochrobactrum spp. strain isolated from horse lungs. Further characterisation needed = by the end of the year.

WP-1 - Canine brucellosis

Samples collected:

- 830 tissue and sera samples from adult dogs
- Foetuses, stillbirths, puppies died soon after birth
- Geolocalisation: Whole France, mainly kennels and individual private dogs.

First results:

- Available serology tests compared
- Isolation tried from 358 samples
- One case of *B. suis* type II isolated and characterised.

COHESIVE AND IDEMBRU prepared

White Paper on *Brucella canis* in Europe

WP-3 - Genomic characterisation of Brucella detected from samples and selected isolates

Compared variables:

Brucella species:

- *B. melitensis* 16M
- *B. abortus* 544
- *B. suis* S2 (Thomsen)

Different storage conditions :

- Day1
- Day4 + 4°C
- Day 4 – 20°C

Type of swab:

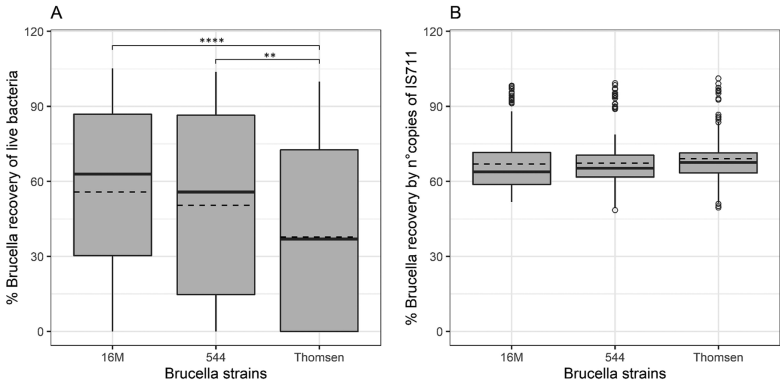
- Dry
- Wet

Tip materials:

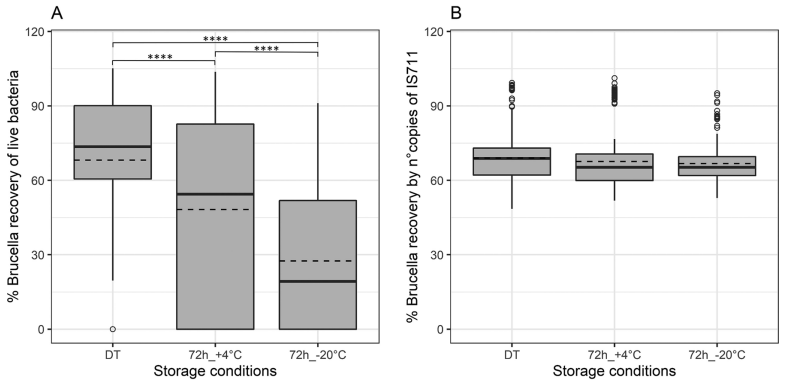
- Viscose
- Cotton-wool
- Carded cotton
- Polyester
- Nylon flocked
- Calcium alginate
- Foam
- Polyester flocked

WP-3 - Genomic characterisation of Brucella detected from samples and selected isolates

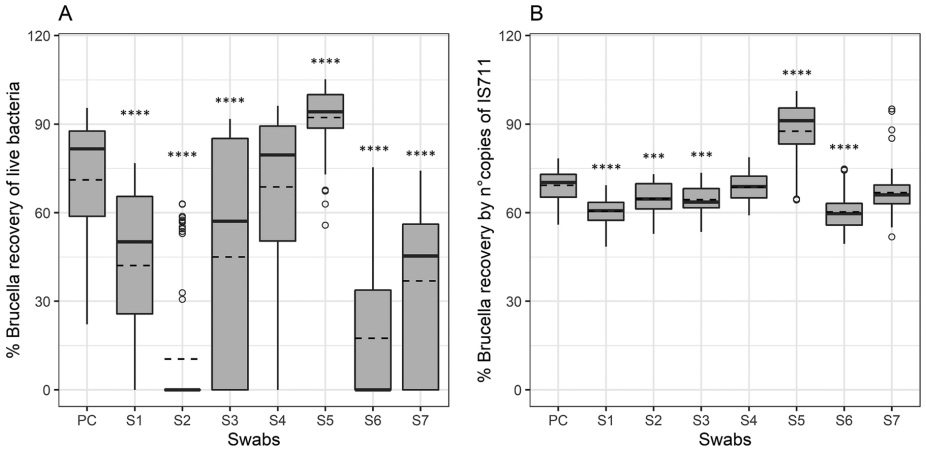
Recovery rates per strain type



Recovery rates per storage condition



Recovery rates per swab type



- ❑ *Brucella* species present different survival capacity
  16M and 544 >>> S2
- ❑ qPCR has is less variable than bacteriology isolation
- ❑ Storage conditions highly impact *Brucella* recovery of live bacteria
  Direct sample treatment >>> + 4°C storage >>> - 20°C storage
- ❑ Flocked wet swab (eSwab) increases the *Brucella* detection and survival
  wet swab >>> dry swab

WP-3 - Genomic characterisation of Brucella detected from samples and selected isolates

- Harmonisation and standardisation of protocols for whole genome sequencing
- Establishment of optimal protocols for the extraction of pathogen DNA from complex matrices (e.g. clinical and environmental samples)
- Multiplex assays development:
  - Ochrobactrum specific targeting:
    Fw primer: GCATTGGCAGCCAGTCTGC
    Rw primer: CGCTTTATCTGTTCAGGCACGACAC
    Probe: GCATTGGCAGCCAGTCTGCATCGCCGAAGCTTGCGC
  - Preamplification – Fluidigm cartridge optimization

WP-4 - Phenotypic characterisation of Brucella detected from samples and selected isolates

- AMR plates use for strain characterization.
  - *B. melitensis* 16M
  - *B. abortus* 544
  - *B. ovis*
  - *B. suis* S2 Thomsen
  - B. microti
  - B. canis

- Standardised bacteriology typing

- MALDI-TOF Preparation SOPs:
  - Growth media
  - Bacterial inactivation
  - Protein isolation
  - Protein preparation for MALDI-TOF

WP-5 - Zoonotic potential and virulence

- *In vitro* cell infection models:

HeLa cells and THP-1 cells tested with:

B. melitensis 16M
B. abortus 544
B. ovis
B. suis S2 Thomsen
B. microti
B. canis

- Comparing:
  - ✓ Cell infection speed
  - ✓ Bacterial multiplication
  - ✓ Cellular RNA seq
  - ✓ Cell activation – cytokine profiling.

WP-7 - Coordination, management and communication

- Reports: 9 months, annual, financial
- Communications: annual conference, EFSA meeting, stakeholders meeting, risk assessment meeting
- Consortium: annual workshops; monthly meetings; individual partner or group meetings
- OH EJP Coordination team communication
- Zenodo publishing
- Data Management Plan

Objectives IDEMBRU - Identification of emerging *Brucella* species: new threats for humans and animals

- Priority topic ET 2.1 →new brucellosis threats
- "One Health" approach, adaptation of existing -omics methods, including next generation sequencing (NGS), proteomics and metabolomics in the context of emerging *Brucella* species.
- Identification and quantification of emerging animal reservoirs and their surrounding environment → improved networks for monitoring new *Brucella* threats across European countries.
- Integration of epidemiological data (human and animal health), phenotypic and genomic data
- To develop a toolkit to detect and characterize emerging *Brucella* species and reservoirs.

Together with OHEJP COHESIVE project a new, emerging threat from classical *Brucella canis* species has been identified

anses — World Organisation for Animal Health — Reference Laboratory for Brucellosis

- Canine brucellosis, mainly caused by *Brucella canis* → abortions and infertility in dogs.

- *B. canis* isolation = very specific, but compromised by intermittent bacteremia
- →serological methods = central role in the diagnosis

Can be a major problem in dog breeding kennels + social impacts

- Performances of serological tests ?
- →false positives and negatives
- **Main RISKS**
  - **FALSE POSITIVES → unjustified treatment / neutering / euthanasia**
  - **FALSE NEGATIVES → dissemination & zoonotic risk**
  - **No legislatives on EU levels for pet animals (AHL)**
  - **Zoonotic disease!!!**
  - **- Daily identification of new infected kennels in EU**

White paper on *B. canis* problem in preparation
Collaboration with:

efsa — European Food Safety Authority

ecdc — EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

Human risks assesment within WP2

WP1 - Recording the situation of brucellosis in emergent wild and environmental reservoirs

WP	WP leader	WP deputy leader	Other involved partners
1	INIAV – AC Ferreira	NDRVMI–BFSA– H Daskalov	ANSES, APHA, BfR, FLI, IZSAM

INIAV Activities were carried out within the scope of WP1 tasks:

- **JRP19-WP1-T1:**
  - Mapping of existing data on emerging *Brucella* spp.
- **JRP19-WP1-T2:**
  - Harmonization and sharing of sampling protocols and epidemiological survey
  - Biological collections (sera, tissues, environmental, strains)
- **JRP19-WP1-T2: JRP19-WP1-T3**:
  - Synthesis and analysis of epidemiological and phenotypic data

JRP19-WP1-T2: Biological collections (sera, tissues, environmental, strains)

❖ Harmonization and sharing of sampling protocols and epidemiological survey

List of shared documents

- OHEJP_JRP19_WP1_SOP1_20200527_sample_treatment
- OHEJP_JRP19_WP1_SOP2_20201105_EpidemiologicalQuestionnaire_final
- OHEJP_JRP19_WP1_SOP3_20201104_sample_collection
- OHEJP_JRP19_WP1_SOP3_20201118_sample_collection - Exterior collectors
- OHEJP_JRP19_WP1_SOP4_20210407_serological diagnosis
- OHEJP_JRP19_WP1_SOP5_DNA extraction from soil and faeces
- OHEJP_JRP19_WP1_SOP6_20210407_bacteriological diagnosis_draft

JRP19-WP1-T2: Biological collections (sera, tissues, environmental, strains)

❖ Establishment of National network to improve sampling

Institutions	Region	Animal host
• Institute of Forests and Nature Conservation	Madeira (Ilhas Desertas)	Sea-lion, dolphin
• Institute for the Conservation of Nature and Forests	North, Trás-os-montes	Bat
• Veterinary Hospital of University of Trás-os-Montes/ Recovery Center	North, Trás-os-montes	Amphibian, rodents, wild carnivores
• Directorate of Food and Veterinary Services of the Central Region	Centre, Castelo Branco	Wild boar, deer
• Faculty of Veterinary Medicine, University of Lisbon	Lisbon region	Dog
• Faculty of Veterinary Medicine, University Lusofona	Lisbon region	Dog, fox, exotic animals
• CRVA-Anicura Atlântico Veterinary Reproduction Centre	Lisbon region	Dog, exotic animals
• Lisbon Animal House / Stray animals Recovery Center	Lisbon region	Dog
• Ria Formosa Wild Animal Rescue and Research Center	Algarve (Olhão)	Fox and rodents

JRP19-WP1-T2: Biological collections (sera, tissues, environmental, strains)

❖ Samples (n=584) and Results

Host species	Biotope	No.tested	Bacteriology	IS711 / bcsp31 qPCR	Serology
Deer	Forest	159	all negative	30 PCR+	not done
Dog	Forest	259	on going	30 PCR+ ; 50 doubt	on going
Fox	Forest	15	all negative	all negative	not done
Hare	Forest	46	all negative	all negative	not done
Rodents	Forest	7	all negative	all negative	not done
Seals	Coastal region	2	all negative	all negative	not done
Snake	Forest	1	*Ochrobactrum anthropi*	negative	not done
Wild boar	Forest	95	3 B. suis bv2	52 PCR+	not done

WP 2 - Recording the situation of brucellosis in human

- WP 2 Task 2.1 Implemented a network of laboratory surveillance of brucellosis and elaboration of a epidemiological questionnaire;

- WP 2 Task 2.2 - Isolation and detection of *Brucella* spp.

- WP 3 Task 2.3 Analysis data and constitutions of biobank

WP2 – T1 Dissemination of the network by all hospitals, and others in the country alerting the eventual emergence of new species of Brucella;

Institutions	Region
• National Hospitals	All
• Private laboratories	All
• Directorate General of Health	All
• Faculty of Veterinary Medicine, University of Lusofona	Lisbon region
• Slaughters of Setubal	South of Contry

WP 2 - T 2 - Isolationn and detection of Brucella spp.

Soure /Type	Number samples	Isolation	Realtime PCR	Serology	WGS
National Hospitals - Blood	53	Not done	All negative	All negative	Not done
National Hospitals – LCR	25	Not done	1 *Brucella melitensis*	Not done	Not done
National Hospitals – strains	6	6 *B. melitensis*	Positive for IS711 / BME		*B. melitensis*
National Hospitals - biopsy	10	1 *B. intermedia* 1 *B. melitensis* 8 negative	Positive for IS711 Positive for IS711 / BME 8 negative	Not done	*B. Intermedia* *B. melitensis*
Reference Laboratory - serum	200	Not done	7 Positive IS711	5 positive	No results
DogBReeders	15	Not done	All negative	All negative	Not done

WP3 - Genomic characterisation of Brucella detected from samples and selected isolates

- Task 2. Harmonisation and standardisation of protocols for whole genome sequencing

  - • Tissue Qiamp (Qiagen)
  - • Kingfisher (Thermofisher)
  - • ZYMO Reasearch – DNA Miniprep Kit

WP3 - Genomic characterisation of Brucella detected from samples and selected isolates

- Task 3. Whole genome Sequencing

  - • MiSeq Illumina platform (Illumina Inc.)

  - • dual-indexed Nextera XT Illumina library preparation

  -

  - • INNUca v3.1 pipeline

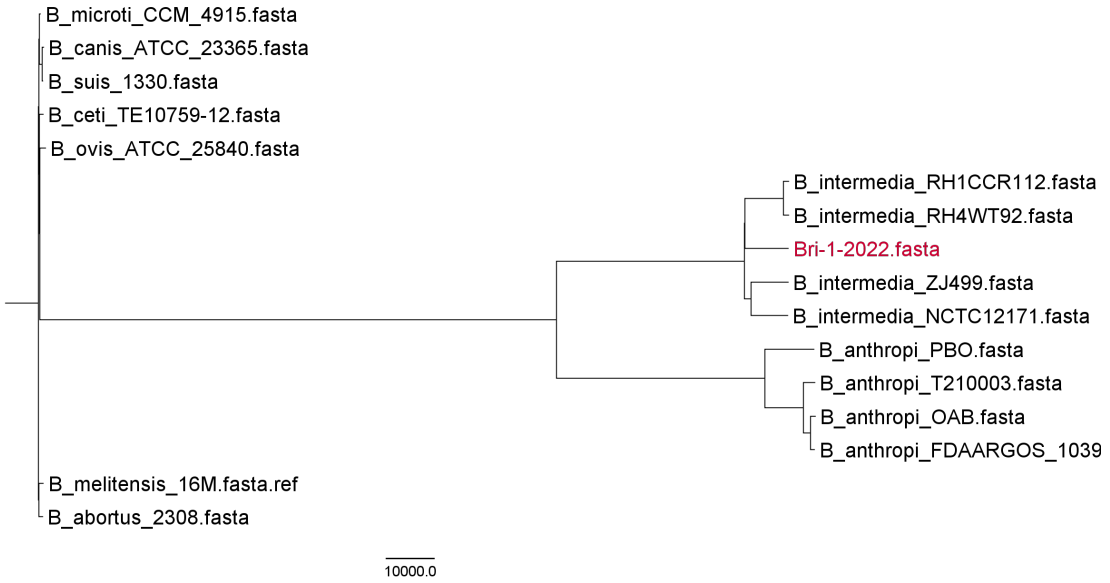
  - • Extraction in silico MLVA and wgMLST

Ana Pelerito ,Alexandra Nunes ,Maria Sofia Núncio ,João Paulo Gomes. Genome–scale approach to study the genetic relatedness among *Brucella melitensis* strains. 2020. Plos One. https://doi.org/10.1371/journal.pone.0229863

WP3 - Genomic characterisation of Brucella detected from samples and selected isolates

Brucela intermedia

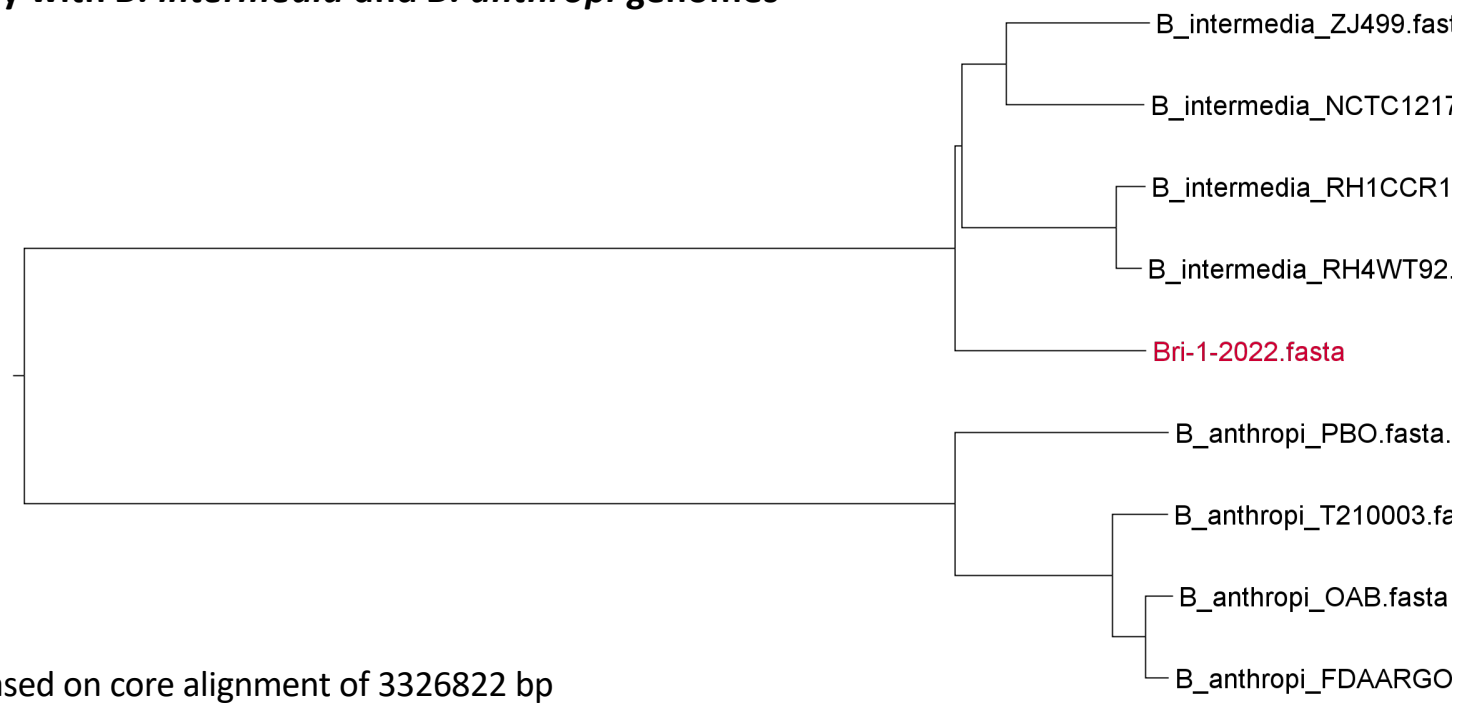
- teenager with a lower limb injury for many years subjected to several surgeries
- after a new surgery and treatment the skin biopsy was already negative



