

Brucella, a bacterial genus in expansion: new species, new reservoirs



秦万里

Claire Ponsart

Luca Freddi, Guillaume Girault, Acacia Ferreira Vicente, Vitomir Djokic