Phylogenetic tree with representative genomes of several Brucella species
(*B. microti, B. canis, B. suis, B. ceti, B. ovis, B. melitensis, B. abortus, B. anthropi and B. intermedia*)

WP3 - Genomic characterisation of Brucella detected from samples and selected isolates

Phylogenetic tree only with *B. intermedia* and *B. anthropi* genomes



Phylogenetic tree based on core alignment of 3326822 bp

WP3 - Genomic characterisation of Brucella detected from samples and selected isolates

ATB Resistance

GENE	PRODUCT	RESISTANCE	%COVERAGE	%IDENTITY
cml_Ochro	Cml family chloramphenicol efflux MFS transporter	CHLORAMPHENICOL	97.91	84
blaOCH-7	class C extended-spectrum beta-lactamase OCH-7	beta-lactams	99.49	82.88

Virulence factors

GENE	PRODUCT	%COVERAGE	%IDENTITY
htrB	(htrB) lipid A biosynthesis lauroyl acyltransferase [LPS (CVF383)] [Brucella melitensis bv. 1 str. 16M]	100	86.91
acpXL	(acpXL) acyl carrier protein [LPS (CVF383)] [Brucella melitensis bv. 1 str. 16M]	100	93.26
fabZ	(fabZ) (3R)-hydroxymyristoyl ACP dehydratase [LPS (CVF383)] [Brucella melitensis bv. 1 str. 16M]	100	82.38
kdsA	(kdsA) 2-dehydro-3-deoxyphosphooctonate aldolase [LPS (CVF383)] [Brucella melitensis bv. 1 str. 16M]	100	81.89
ricA	(ricA) Rab2 interacting conserved protein A [RicA (VF0414)] [Brucella melitensis bv. 1 str. 16M]	100	80.87
wbpZ	(wbpZ) mannosyltransferase C [LPS (CVF383)] [Brucella melitensis bv. 1 str. 16M]	98.94	89.66
manAoAg	(manAoAg) mannose-6-phosphate isomerase [LPS (VF0367)] [Brucella melitensis bv. 1 str. 16M]	100	90.55
manCoAg	(manCoAg) mannose-1-phosphate guanylyltransferase [LPS (VF0367)] [Brucella melitensis bv. 1 str. 16M]	83.72	90.53
kdsB	(kdsB) 3-deoxy-manno-octulosonate cytidylyltransferase [LPS (CVF383)] [Brucella melitensis bv. 1 str. 16M]	81.76	84.99
pgm	(pgm) phosphoglucomutase [LPS (VF0367)] [Brucella melitensis bv. 1 str. 16M]	100	86.01
cgs	(cgs) cyclic beta 1-2 glucan synthetase [C<beta>G (VF0366)] [Brucella melitensis bv. 1 str. 16M]	99.48	86.12
acpXL	(acpXL) acyl carrier protein [LPS (CVF383)] [Brucella melitensis bv. 1 str. 16M]	99.16	96.6

WP4 - Phenotypic characterisation of Brucella detected from samples and selected isolates

Task 2 - Antimicrobial Susceptibility Testing (EMERGE project partner)

- 56 samples *Brucella spp.* tested

- BMD tests were performed with user-defined commercial microdilution plates (MICRONAUT, MERLIN Diagnostika, Berlin, Germany)

WP4 - Phenotypic characterisation of Brucella detected from samples and selected isolates

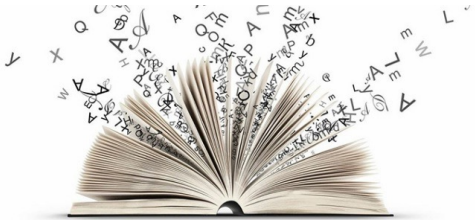
Strain	Species	Geographic origin	Biological Product	Host	Biovar	RAM_MIC	DOX_MIC	TET_MIC	GEN_MIC	STR_MIC	CMP_MIC	LEV_MIC	T/S_MIC	CIP_MIC
120/99E	B. melitensis	Cordoba	Sangue	Humana	1	0,5	0,25	<0,016	1	2	2	0,25	0,016/0,2	>4
167/00E	B. melitensis	Cordoba	sangue	Humana	1	0,38	0,125	0,032	1	1	2	0,125	0,016/0,2	2
194/00E	B. melitensis	Cordoba	Sangue	Humana	1	0,5	0,064	0,023	1	0,5	2	0,5	0,016/0,2	0,25
457/06E	B. melitensis	A Coruna	Sangue	Humana	2	0,5	0,125	0,032	1	2	2	0,125	0,016/0,2	0,25
213/03E	B. melitensis	Teruel	Sangue	Humana	1	0,38	0,047	<0,016	1	2	2	0,125	0,031/0,5	0,25
170/04E	B. melitensis	Valencia	Sangue	Humana	2	0,5	0,19	0,047	1	1	2	0,25	0,25/4,76	2
238/04E	B. melitensis	Teruel	Sangue	Humana	2	0,75	0,125	0,047	2	2	2	0,25	0,25/4,76	2
723/07E	B. melitensis	Burgos	Liquido arti	Humana	2	0,75	0,094	0,023	1	1	2	0,25	0,25/4,76	2
104-11RK	B. melitensis	Germany	Desconheci	Desconhe	3	1,5	0,5	0,19	1	4	2	0,25	0,25/4,76	2
104-12 RK	B. melitensis	Germany	Desconheci	Desconhe	3	1,5	0,19	0,25	2	4	2	0,25	0,25/4,76	2
104-13RK	B. melitensis	Germany	Desconheci	Desconhe	3	1	0,38	0,074	1	4	2	0,25	0,25/4,76	2
183-4RK	B. melitensis	Hungary	Desconheci	Desconhe	3	2	4	2	1	4	2	0,25	0,25/4,76	2
183-6RK	B. suis	Hungary	Desconheci	Desconhe	2	0,75	0,75	0,125	1	2	2	0,25	0,25/4,76	0,25
183-7RK	B. ovis	Hungary	Desconheci	Desconhe	2	1	0,19	0,094	2	1	2	0,25	0,25/4,76	0,25
148-9RK	B. melitensis	Belgium	Desconheci	Desconhe	3	12	1,5	0,38	1	1	2	0,25	0,25/4,76	0,25
146-10RK	B. melitensis	Spain	Desconheci	Desconhe	3	2	1	1,5	1	1	2	0,25	0,25/4,76	0,25
146-11RK	B. abortus	Spain	Desconheci	Desconhe	2	1,5	0,38	-	1	4	2	0,25	0,25/4,76	0,5
146-12RK	B. melitensis	Spain	Desconheci	Desconhe	3	1,5	0,5	0,19	2	2	2	0,25	0,25/4,76	0,5

WP4 - Phenotypic characterisation of Brucella detected from samples and selected isolates

Strain	Species	Geographic origin	Biological Product	Host	Biovar	RAM_MIC	DOX_MIC	TET_MIC	GEN_MIC	STR_MIC	CMP_MIC	LEV_MIC	T/S_MIC	CIP_MIC
1P	B. melitensis	Portuguesa	Estirpe	Humana	2	1	0,023		2	2	2	0,25	0,031/0,5	0,5
35P	B. melitensis	Portuguesa	Estirpe	Humana	2	>32	0,19		1	1	2	0,25	0,25/4,76	0,5
36P	B. melitensis	Portuguesa	Estirpe	Humana	2	1	0,19		1	1	1	0,125	0,031/0,5	0,5
38P	B. melitensis	Portuguesa	Estirpe	Humana	2	0,5	1,5		1	1	1	0,5	0,031/0,5	0,5
40P	B. melitensis	Portuguesa	Estirpe	Humana	1	2	0,047		1	1	1	0,25	0,031/0,5	0,5
41P	B. melitensis	Portuguesa	Estirpe	Humana	2	0,75	0,19		2	1	1	0,25	0,016/0,2	0,25
43P	B. melitensis	Portuguesa	Estirpe	Humana	2	1,5	0,125	0,032	2	1	1	0,25	0,016/0,2	0,25
44P	B. melitensis	Portuguesa	Estirpe	Humana	2	1,5	0,094		1	1	2	0,25	0,016/0,2	0,25
66 P(127624)	B. melitensis	Portuguesa	Estirpe	Humana	2	1,5	0,19		2	4	2	0,25	0,016/0,2	0,25
71P	B. melitensis	Portuguesa	Estirpe	Humana	3	1,5	0,094		1	1	2	0,125	0,016/0,2	0,25
147P	B. melitensis	Portuguesa	Estirpe	Humana	1	1,5	0,25	0,094	2	4	4	0,5	0,016/0,2	0,25
153P	B. melitensis	Portuguesa	Estirpe	Humana	1				1	2	2	1	0,016/0,2	0,25
165P	B. melitensis	Portuguesa	Estirpe	Humana	3	>32	0,125		2	2	1	0,25	0,016/0,2	0,25
166P (SP)	B. melitensis	Portuguesa	Estirpe	Humana	2	2	<0,016		2	2	2	0,25	0,016/0,2	0,25
167P (XX)	B. melitensis	Portuguesa	Estirpe	Humana	2	1,5	0,023		2	2	4	0,25	0,016/0,2	0,25
168 P(2)	B. melitensis	Portuguesa	Estirpe	Humana	2	2	0,19		1	1	2	0,25	0,031/0,5	0,25
187P	B. melitensis	Portuguesa	Estirpe	Humana	3	0,19	1,5	0,032	2	4	2	0,25	0,25/4,76	0,25
194P	B. melitensis	Portuguesa	Estirpe	Humana	2	0,19	0,125	0,047	1	2	1	0,125	8/152	1
209P	B. melitensis	Portuguesa	Estirpe	Humana	3	0,25	0,094	0,032	1	2	2	0,031	0,031/0,5	0,031/0,5
228P	B. melitensis	Portuguesa	Estirpe	Humana	3	1,5	0,125	0,032	2	4	2	0,25	0,031/0,5	2
234P	B. melitensis	Portuguesa	Estirpe	Humana	3	1	0,19	0,032	1	2	2	0,25	0,031/0,5	0,016
240P	B. melitensis	Portuguesa	Estirpe	Humana	3	0,5	0,19	0,032	1	2	2	0,25	16/304	1
258P	B. melitensis	Portuguesa	Estirpe	Humana	3	0,5	<0,016	0,094	1	2	2	0,031	0,031/0,5	1
261P	B. melitensis	Portuguesa	Estirpe	Humana	3	0,5	<0,016	0,094	1	2	2	0,25	0,031/0,5	1

Planned Activities

- ♦ Research the Brucella in Fishermans;
- ♦ Research the Brucella in workers of the Slaughters
- ♦ Development of a decision-tree as a tool to support decision-making concerning emergent *Brucella* species infections



Publications

- Pelerito, A., *et al*. (2021). **Genetic characterization of *Brucella* spp: whole genome sequencing – based approach for determination of Multiple Locus Variable Number Tandem Repeat profiles**. Front. Microbiol. 12:740068. doi: 10.3389/fmicb.2021.740068

- .Tscherne A, Mantel E, Boskani T, Budniak S, Elschner M, Fasanella A, Feruglio SL, Galante D, Giske CG, Grunow R, Henczko J, Hinz C, Iwaniak W, Jacob D, Kedrak-Jablonska A, Jensen VK, Johansen TB, Kahlmeter G, Manzulli V, Matuschek E, Melzer F, Nuncio MS, Papaparaskevas J, Pelerito A, Solheim M, Thomann S, Tsakris A, Wahab T, Weiner M, Zoeller L, Zange S; EMERGE AST Working Group. (2022). **Adaptation of Brucella melitensis Antimicrobial Susceptibility Testing to the ISO 20776 Standard and Validation of the Method**. Microrganisms. 10.3390/microorganisms10071470

WP3 - Genomic characterisation of *Brucella* detected from samples and selected isolates

WP	WP leader	WP deputy leader	Other involved partners
3	APHA - R. Ashford	IZSAM – G. Garofolo	ANSES, BfR, FLI, INIAV, INSA, WBVR

INIAV Activities within the scope of WP3 tasks:

- Task 1. Optimisation of routine methods used in INIAV for the extraction of *Brucella* DNA from clinical and environmental samples.
- Task 2. Implementation of WGS - Illumina MiSeq, paired-end protocolo, 150 bp reads
  - Illumina Nextera XT DNA Library Preparation Kit
  - Nextera XT Index Kit
  - MiSeq Reagent Micro Kit v2 /v3
- Task 3. All strains tested in Multiple locus VNTR analysis (MLVA).

WP4 - Phenotypic characterisation of *Brucella* detected from samples and selected isolates

WP	WP leader	WP deputy leader	Other involved partners
4	FLI – F Melzer	BfR – S Al Dahouk	ANSES, APHA, IZSAM, INIAV, INSA

INIAV Activities within the scope of WP4 task 2:

- AMR testing → total of 80 strains tested
  - 75 *Brucella* spp → REF and field strains
  - 1 *Ochrobactrum anthropi*

AMR data – reference strains

strain _id	species	biovar	host	GEN_MIC	STR_MIC	DOX_MIC	TET_MIC	CMP_MIC	RAM_MIC	T/S_MIC	CIP_MIC	LEV_MIC
REF S.aureus ATCC 29213	na	na	na	0,5	8	0,5	1	>8	<0,125	0,062/1,187	0,25	0,25
REF E. coli ATCC 25922	na	na	na	1	4	0,5	0,5	4	4	0,062/1,187	0,0313	0,03125
REF Ba1 str 544	B. abortus	1	cattle	0,25	2	0,125	0,25-0,125	2	<0,125	0,125/2,375	2	2
REF Ba1 str 99W	B. abortus	1	cattle	0,25	0,5	0,25	0,125	<0,5	0,5	<0,002/0,037	0,25	0,25
REF Ba3	B. abortus	3	cattle	0,25	0,5	0,25	0,125	1	1	>0,002/0,037	0,5	0,5
REF Ba4	B. abortus	4	cattle	0,25	0,5	0,0625	0,0625	1	1	0,004/0,074	0,25	0,5
REF Ba5	B. abortus	5	cattle	0,25	16	0,25	0,125	0,5	0,5	<0,002/0,038	0,25	0,25
REF Ba6	B. abortus	6	cattle	0,25	1	1	0,125	1	2	<0,002/0,039	0,5	0,5
REF Ba9	B. abortus	9	cattle	0,25	0,5	0,25	0,125	1	0,5	<0,002/0,040	0,25	0,25
REF Bm1 str 16M	B. melitensis	1	goat	0,25	1	0,0625	0,125-0,0625	1	0,5	0,031/0,593	0,25	0,25
REF Bm3 str Tulya	B. melitensis	3	sheep	0,125	1	0,0625	0,0625	<0,5	1	<0,002/0,037	0,25	0,25
REF Bs1 str 1330	B. suis	1	swine	0,25	1	0,0313	0,125-0,0625	4	1	0,015/0,297	0,25	0,25
REF Bs2 str Thomsen	B. suis	2	hare	0,25	2	0,125	0,125	>8	0,5	0,125/2,375	1	1
REF Bs3 str 686	B. suis	3	swine	0,125	0,5	0,0313	0,0313	2	0,25	0,031/0,593	0,25	0,25
REF Bs4 str 40	B. suis	4	reindeer	0,125	0,5	0,0313	0,125	1	0,25	0,015/0,297	0,25	0,25
REF Bs5 str 513	B. suis	5	mouse	0,125	0,5	0,0625	0,125	<0,5	0,5	0,125/2,375	0,5	0,5
REF B.canis str RM6/66	B. canis	na	dog	0,125	0,25	0,125	0,125	1	0,5	0,07/0,148	0,25	0,25
REF B.ovis str BOW	B. ovis	na	ram	0,0625	8	>8	>8	>8	2	0,062/1,187	0,25	0,5
REF B.ceti	B.ceti	na	dolphin	0,25	0,25	0,125	0,25	1	1	0,015/0,297	0,5	0,5
REF B.pinnipedialis	B.pinnipedialis	na	seal	0,25	0,25	0,25	0,125	2	1	0,015/0,297	0,25	0,25
REF B.vulpis	B. vulpis	na	fox	0,5	2	2	2	8	>8	0,125/2,375	0,0625	0,0625

AMR data – field strains

strain _id	species	biovar	host	GEN_MIC	STR_MIC	DOX_MIC	TET_MIC	CMP_MIC	RAM_MIC	T/S_MIC	CIP_MIC	LEV_MIC
jiboia_Oa_27142-21	O. anthropi	na	Boa constrictor	4	16	0,25	1-0,5	>8	8	0,25/4,75	0,5-0,25	0,25
499Ba1	B. abortus	1	cattle	0,25	1	0,125	0,125	2	1	0,015/0,298	0,25	0,25
487Ba1	B. abortus	1	catle	0,25	0,5	0,0625	0,125	2	1	>0,002/0,037	0,25	0,25
BS_16-198	B. suis	1	goat	0,25	1	0,0625	0,125	2	1	0,5/9,5	1	1
BS_09-164	B. suis	2	swine	0,125	0,25	0,0313	0,0625	1	1	0,062/1,187	0,25	0,125
BS_00-001	B. suis	2	swine	0,25	0,25	0,0313	0,0625	1	0,5	0,062/1,187	0,25	0,25
BS_09-129	B. suis	2	wildboar	0,125	0,25	0,0313	0,0625	1	0,5	0,062/1,187	0,125	0,125
24Bs	B. suis	2	swine	0,5	1	0,0625	0,125	2	1	0,007/0,152	0,25	0,25
BM_17-2123	B. melitensis	2	sheep	0,25	2	0,0625	0,0625	2	0,5	0,007/0,148	0,25	0,25
24Bm	B. melitensis	3	sheep	0,25	0,5	0,0625	0,125	2	0,5	0,015/0,297	0,25	0,25
30144/10-ME16524	B. melitensis	3	humam	0,25	1	0,0625	0,125	2	0,5	0,004/0,074	0,25	0,25
8217/01	B. melitensis	3	humam	0,25	1	0,125	0,125	2	0,5	0,007/0,148	0,5	0,5
2.90_383/03	B. melitensis	3	humam	0,25	1	0,125	0,125	2	0,5	0,007/0,149	0,5	0,5
6.479_29730/05	B. melitensis	3	humam	0,25	1	0,125	0,125	2	0,5	0,007/0,150	0,25	0,25
6.480_29731/05	B. melitensis	3	humam	0,5	4	>8	>8	8	1	0,25/4,75	0,5	0,5
6.481_29732/05	B. melitensis	3	humam	0,25	1	0,125	0,125	2	0,5	0,007/0,150	0,5	0,5
6.482_30795/05	B. melitensis	3	humam	0,25	1	0,125	0,125	2	0,5	0,004/0,074	0,25	0,25
6.483_30796/05	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	0,007/0,150	0,5	0,5
6.484_30797/05	B. melitensis	3	humam	0,25	1	0,125	0,125	4	0,5	0,031/0,593	0,5	0,5
6.485_31978/05	B. melitensis	3	humam	0,25	0,5	0,125	0,125	2	0,5	0,007/0,150	0,25	0,25
6.486_31979/05	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	0,007/0,150	0,25	0,25
7.490_41786/05	B. melitensis	3	humam	0,25	1	0,0625	0,125	2	0,5	0,007/0,151	0,25	0,25
7.505_3292/06	B. melitensis	3	humam	0,25	1	0,0625	0,125	2	0,5	0,007/0,152	0,25	0,25
7.520_12411/05	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	0,004/0,074	0,25	0,25
7.563_30570/06	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	0,004/0,074	0,25	0,25
8.630_7242/07	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	0,007/0,150	0,25	0,25
9.694_02/4222	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	0,007/0,150	0,25	0,25
9.695_02/5258	B. melitensis	3	humam	0,25	1	0,0625	0,125	2	0,5	0,007/0,150	0,25	0,25
9.696_02/6425	B. melitensis	rough	humam	0,25	0,5	0,0625	0,0625	1	1	0,015/0,297	0,25	0,25

AMR data – field strains

strain _id	species	biovar	host	GEN_MIC	STR_MIC	DOX_MIC	TET_MIC	CMP_MIC	RAM_MIC	T/S_MIC	CIP_MIC	LEV_MIC
9.697_02/6501	B. melitensis	1	humam	0,25	2	0,125	0,125	2	0,5	0,004/0,074	0,25	0,25
9.698_02/6907	B. melitensis	1	humam	0,5	1	0,125	0,125	2	1	>0,002/0,037	0,25	0,25
9.699_02/7384	B. melitensis	3	humam	0,25	0,5	0,125	0,125	2	0,5	>0,002/0,037	0,25	1
9.700_03/0342	B. melitensis	3	humam	0,5	4	0,5	0,25	8	0,5	1/19	1	0,25
9.701_03/0346	B. melitensis	3	humam	0,25	0,5	0,125	0,25	2	0,5	0,007/0,150	0,25	0,25
9.702_03/2230	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	0,007/0,150	0,25	0,25
9.703_03/3515	B. melitensis	3	humam	0,5	16	>8	>8	>8	1	0,007/0,151	0,5	0,5
9.704_03/4805/7430	B. melitensis	3	humam	0,5	4	0,25	0,25	2	0,5	0,5/9,5	1	1
9.705_04/2179	B. melitensis	1	humam	0,25	1	0,125	0,125	2	0,5	>0,002/0,037	0,25	0,5
9.707_05/3515	B. melitensis	3	humam	0,25	0,5	0,125	0,125	2	0,5	0,007/0,150	0,5	0,5
9.708_05/3565	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	1	0,015/0,297	0,25	0,25
9.709_05/4465	B. melitensis	3	humam	0,25	0,5	0,0625	0,0625	2	0,5	0,007/0,150	0,25	0,25
9.710_05/3111	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	>0,002/0,037	0,25	0,25
9.711_05/3592	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	0,007/0,150	0,25	0,25
9.712_05/3731	B. melitensis	3	humam	0,125	0,25	0,0625	0,0625	2	0,5	0,007/0,150	0,25	0,25
9.713_05/0716	B. melitensis	1	humam	0,25	1	0,0625	0,125	2	0,5	>0,002/0,037	0,25	0,25
9.714_05/3819	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	1	0,5	0,007/0,150	0,25	0,25
9.715_06/0444	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	>0,002/0,037	0,25	0,25
9.716_06/1576	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	0,007/0,150	0,25	0,25
11.839_20941/08-1002	B. melitensis	3	humam	0,25	0,5	0,0625	0,125	2	0,5	0,007/0,151	0,25	0,25
11.860_1002	B. melitensis	3	humam	0,25	0,5	0,125	0,0625	1	1	0,007/0,152	0,25	0,25
11.861_993081	B. melitensis	3	humam	0,125	0,5	0,0625	0,125	1	0,5	>0,002/0,037	0,25	0,25
11.862_994004	B. melitensis	3	humam	0,25	1	0,0625	0,125	2	0,5	>0,002/0,037	0,25	0,25
12.912_30144/10-ME1074	B. melitensis	3	humam	0,25	0,5	0,125	0,125	2	1	0,031/0,593	0,5	0,5
12.917_ME25620A (Oeiras)	B. melitensis	3	humam	0,25	0,5	0,125	0,125	2	0,5	>0,002/0,037	0,25	0,25
12.924_IRJ440-250811	B. melitensis	3	humam	0,25	0,5	0,0625	0,0625	2	0,5	0,015/0,297	0,25	0,25

Planned Activities

- Validation of WGS procedure (MiSeq platform) → library preparation, quality, number of reads, coverage, bioinformatics protocols.
- Acquisition of AMR microplates for characterization of *B. abortus* strains and implement the method in INIAV.
- INIAV aims to organize, in January 2023, a workshop to introduce stakeholders in the animal health, public health and food security sectors, to the FoodChainLab software, developed within Cohesive project, for foodborne diseases outbreak investigation.

Publications

- Ferreira A.C., *et al*. (2022). Insights into the seroprevalence of *Brucella canis* infection in dogs in Portugal. Brucellosis 2022 International Conference, Giulianova, Teramo, Itália.
- Ferreira A.C., *et al*. (2022). PORIFERA - Core-genome-based analytical pipeline for prediction of new markers to rapid identification of emerging *Brucella* species. Brucellosis 2022 International Conference, Giulianova, Teramo, Itália.
- Ponsart, C., *et al*. (2021). Identification of emerging *Brucella* species: new threats for human and animals (IDEMBRU). One Health EJP Annual Scientific Meeting 2021 (Online Event), 9-11 maio, Statens Serum Institut, DTU, Copenhaga.
- Pelerito, A., *et al*. (2021). Genetic characterization of *Brucella* spp: whole genome sequencing – based approach for determination of Multiple Locus Variable Number Tandem Repeat profiles. Front. Microbiol. 12:740068. doi: 10.3389/fmicb.2021.740068

OBJECTIVES

The major aim of WP4 is to identify diagnostically relevant features to characterize and differentiate novel emerging *Brucella* spp. from the classical species.

Objectives

T1: Phenotyping of emerging *Brucella* spp.

- classical microbiology
- electron microscopy to investigate structural differences (*Brucella*-like organisms often reveal motility, e.g. a potential flagellum)

T2: Antibiotic Resistance Testing (AMR)

- Microdilution (Mikronaut ™)

FLI also delivered results to WP1 (field samples ~500 mice spleen DNA-extraction+qPCR but negative result), WP3 (sequencing) and did experiments for WP5 (cell culture)

Objectives

T3: A combined RNASeq and peptidomic (mass spectrometry) approach on a panel of emerging *Bruc*ella spp.

- month 49 until month 54 (60)

Species-specific metabolic pathways will reveal a correlation in transcription and translation. This is of particular importance when emerging and classical species are compared since some of the novel species are genetically very close to the classical ones. Hence, the phenotypic differences are a matter of gene regulation rather than a matter of gene configuration.

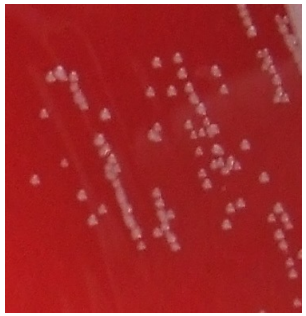
T4: Translational aspects of RNA-Seq for Brucellosis diagnostics in humans - month 25 until month 54 (60)

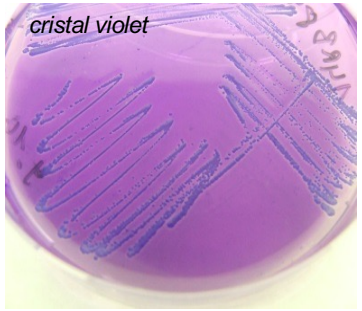
Data-will be analysed and may allow the identification of transcribed and translated virulence factors specific for a species. RNASeq might help to diagnose chronically infected hosts when culture methods often fail. We will also analyse whether the detection of RNA in clinical samples which has a higher abundance is more sensitive than the detection of genomic *Brucella* DNA. For this purpose, the efficacy of RNASeq and 16S metagenomics for the detection of *Brucella* in human and animal samples will be compared.

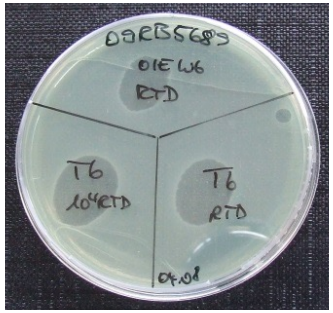
Task 1-Phenotyping of emerging Brucella spp

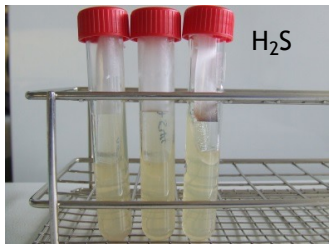
classical microbiology

- Gram-staining
- CO_2 – requirement
- Hemolysis
- Oxidase
- Catalase
- H_2S production
- Urease
- Crystal violet staining
- Growth on Basic fuchsin
- Growth on Thionin
- Lysis by phages





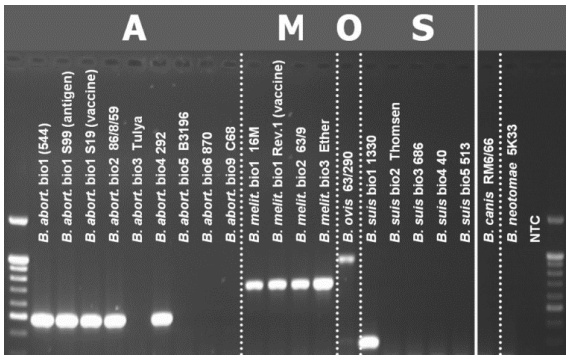


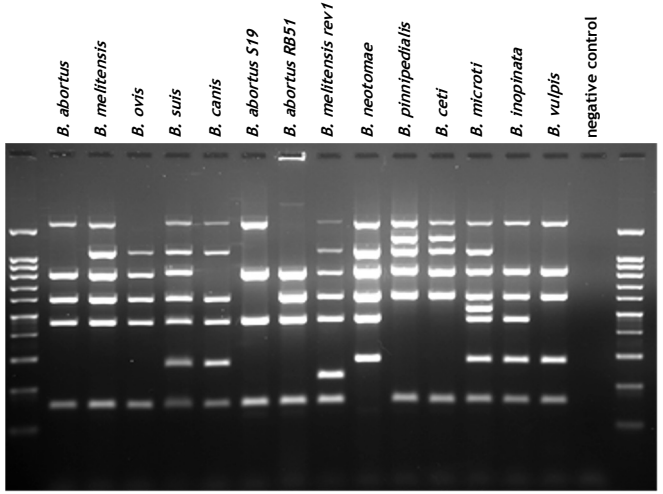


Task 1-Phenotyping of emerging Brucella spp

PCR methods used for routine identification of *Brucella* spp. isolates

- BCSP-31 conventinal PCR
- AMOS-PCR
- Ladder-PCR





Task 1: Results – Phentotyping (classical Microbiology)

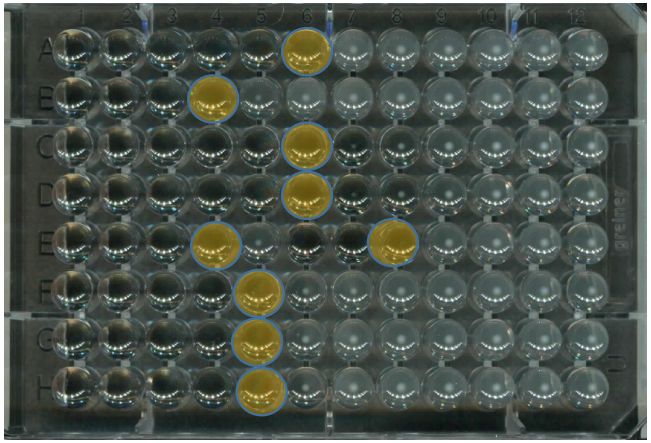
Lab number partner	Strain	Source	Bucella-PCR BCSP31	Amos-PCR	Ladder-PCR bands size	Maldi	Gram staining	CO_2-requirement	Hemolysis	Motility at 37 °C	Oxidase	Catalase	H_2S production	Urease	Crystal violet staining	Disociation Trypaflavin	Thionin	Fuchsin	monospez.Serum A	monospez.Serum M	monospez.Serum R	Lysis F25 RTD	Lysis Wb RTD	Lysis Tb RTD	Lysis Tb 10xRTD	Api20E/NE	Agglutination with Brucellosis positive reference serum
09RB8471	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
13RB5064	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	r	r	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
14RB5420	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	r	r	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
18RB16866	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	r	r	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
20RB21708	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	neg	pos	r	r	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
20RB22556	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	neg	pos	r	r	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
20RB22606	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	neg	pos	r	r	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9205	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9206	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9207	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9208	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9209	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9210	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9211	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9212	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9213	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9214	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9215	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9216	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
10RB9217	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	s	s	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
09RB8910	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	pos	pos	r	r	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
09RB8915	New emerging Brucella	frog	pos	neg	152, 272, 450, 587, 794 bp	Brucella	neg	no	neg	yes	pos	pos	neg	pos	r	r	pos	pos	neg	neg	neg	neg	neg	neg	neg	Ochro/Bruc.	neg
06RB0264	B. microti	Common Vole	pos	neg	*B. microti*	Brucella	neg	no	neg	no	pos	pos	pos	pos	s	s	pos	pos	neg	pos	neg	pos	pos	pos	pos	n.d.	n.d.
09RB4616	B. ceti	Dolphin	pos	neg	*B. maris / pinnipedialis*	Brucella	neg	no	neg	no	pos	pos	neg	pos	r	r	pos	pos	pos	neg	neg	neg	neg	neg	neg	n.d.	n.d.
09RB4620	B. pinnipedialis	Seal	pos	neg	*B. maris / pinnipedialis*	Brucella	neg	yes	neg	no	pos	pos	neg	pos	s	r	pos	pos	pos	pos	pos	pos	pos	pos	pos	n.d.	n.d.
09RB4625	B. suis 2	Wild boar	pos	neg	*B. suis*	Brucella	neg	yes	neg	no	pos	pos	neg	pos	s	s	pos	neg	pos	neg	pos	neg	neg	neg	neg	n.d.	n.d.
03RB0217	B. suis 1330	reference	pos	pos	*B.suis*	Brucella	neg	no	neg	no	pos	pos	pos	pos	s	s	pos	neg	pos	neg	neg	pos	n.d.	neg	pos	n.d.	n.d.
10RB9822	B. vulpis	Fox	pos	neg	*B.vulpis*	Brucella	neg	no	neg	no	pos	pos	neg	pos	s	s	pos	pos	pos	neg	neg	pos	pos	pos	pos	n.d.	n.d.
03RB0215	B. abortus 544	reference	pos	pos	*B.abortus*	Brucella	neg	yes	neg	no	pos	pos	pos	pos	s	s	neg	pos	pos	neg	neg	pos	n.d.	pos	pos	n.d.	n.d.
03RB0216	B. melitensis 16M	reference	pos	pos	*B.melitensis*	Brucella	neg	no	neg	no	pos	pos	neg	pos	s	s	neg	pos	neg	pos	neg	pos	n.d.	neg	neg	n.d.	n.d.
03RB0213	B.canis RM 6/66	reference	pos	neg	*B.canis*	Brucella	neg	no	neg	no	pos	pos	neg	pos	r	r	pos	neg	neg	neg	pos	neg	n.d.	neg	neg	n.d.	n.d.
03RB0215	B. ovis 63/290	reference	pos	pos	*B.ovis*	Brucella	neg	yes	neg	no	neg	neg	neg		r	r	pos	neg	neg	neg	pos	neg	n.d.	neg	neg	n.d.	n.d.

Obtained results -T2 Antibiotic Resistance Testing (AMR)

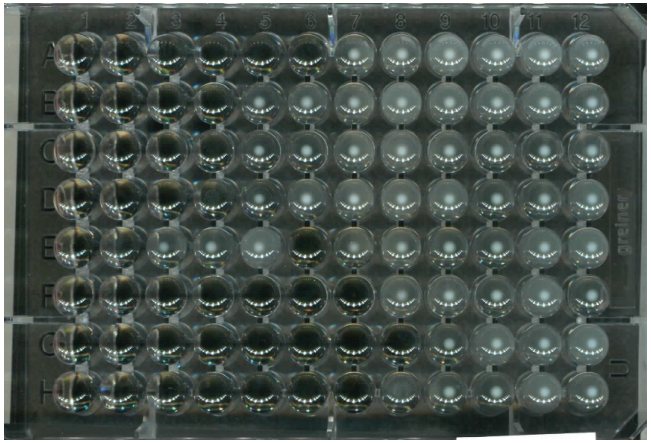
Task 2: Microdilution (Mikronaut ™) antibiotic concentration of each well in mg/L

Well	1	2	3	4	5	6	7	8	9	10	11	12
A -GEN	8	4	2	1	0,5	0,25	0,125	0,0625	0,031	0,016	0,008	0,004
B -STR	16	8	4	2	1	0,5	0,25	0,125	0,0625	0,031	0,016	0,008
C -DOX	8	4	2	1	0,5	0,25	0,125	0,0625	0,031	0,016	0,008	0,004
D -TET	8	4	2	1	0,5	0,25	0,125	0,0625	0,031	0,016	0,008	0,004
E - CMP/RAM	CMP 8	CMP 4	CMP 2	CMP 1	CMP 0,5	RAM 8	RAM 4	RAM 2	RAM 1	RAM 0,5	RAM 0,25	RAM 0,125
F - T/S	4/76	2/38	1/19	0,5/9,5	0,25/4,76	0,125/ 2,375	0,0625/ 1,187	0,031/ 0,594	0,016/ 0,297	0,0078/ 0,148	0,0039/ 0,074	0,00195/ 0,037
G -CIP	4	2	1	0,5	0,25	0,125	0,0625	0,031	0,016	0,008	0,004	0,002
H -LEV	4	2	1	0,5	0,25	0,125	0,0625	0,031	0,016	0,008	0,004	GC

CMP: Chloramphenicol
CIP: Ciprofloxacin
DOX: Doxycyclin
GEN: Gentamycin
LEV: Levofloxacin
RAM: Rifampicin
STR: Streptomycin
TET: Tetracyclin
T/S: Trimethoprim/Sulfamethoxazole
GC: Growth Control



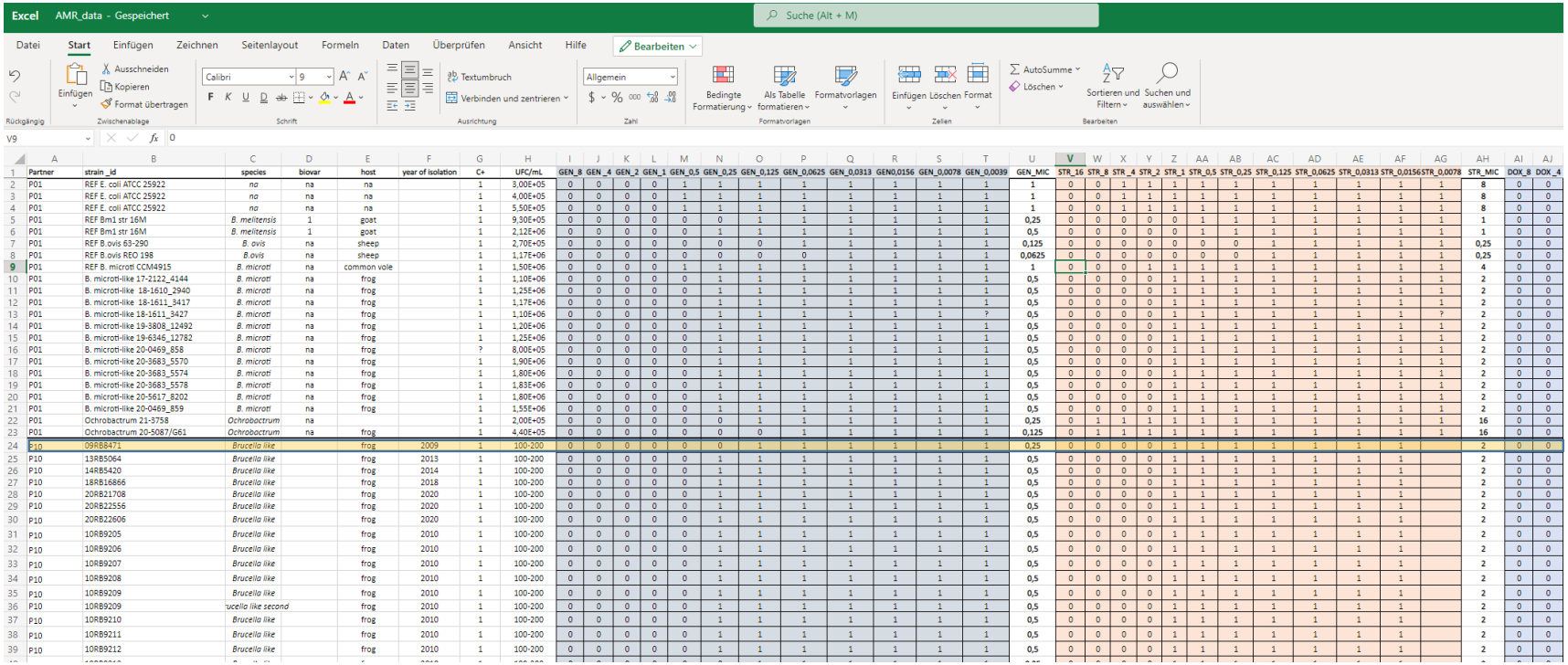
09RB8471_GN1_24h



Ecoli_08BR3642_GN1_18h_220127

Obtained results

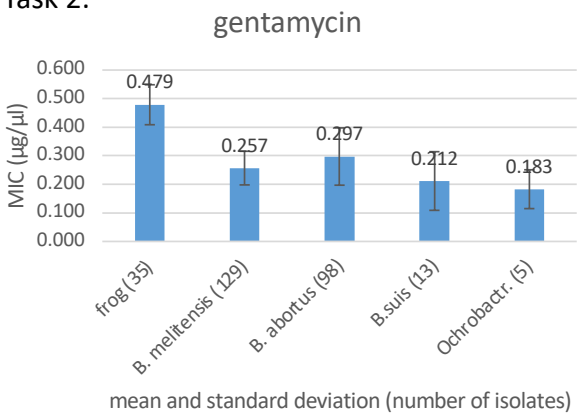
Task 2:

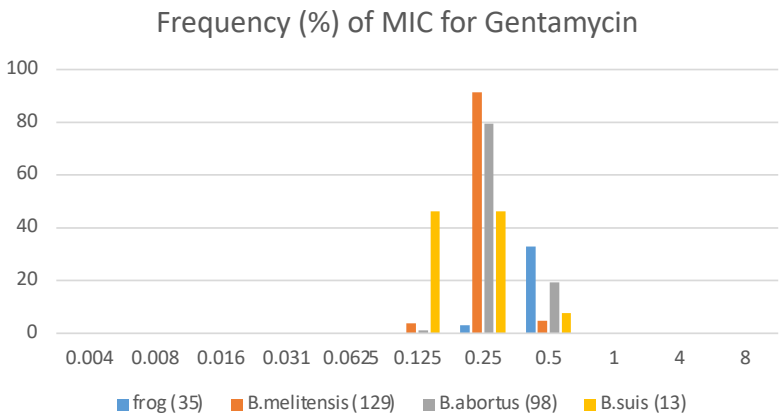


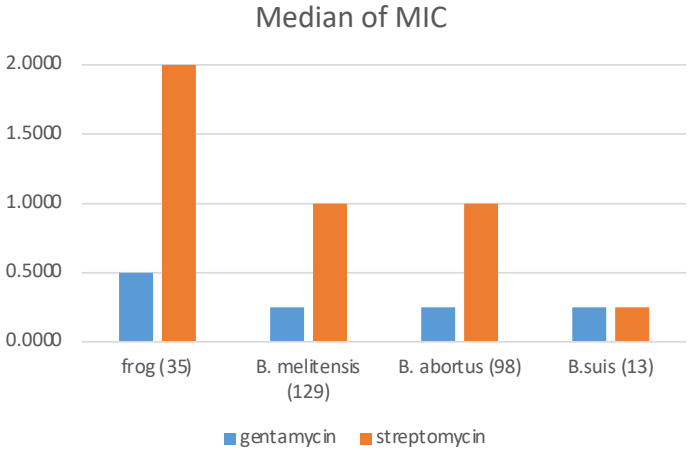
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
1	Partner	strain_id	species	biovar	host	year of isolation	C+	UFC/mL	GEN_8	GEN_4	GEN_2	GEN_1	GEN_0,5	GEN_0,25	GEN_0,125	GEN_0,0625	GEN_0,0313	GEN0,0156	GEN_0,0078	GEN_0,0039	GEN_MIC	STR_16	STR_8	STR_4	STR_2	STR_1	STR_0,5	STR_0,25	STR_0,125	STR_0,0625	STR_0,0313	STR_0,0156	STR_0,0078	STR_MIC	DOX_8	DOX_4
2	P01	REF E. coli ATCC 25922	na	na	na		1	3,00E+05	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	8	0	0
3	P01	REF E. coli ATCC 25922	na	na	na		1	4,00E+05	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	8	0	0
4	P01	REF E. coli ATCC 25922	na	na	na		1	5,50E+05	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	8	0	0
5	P01	REF Brm1 str 16M	B. melitensis	1	goat		1	9,30E+05	0	0	0	0	0	0	1	1	1	1	1	1	0,25	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0
6	P01	REF Brm1 str 16M	B. melitensis	1	goat		1	2,12E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0
7	P01	REF B.ovis 63-290	B. ovis	na	sheep		1	2,70E+05	0	0	0	0	0	0	0	1	1	1	1	1	0,125	0	0	0	0	0	0	0	1	1	1	1	1	0,25	0	0
8	P01	REF B.ovis REO 198	B.ovis	na	sheep		1	1,17E+06	0	0	0	0	0	0	0	0	1	1	1	1	0,0625	0	0	0	0	0	0	0	1	1	1	1	1	0,25	0	0
9	P01	REF B. microti CCM4915	B. microti	na	common vole		1	1,50E+06	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	4	0	0
10	P01	B. microti-like 17-2122_4144	B. microti	na	frog		1	1,10E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
11	P01	B. microti-like 18-1610_2940	B. microti	na	frog		1	1,25E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
12	P01	B. microti-like 18-1611_3417	B. microti	na	frog		1	1,17E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
13	P01	B. microti-like 18-1611_3427	B. microti	na	frog		1	1,10E+06	0	0	0	0	0	1	1	1	1	1	1	?	0,5	0	0	0	0	1	1	1	1	1	1	1	?	2	0	0
14	P01	B. microti-like 19-3808_12492	B. microti	na	frog		1	1,20E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
15	P01	B. microti-like 19-6346_12782	B. microti	na	frog		1	1,25E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
16	P01	B. microti-like 20-0469_858	B. microti	na	frog		?	8,00E+05	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
17	P01	B. microti-like 20-3683_5570	B. microti	na	frog		1	1,90E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
18	P01	B. microti-like 20-3683_5574	B. microti	na	frog		1	1,80E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
19	P01	B. microti-like 20-3683_5578	B. microti	na	frog		1	1,83E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
20	P01	B. microti-like 20-5617_8202	B. microti	na	frog		1	1,80E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
21	P01	B. microti-like 20-0469_859	B. microti	na	frog		1	1,55E+06	0	0	0	0	0	1	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1	1	2	0	0
22	P01	Ochrobactrum 21-3758	Ochrobactrum	na			1	2,00E+05	0	0	0	0	0	0	1	1	1	1	1	1	0,25	0	0	0	0	1	1	1	1	1	1	1	1	16	0	0
23	P01	Ochrobactrum 20-5087/G61	Ochrobactrum	na	frog		1	4,40E+05	0	0	0	0	0	0	0	1	1	1	1	1	0,125	0	1	1	1	1	1	1	1	1	1	1	1	16	0	0
24	P10	09RB8471	Brucella like		frog	2009	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,25	0	0	0	0	1	1	1	1	1	1	1		2	0	0
25	P10	13RB5064	Brucella like		frog	2013	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
26	P10	14RB5420	Brucella like		frog	2014	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
27	P10	18RB16866	Brucella like		frog	2018	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
28	P10	20RB21708	Brucella like		frog	2020	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
29	P10	20RB22556	Brucella like		frog	2020	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
30	P10	20RB22606	Brucella like		frog	2020	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
31	P10	10RB9205	Brucella like		frog	2010	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
32	P10	10RB9206	Brucella like		frog	2010	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
33	P10	10RB9207	Brucella like		frog	2010	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
34	P10	10RB9208	Brucella like		frog	2010	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
35	P10	10RB9209	Brucella like		frog	2010	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
36	P10	10RB9209	rucella like second		frog	2010	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
37	P10	10RB9210	Brucella like		frog	2010	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
38	P10	10RB9211	Brucella like		frog	2010	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0
39	P10	10RB9212	Brucella like		frog	2010	1	100-200	0	0	0	0	0	0	1	1	1	1	1	1	0,5	0	0	0	0	1	1	1	1	1	1	1		2	0	0

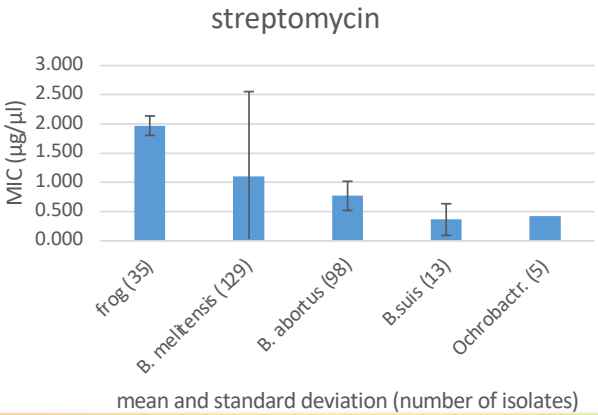
Obtained results

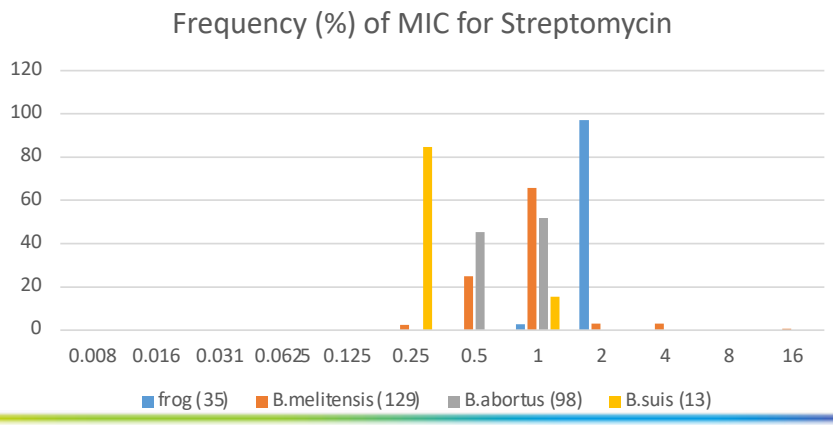
Task 2:





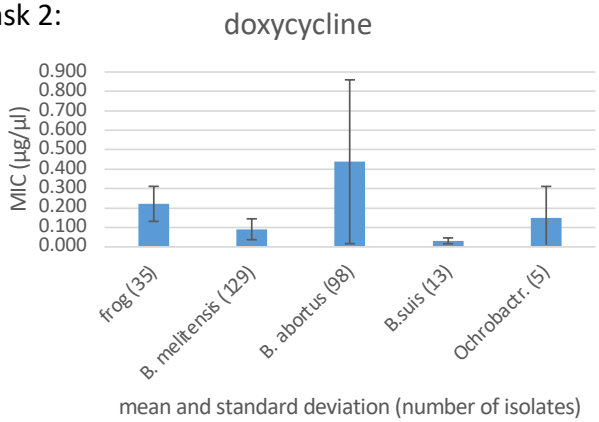


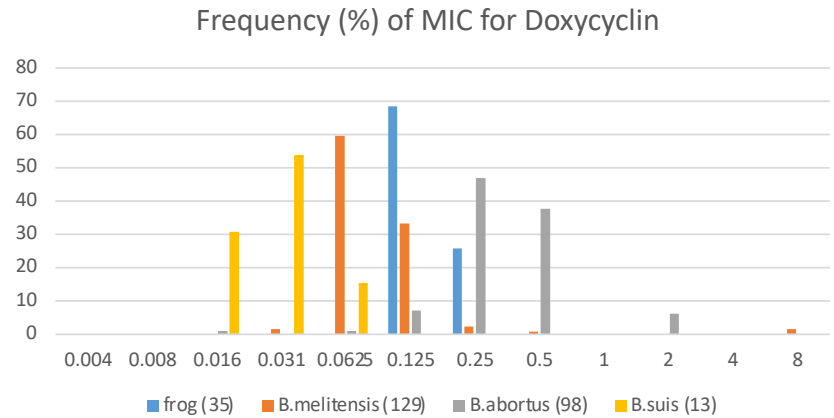


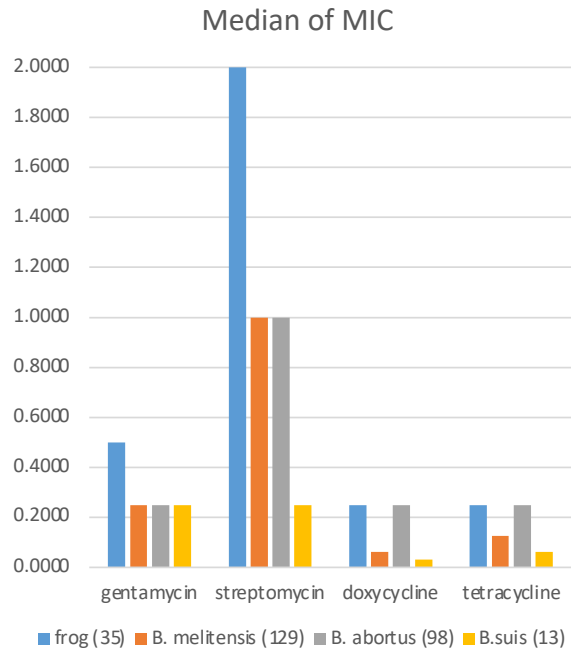


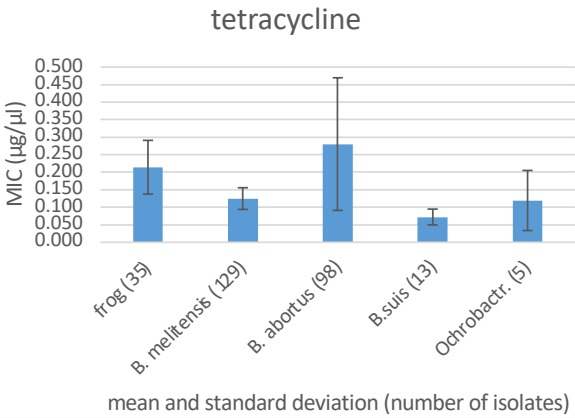
Obtained results

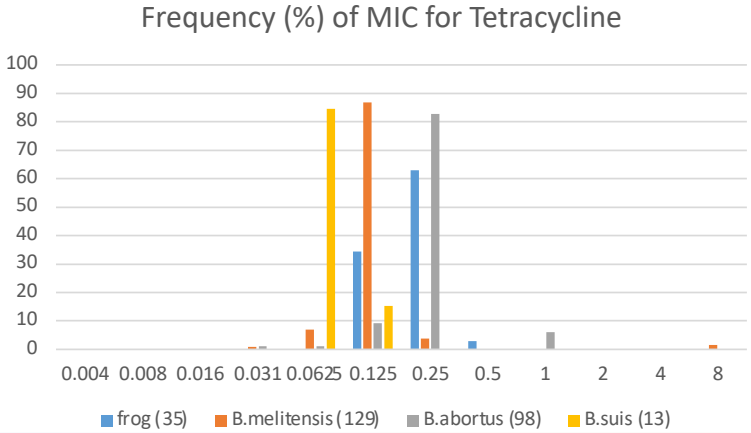
Task 2:











Obtained results

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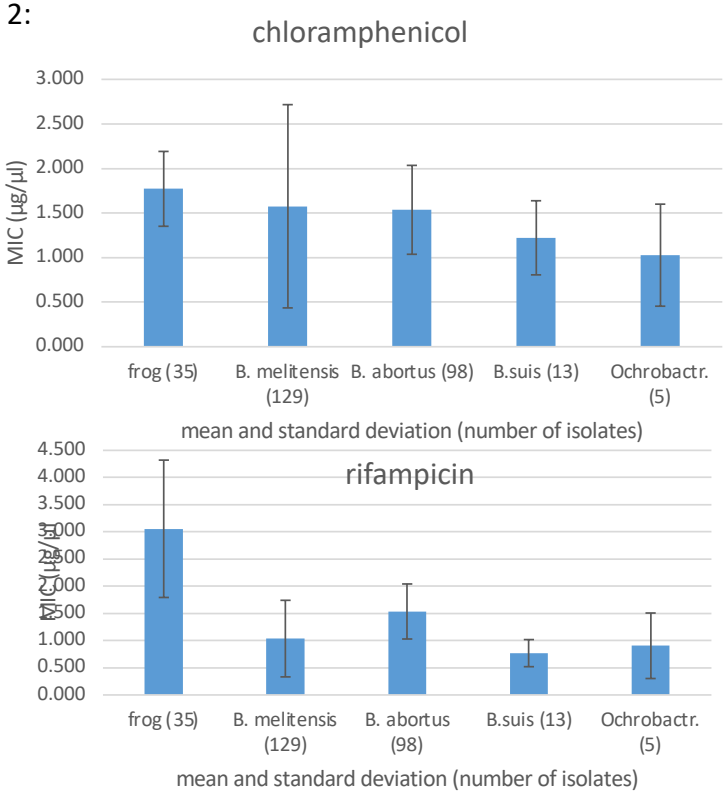

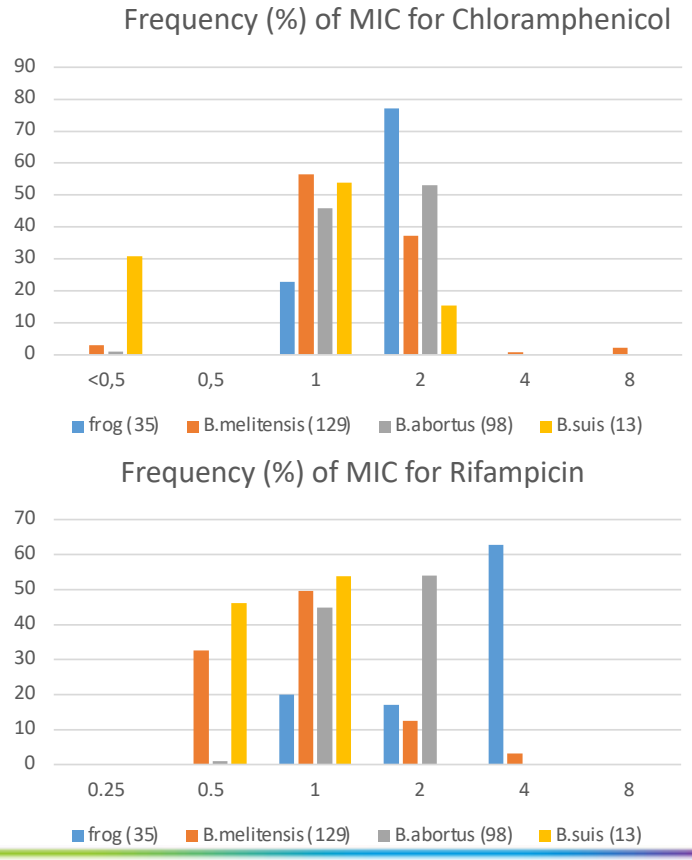

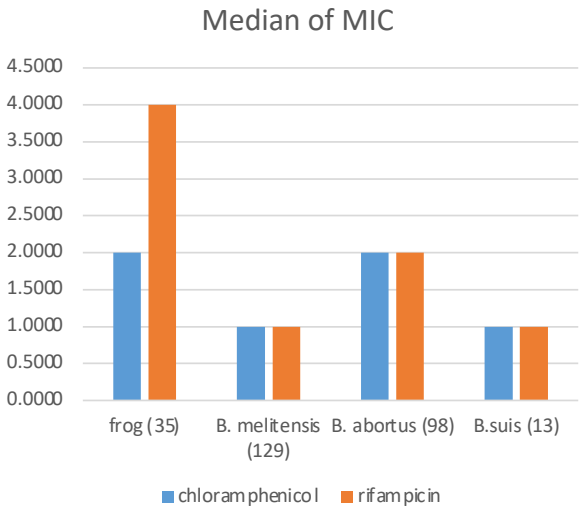

Obtained results

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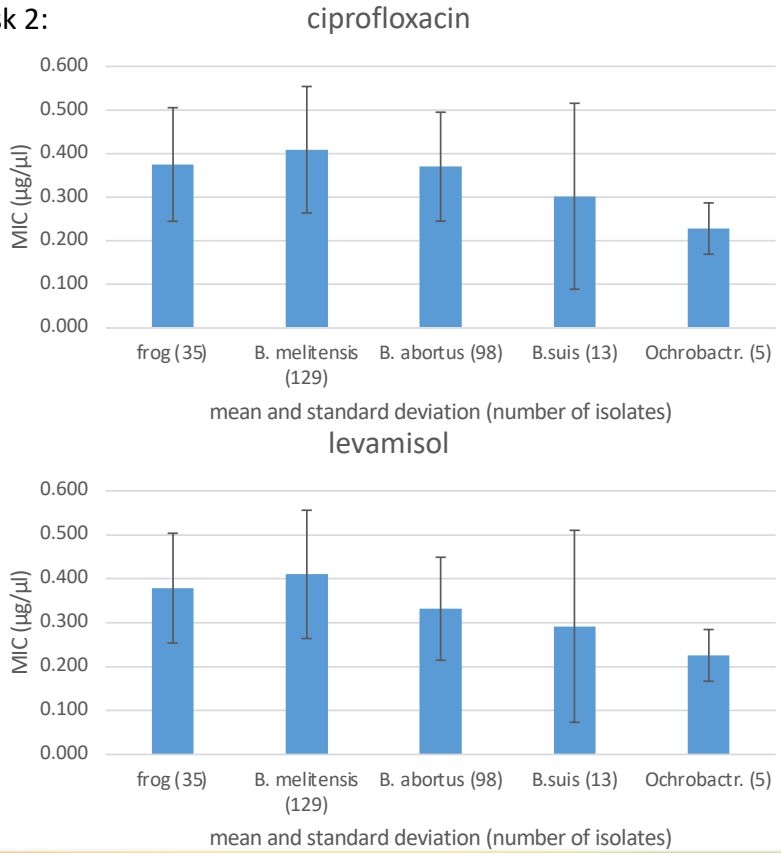

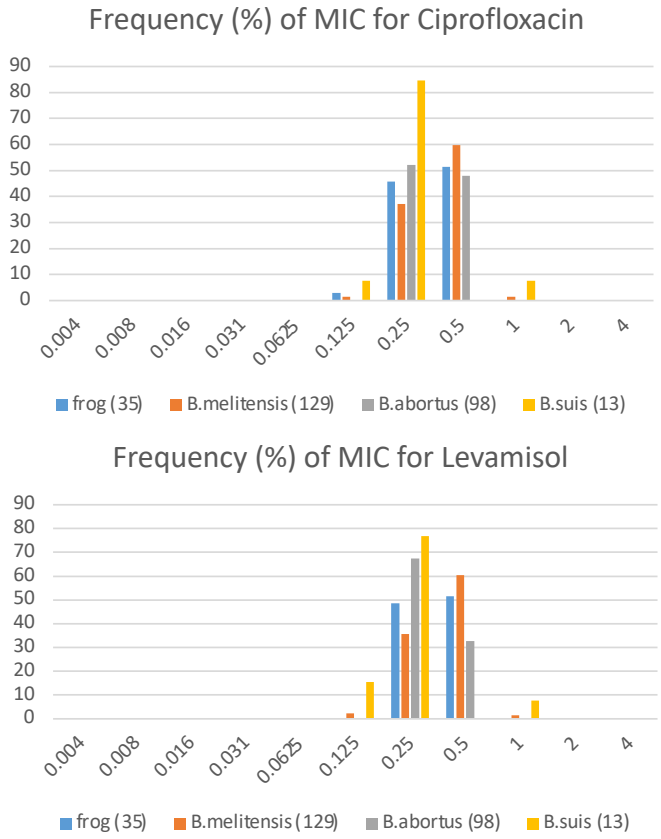

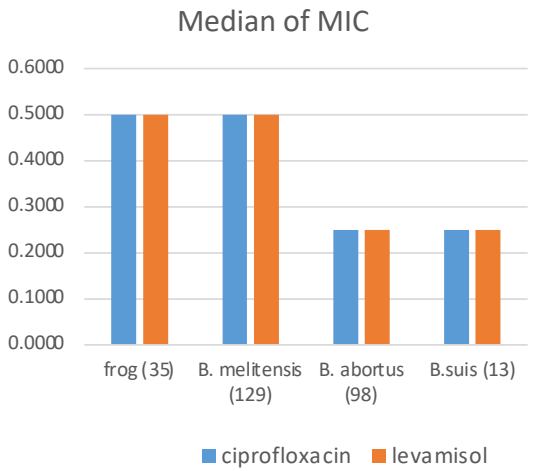

Obtained results

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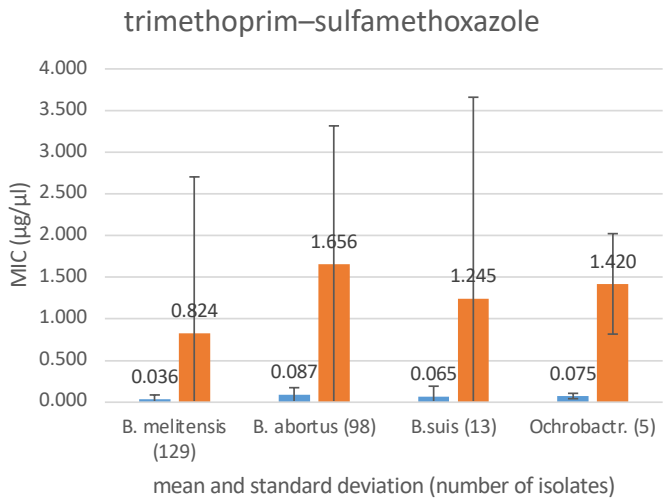
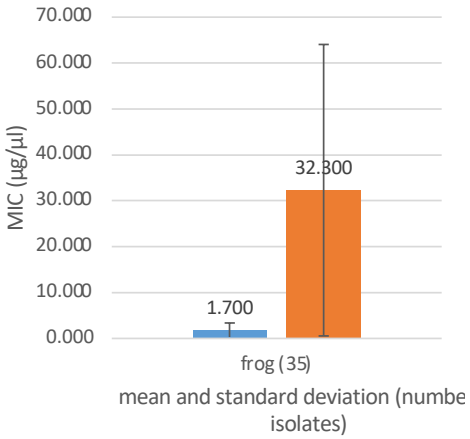
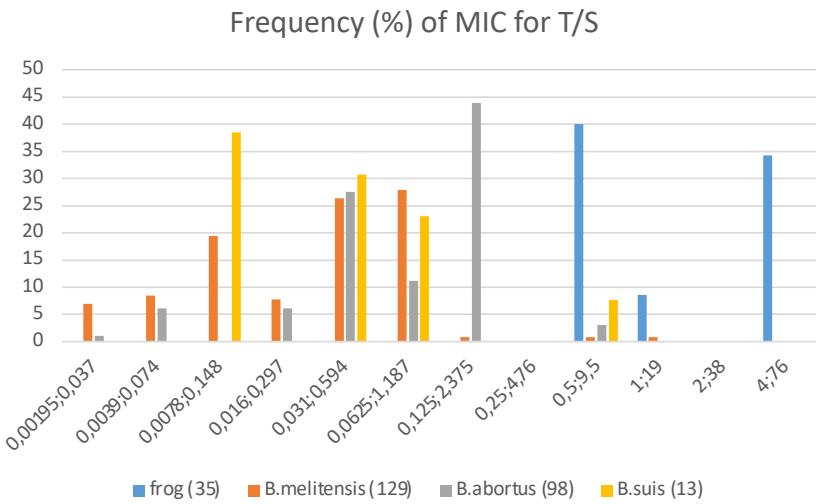
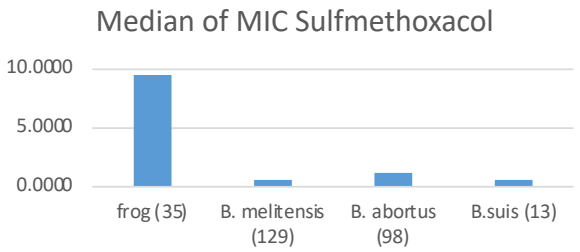






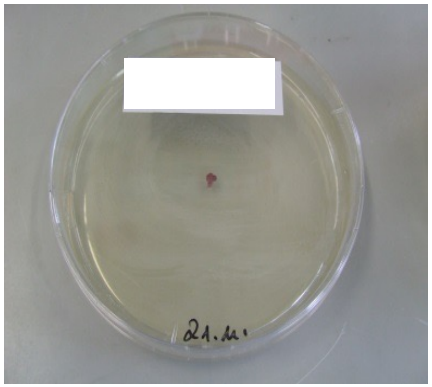


Principal results for WP6 - Toolkit

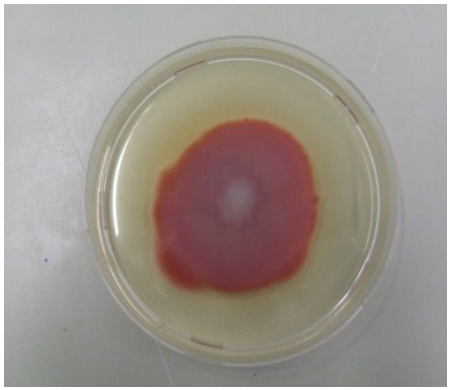
Principal results for WP6 - Toolkit

- Result 1: Motility seems to be a typical property of the novel emerging brucellae (so far from frogs all isolates were motile)

motility *B.melitensis* after 72 h



motility new emerging Brucella after 48 h



Principal results for WP6 - Toolkit

- Result 2: differences in AMR between novel emerging brucellae and classical strains (trimethoprim/sulfamethoxazole, rifampicin, gentamycin, streptomycin)

but: -more new emerging brucella isolates are necessary to do a good statistical analysis
-results based on two different labs so far

What has to be done

- Final evaluation of the data
- Electron microscopy (delays, as vibrations had been occurring for a year due to a major construction site and last two months S3-lab was closed)
- Phenotyping scheme to differentiate emerging Brucella sp. from classical species (based on motility and AMR)
- AMR testing protocol for phenotyping
- Based on the small number of new emerging Brucella we can not prepare proposals for ECOFFs

For final toolkit

- Motility examination based on a specific agar (protocol will be delivered)
- AMR testing protocol for phenotyping (Mikronaut plate for Gram negative bacteria could be used)
- Based on the small number of new emerging Brucella we can not prepare proposals for ECOFFs

Sascha

- Substrates like Rhamnose
- MALDI specific biomarkers for non core clade vs. core clade Brucella
- RNA sequencing (problems)

Ana

WP4 Phenotyping

What is the starting point?

- Isolate with suspicion of beeing Brucella spp.

Which methods to do first identification and differentiation?

- Brucella-PCR, Ladder-PCR, Ochrobactrum-PCR (may be could be implemented in the Ladder)
- MALDI for PCR positives
- For MALDI positives: Classical microbiology just for Brucella spp. + motility + Anti-M/A/R + Aggl. with positive reference serum
- AMR-testing using Mikronaut or any other system for Gram neg. bacteria
- Specific substrates utilisation like Rhamnose

If results positive for new emerging Brucella, more especially genotyping should be done.
If reasonable investigation should be started (environment, other animals).

Non core *Brucellae* – Learn basic knowledge about the suspected organism

- Adrian to write up what non core *Brucellae* are

- Review papers on atypical *Brucellae* - Adrian

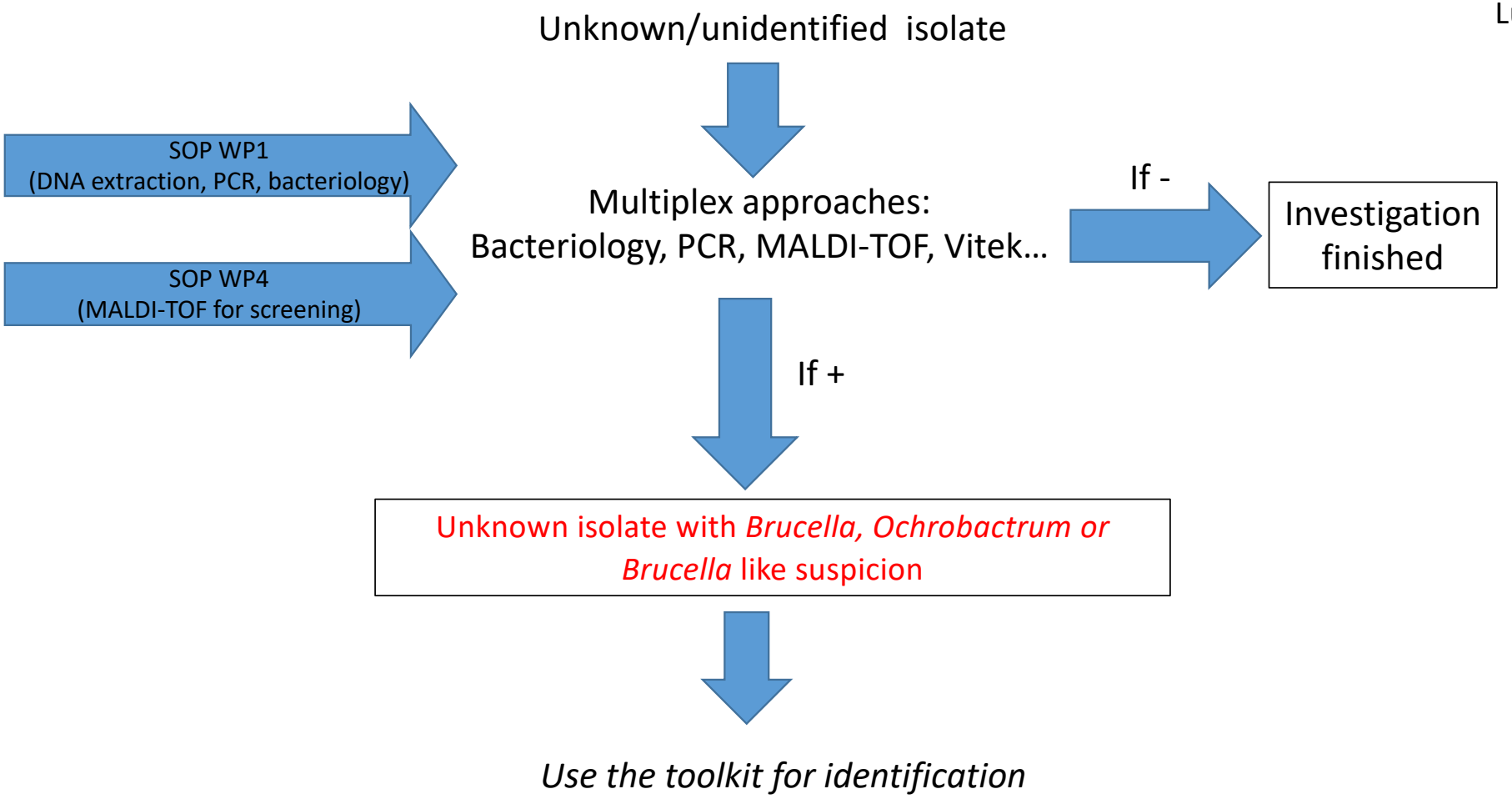
- Examples the animal, human and environment cases of atypical *Brucella* infections - Adrian

Non core *Brucellae* – Organise the data collection with tools

- List of metadata to be added from this project
- Data base example
- Epidemiological questionnaire
- PADI Web:
  - Vitomir will communicate list of words used in English and French.
  - XX, XX, XXX
  -
  - Each partner should provide the list of key words in their respective language for Google news search.

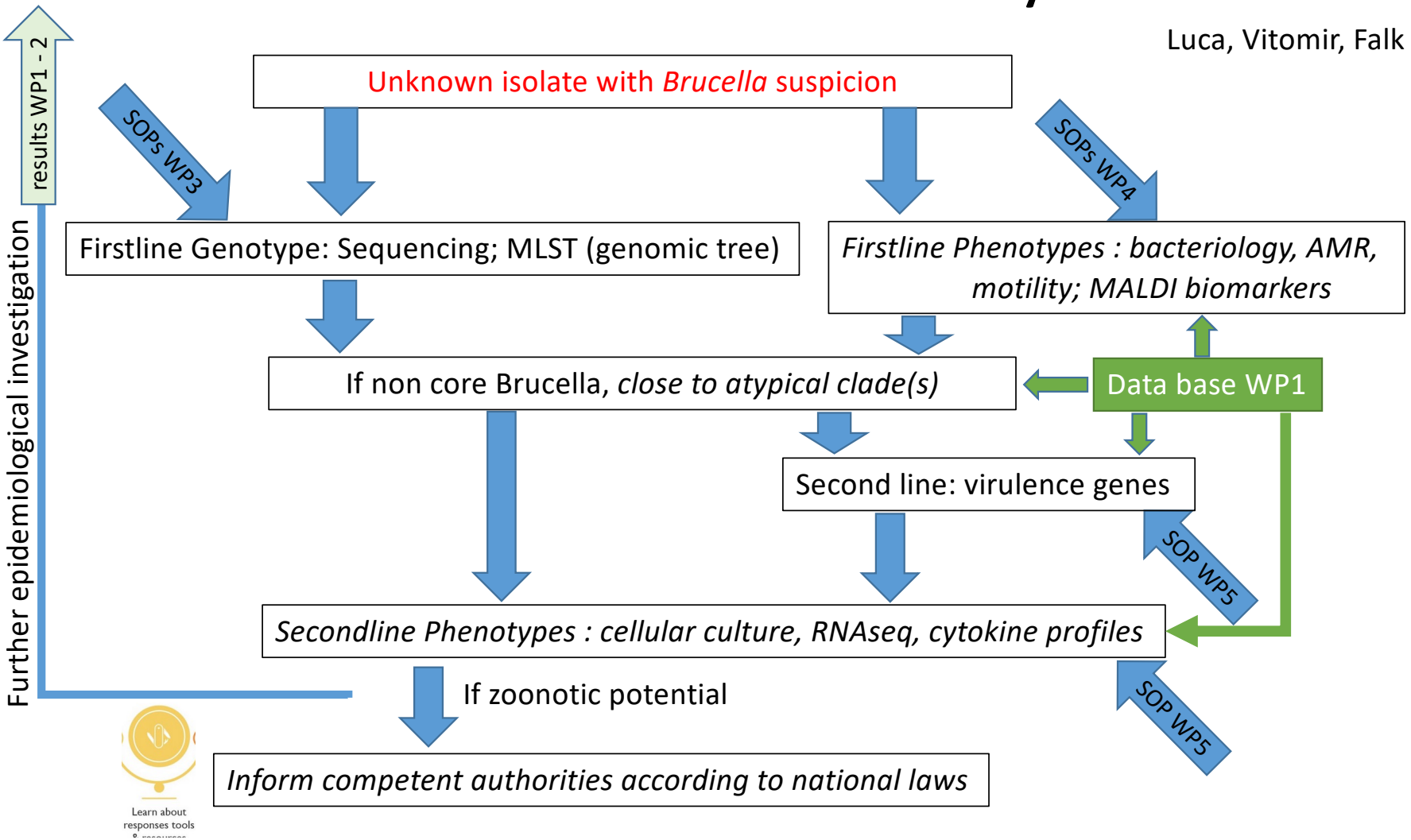
Non core *Brucellae* – **Diagnostic laboratory : SOPs, Flowchart**

Luca, Vitomir



Non core *Brucellae* – **Confirm the identification with laboratory confirmation**

Luca, Vitomir, Falk



Brucella canis - Learn basic knowledge about the suspected organism

Claire – write short introduction

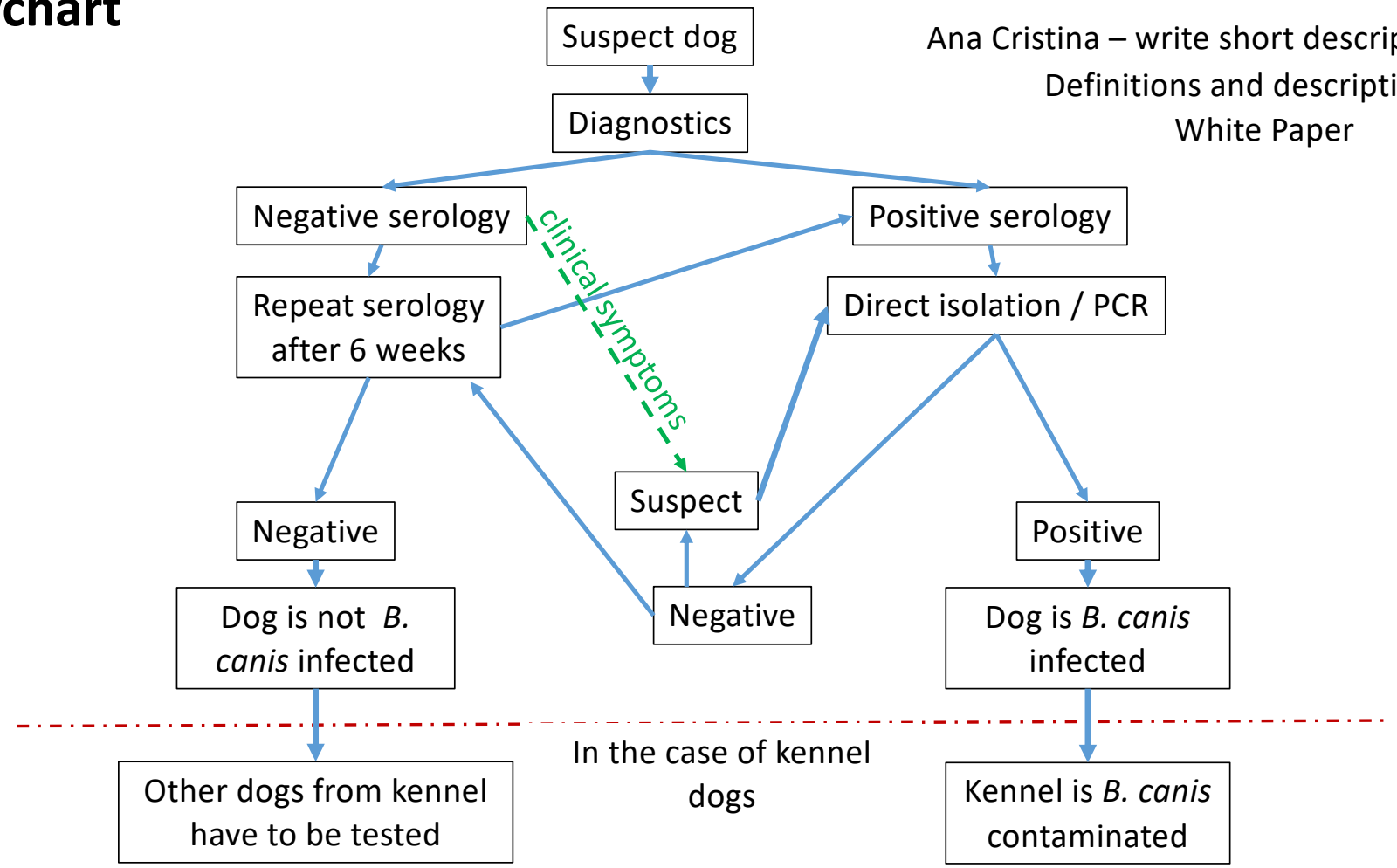
- White Paper
- HAIRS report
- Fact sheets from APHA
- ANSES *B. canis* fact sheet
- IZSAM article review on *B. canis*
- List of literature review - Claire
- EFSA EREN note *on B. canis*

Brucella canis – Develop the case definition

- White paper - Vitomir

Brucella canis – Confirm the outbreak with laboratory confirmation **SOPs, Diagnostics Flowchart**

Ana Cristina – write short description
Definitions and descriptions in White Paper



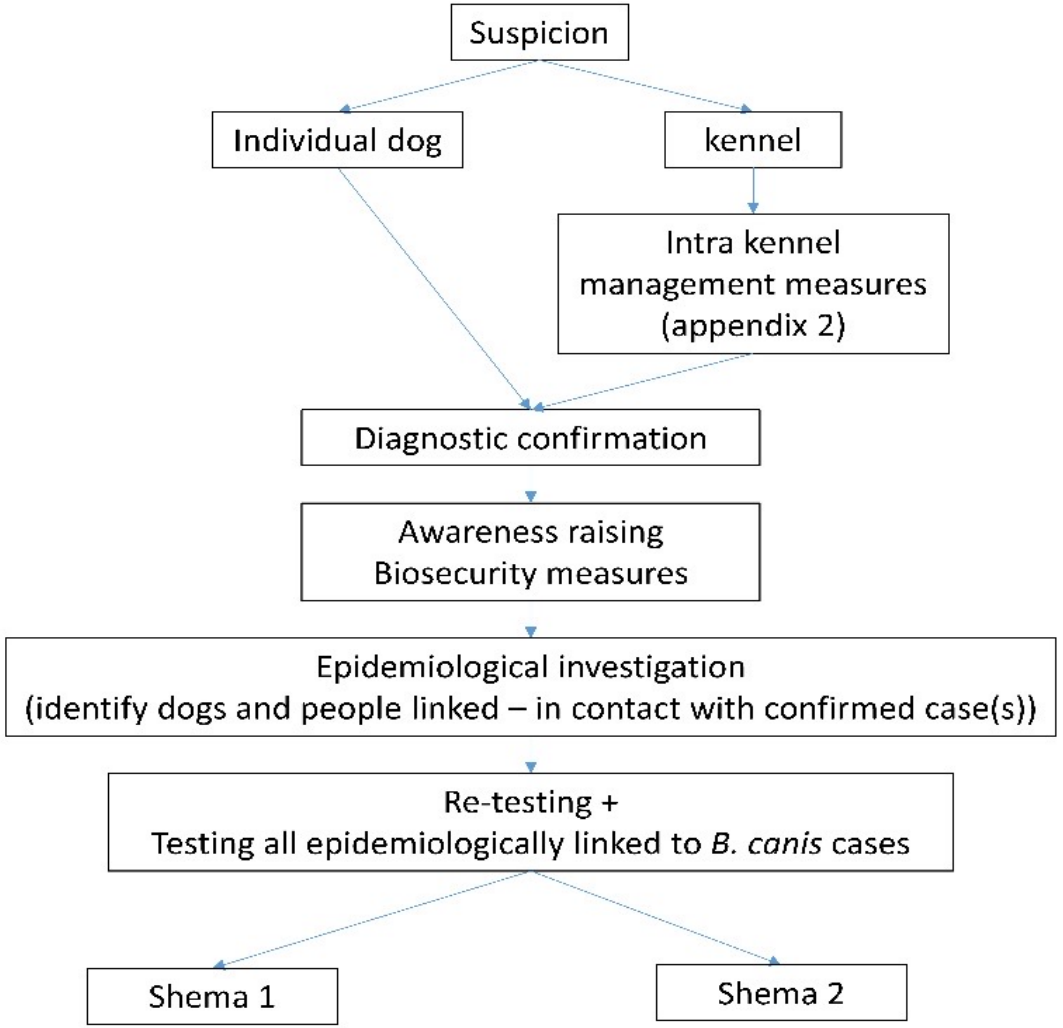
Brucella canis – Organise the data collection with tools

- Standardized data collection form – Claire and Fabrizio.
- PADI Web
  - Keywords : XX, XX

Brucella canis – Learn about responses tools & resources – outbreak management

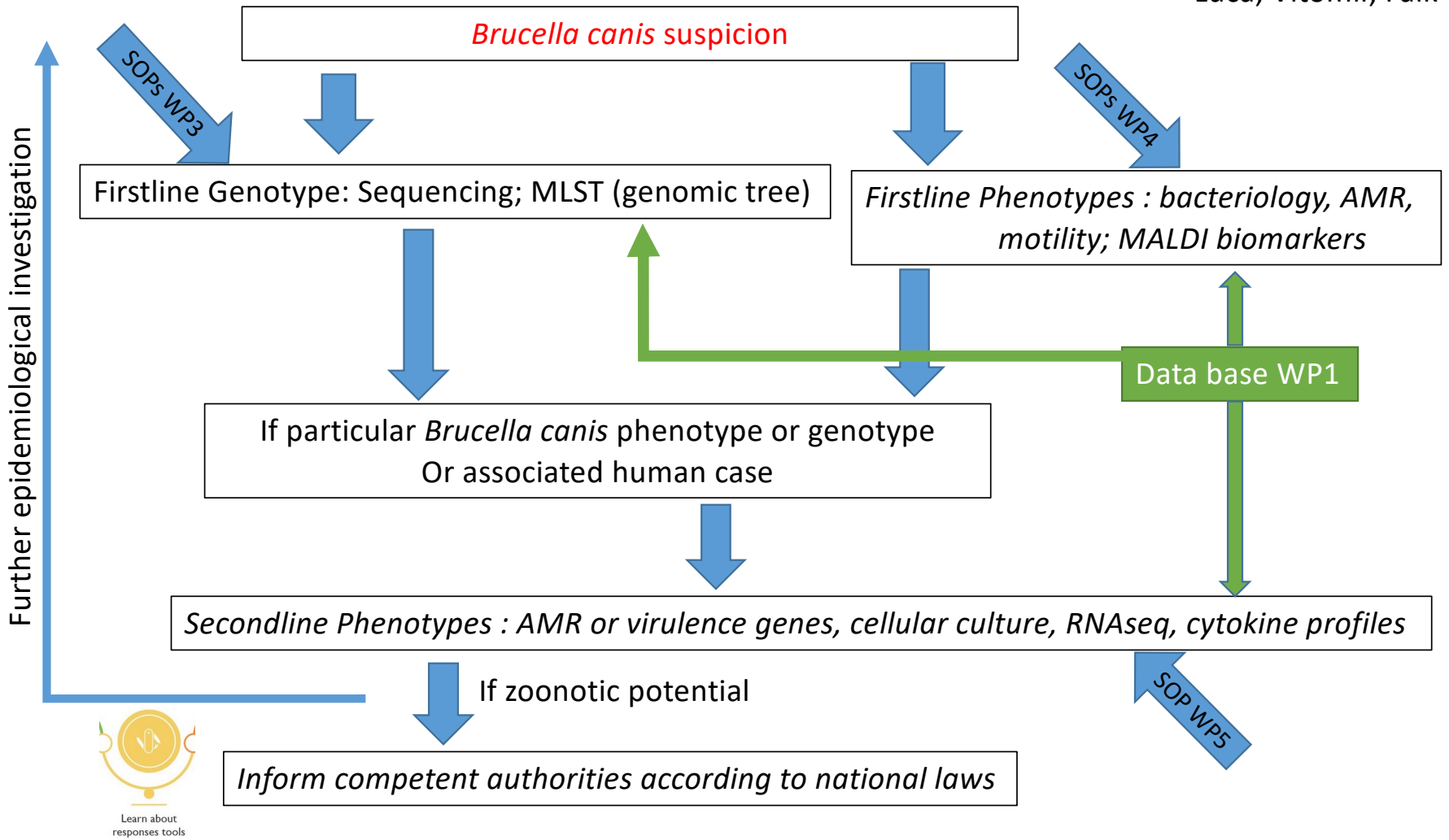
- White paper

Ana Cristina – write short description

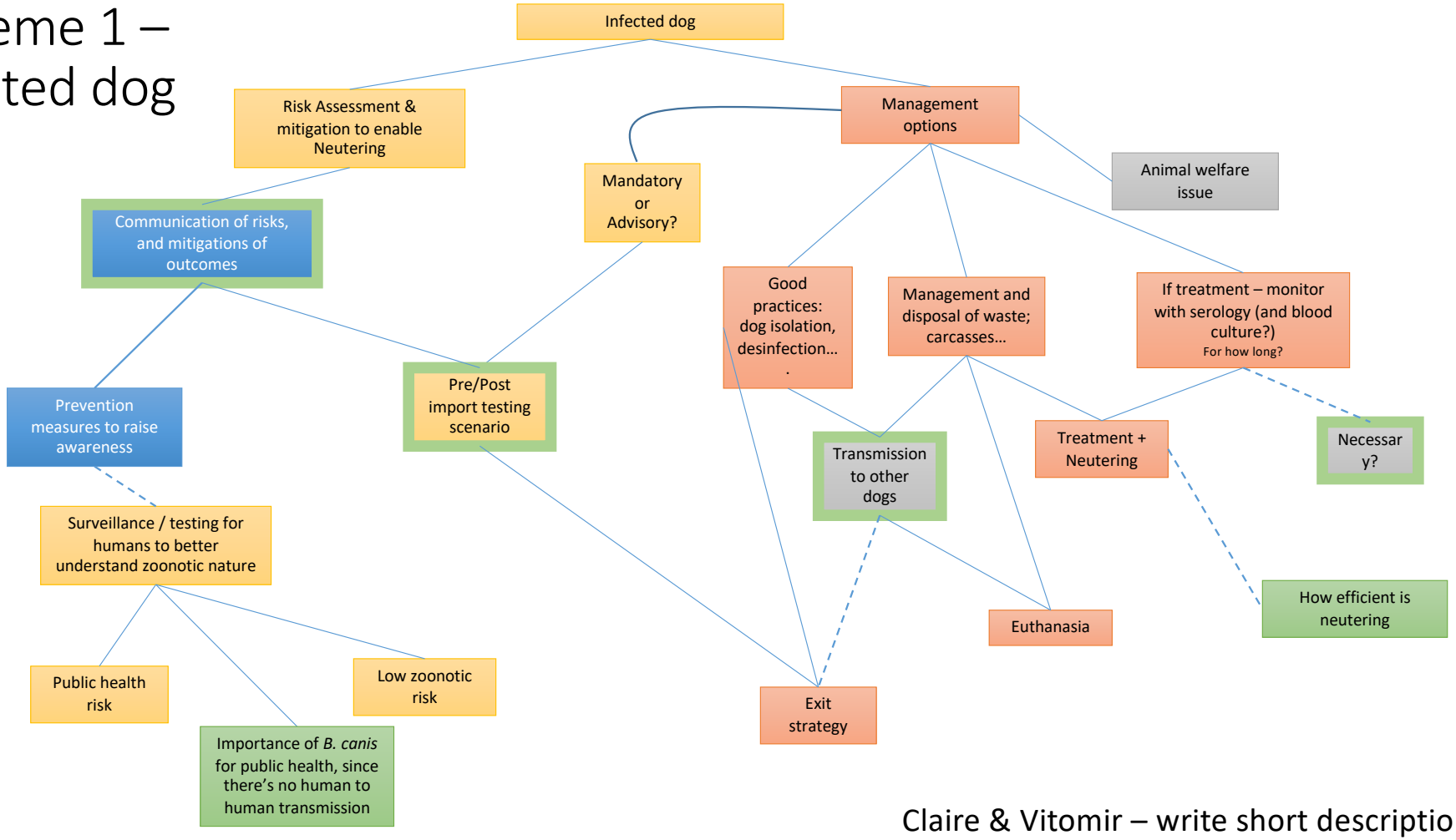


Brucella canis – Confirm the outbreak with laboratory confirmation

Luca, Vitomir, Falk

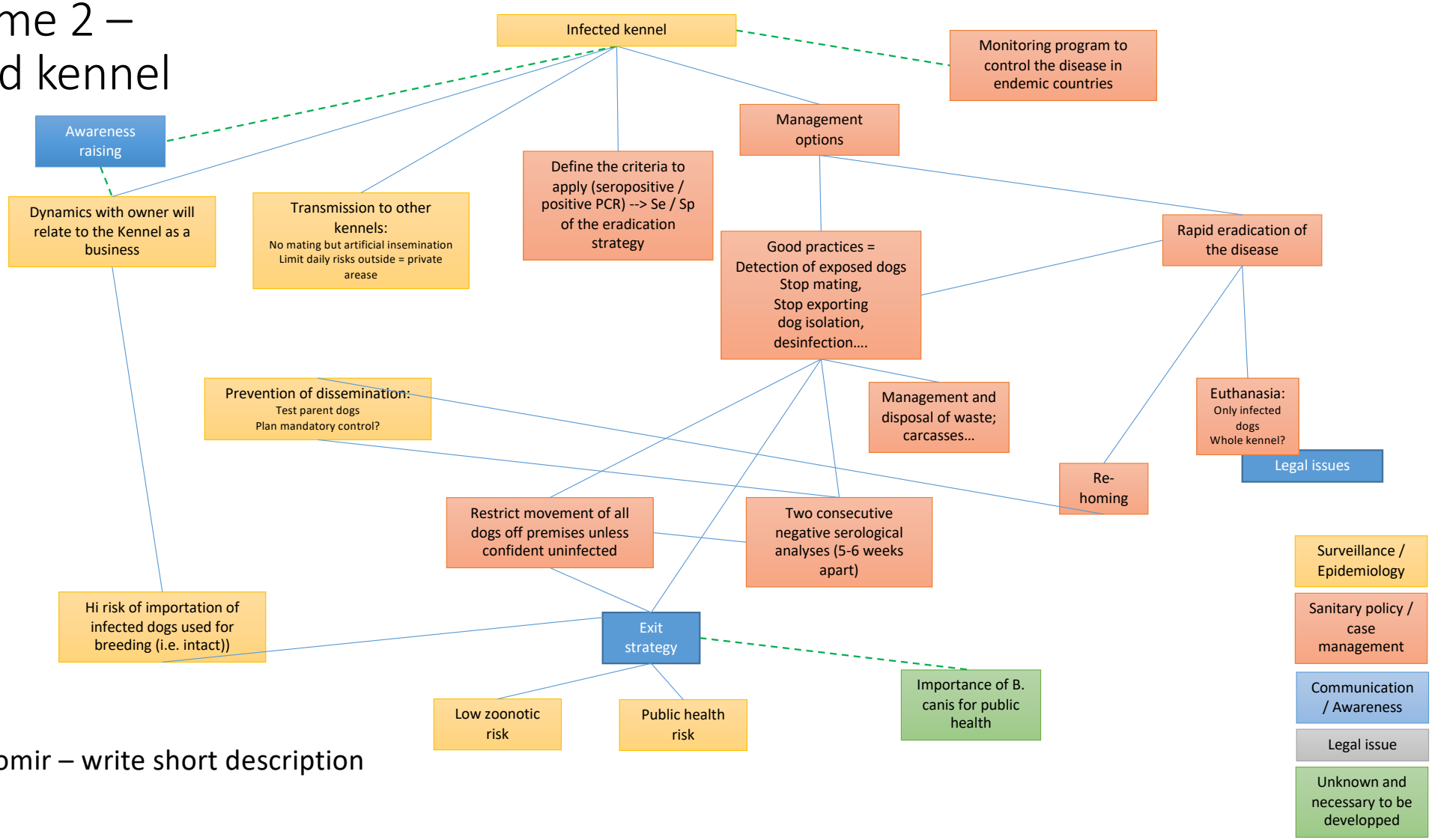


Scheme 1 – Isolated dog



Claire & Vitomir – write short description

Scheme 2 – Infected kennel



Claire & Vitomir – write short description

B. canis - Watch online training

- Notes
- EFSA presentations - Claire
- Training sessions – France (French, English), UK
- Falk to translate to German;
- Ana Cristina to translate to Portugeese
- Fabrizio to Italian
- Liliia to Russian and Ukranian

Brucella in non prefered host – Learn basic knowledge about the suspected organism

- Short introduction

- Review papers on Brucellae in non prefered host / wildlife – Claire

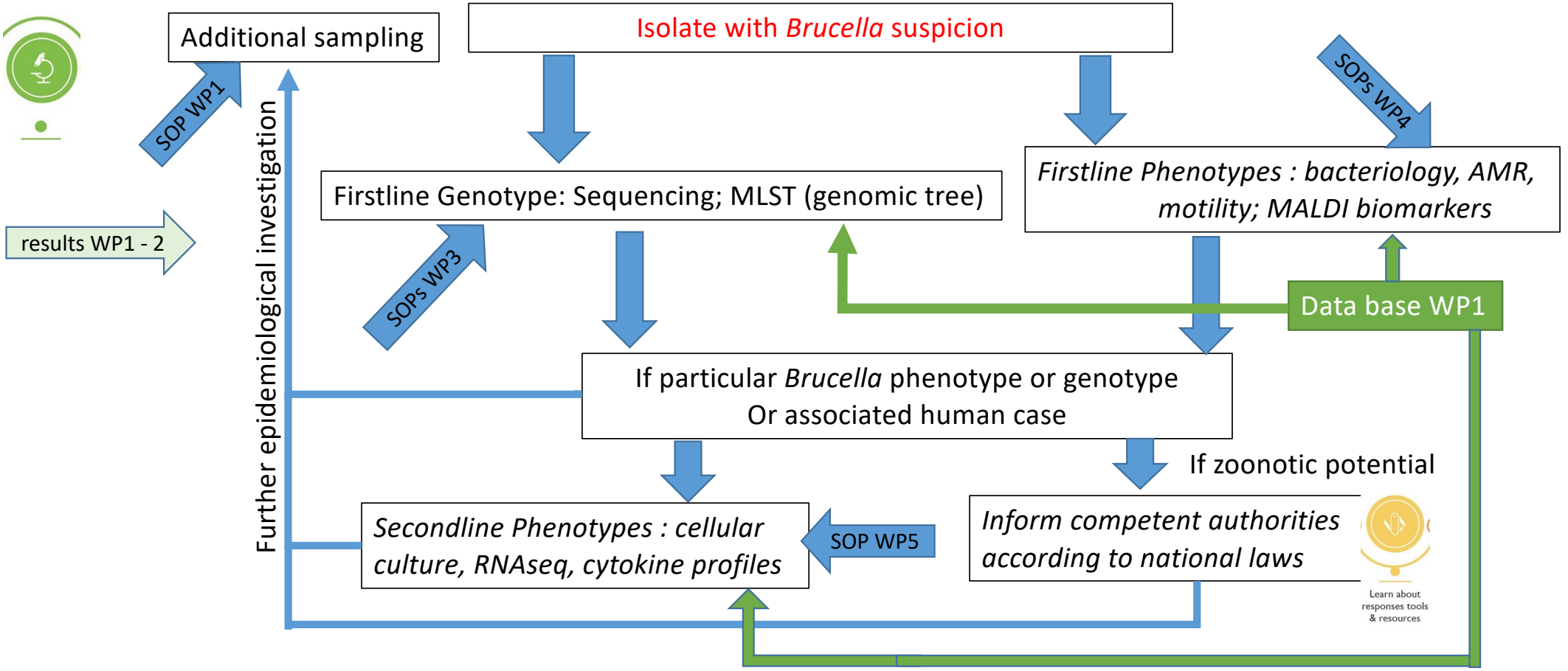
- Examples: B. suis biovar 2 in cattle (Belgique, France, Portugal, Cristina), B. melitensis in ibex

Brucella in non prefered host – Organise the data collection with tools

- List of metadata to be added from this project
- Data base example
- Epidemiological questionnaire

Brucella in non prefered host – **Confirm the identification with laboratory confirmation**

Claire and Vitomir



Padi-web EXAMPLES OF RECENT EPIDEMIOLOGICAL RESEARCH

Elena Arsevska, Cirad UMR ASTRE, France

Veterinary epidemiologist

Examples of Padi-web epi research

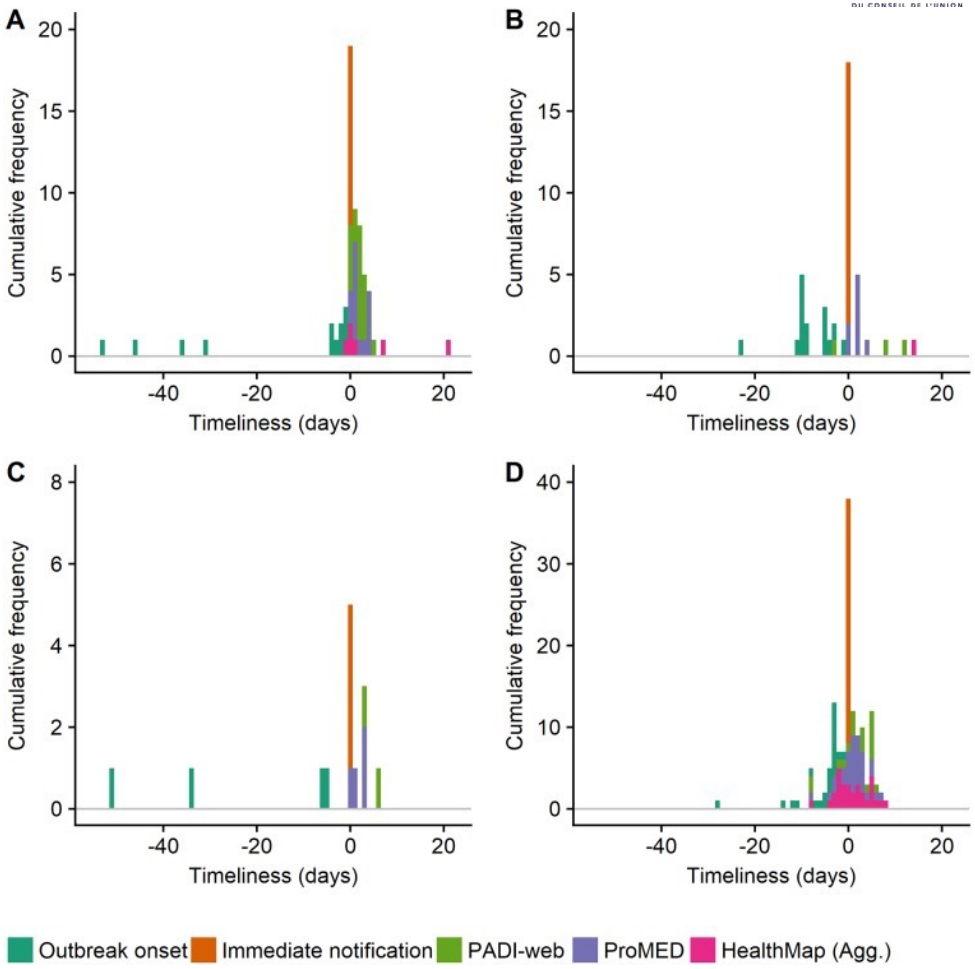
Timeliness in outbreak detection

A. African swine fever (ASF), B. Foot-and-mouth disease (FMD), C. Bluetongue (BTV), D. Avian influenza (AI)

Lag in days from the onset of a primary outbreak, its immediate notification to the OIE and its detection by PADI-web, ProMED, and HealthMap from January to June 2016

The figures show the range of outbreak detection from 55 days before to 25 days after immediate notification (day 0)

PADI-web timely detected primary outbreaks of AI and FMD disease in Asia, i.e. they were detected 8 and 3 days before immediate notification to OIE, respectively



Arsevska, et al. 2018. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0199960

Examples of Padi-web epi research

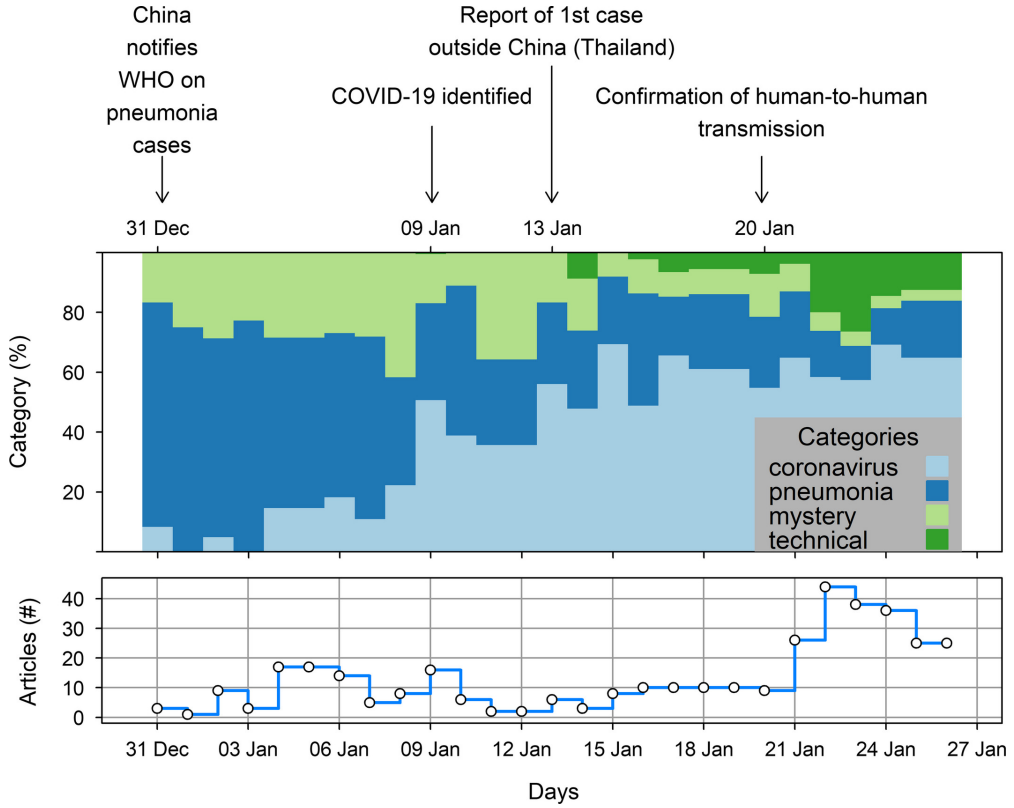
Vocabulary evolution in news media

Detection of early signals for COVID-19 emergence and change of vocabulary of disease description in media during the evolution of the pandemics

At the early stage of the pandemics, the specific vocabulary used was related to ‘pneumonia symptoms’ and ‘mystery illness’

Once COVID-19 was identified, the vocabulary changed to virus family and specific COVID-19 acronyms

Recommandations/usefulness for syndromic surveillance of disease-x



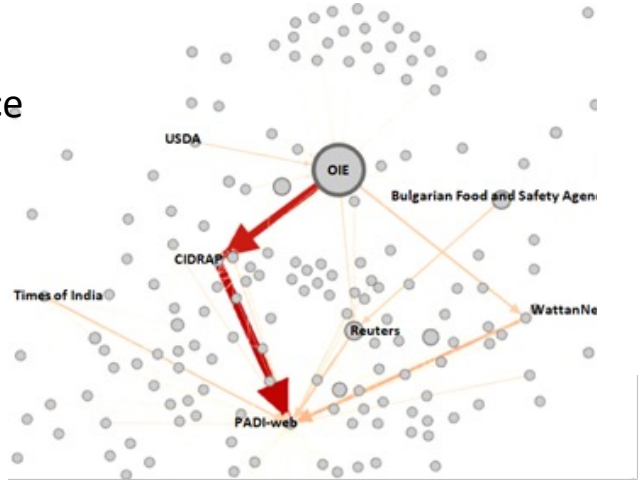
Valentin, et al. 2020. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0199960

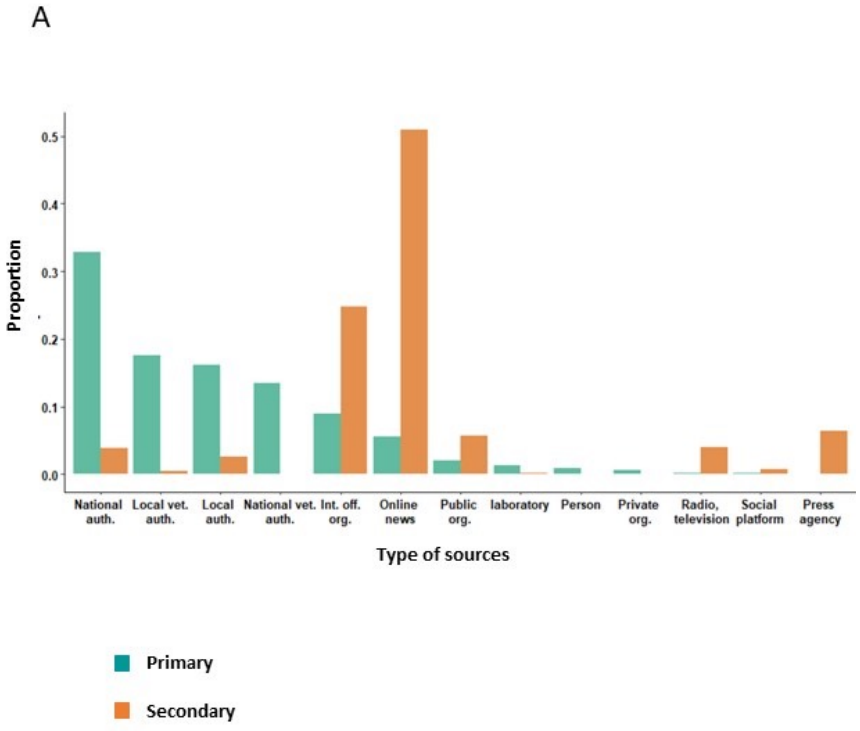
Examples of Padi-web epi research

Typology of sources in digital disease surveillance for Highly Pathogenic Avian Influenza (HPAI)

How outbreak-related information disseminates from a primary source (transmitter) to a definitive source (an EBS tool) during a 1y period of HPAI emergence, 2018-2019

Helpful to set priority sources to improve digital disease surveillance





[Valentin, et al. 2022 ongoing work]

MOOD: RESEARCH AND INNOVATION IN EPIDEMIC INTELLIGENCE

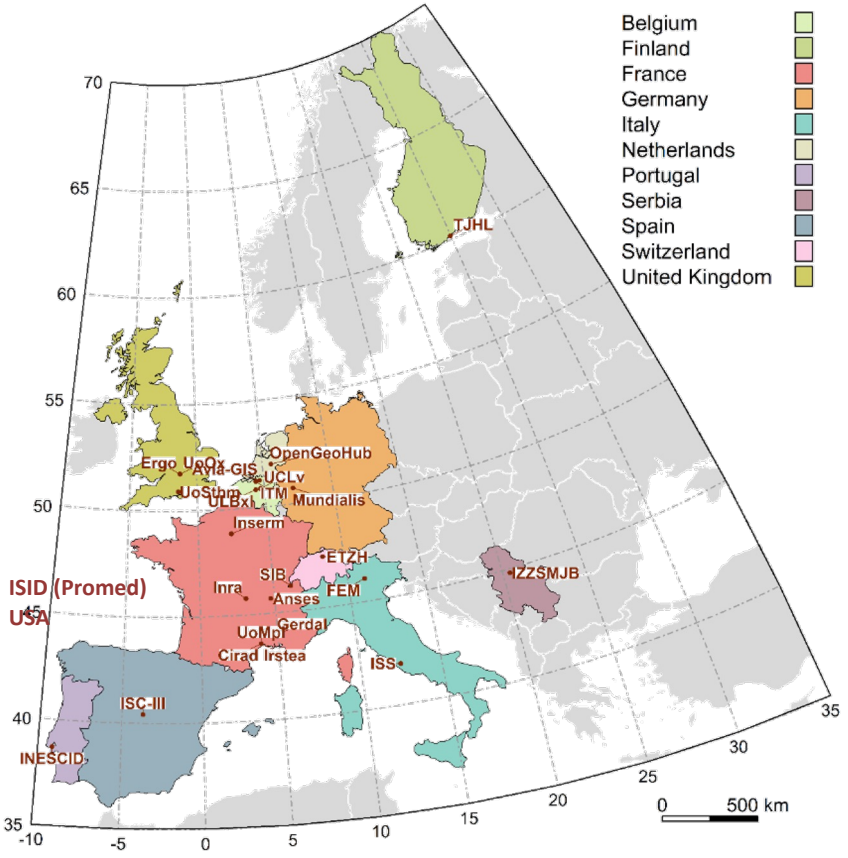
Elena Arsevska, Cirad UMR ASTRE , France

H2020 MOOD project coordinator

H2020 Project MOOD

MONITORING OUTBREAK EVENTS FOR DISEASE SURVEILLANCE IN A DATA SCIENCE CONTEXT

- Horizon 2020 (2020-2023)
- Research and innovation, health
- 14 ME, 25 partners, 13 countries
- Public and veterinary health agencies from 5 European countries
  - France
  - Italy
  - Spain
  - Serbia
  - Finland



H2020 Project MOOD

OBJECTIVES

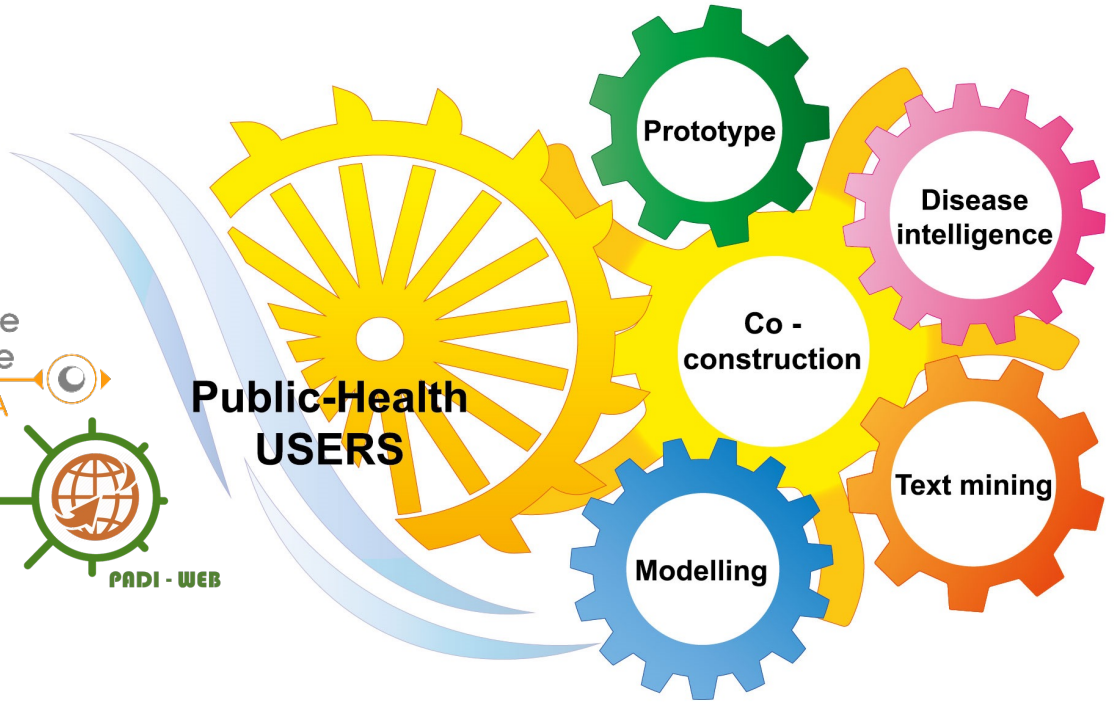
Develop innovative tools and services for the early detection, assessment, and monitoring of infectious disease emergence from Big Data in a “One Health” context

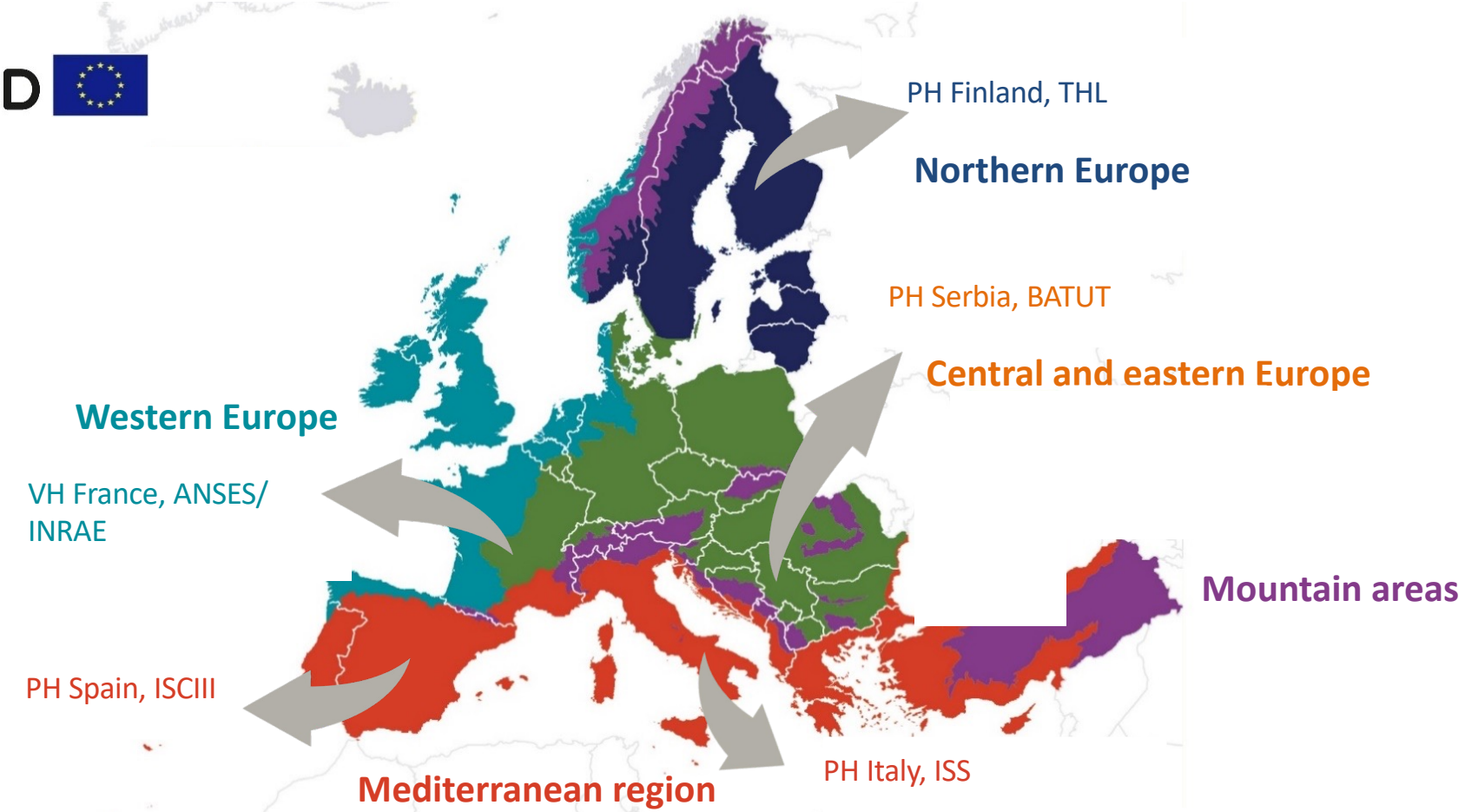
APPROACH

Co-conception of tools and services based on end-user needs: national and supra-national human and veterinary public-health agencies

Veille Sanitaire Internationale
Plateforme ESA

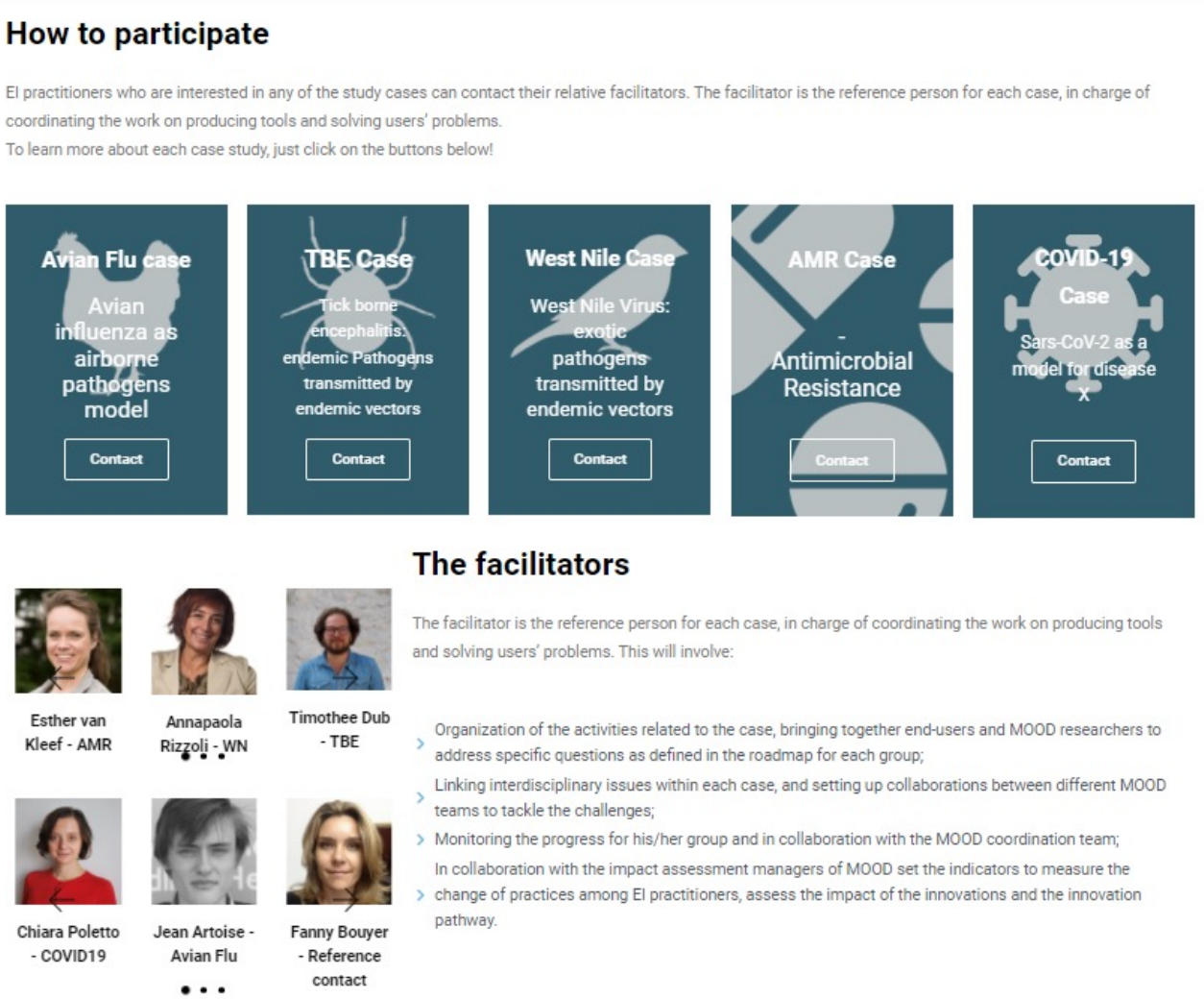
PADI - WEB



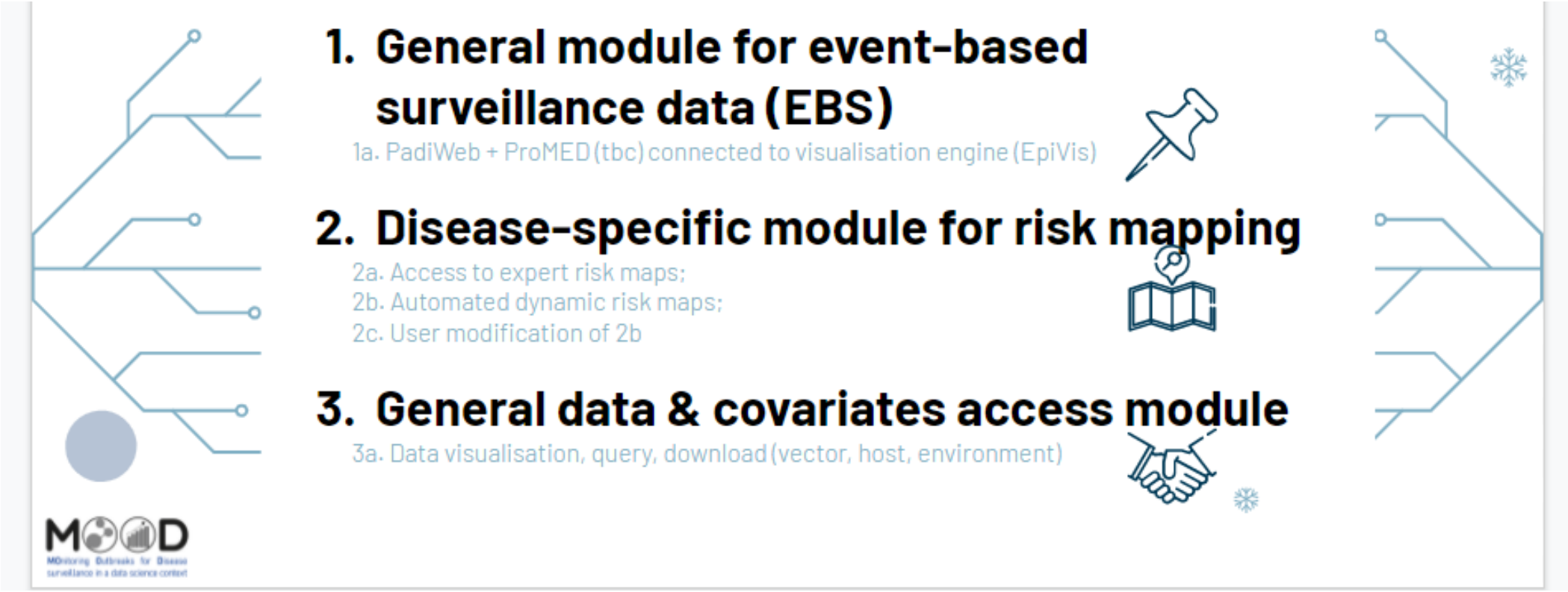


MOOD end-users at PH/VH agencies in Europe

MOOD disease case studies and facilitators



MOOD platform with tools and modules for PH/VH agencies



Padi-web developments in MOOD

- **New functionalities of visualisation and monitoring of a disease X** in Padi-web – user groups co-conception session (you are welcome to join)
- **Improved news detection and infrmation extraction of Padi-web** – disease expert groups (you are welcome to contribute)
- **Risk mapping using media data**
- **Impact evaluation** of innovations in epidemic intelligence systems and One health collaborations
- **Training** for the use of PADI-web

Follow our activities

H2020 MOOD project
Coordination Cirad, UMR ASTRE
mood-coordination@cirad.fr

Web site: https://mood-h2020.eu/

MOOD (H2020) - twitter

MOOD webinars & MOOD newsletter subscribe here: https://mood-h2020.eu/newsletter/

PADI-web tool for web disease monitoring
padi-web@cirad.fr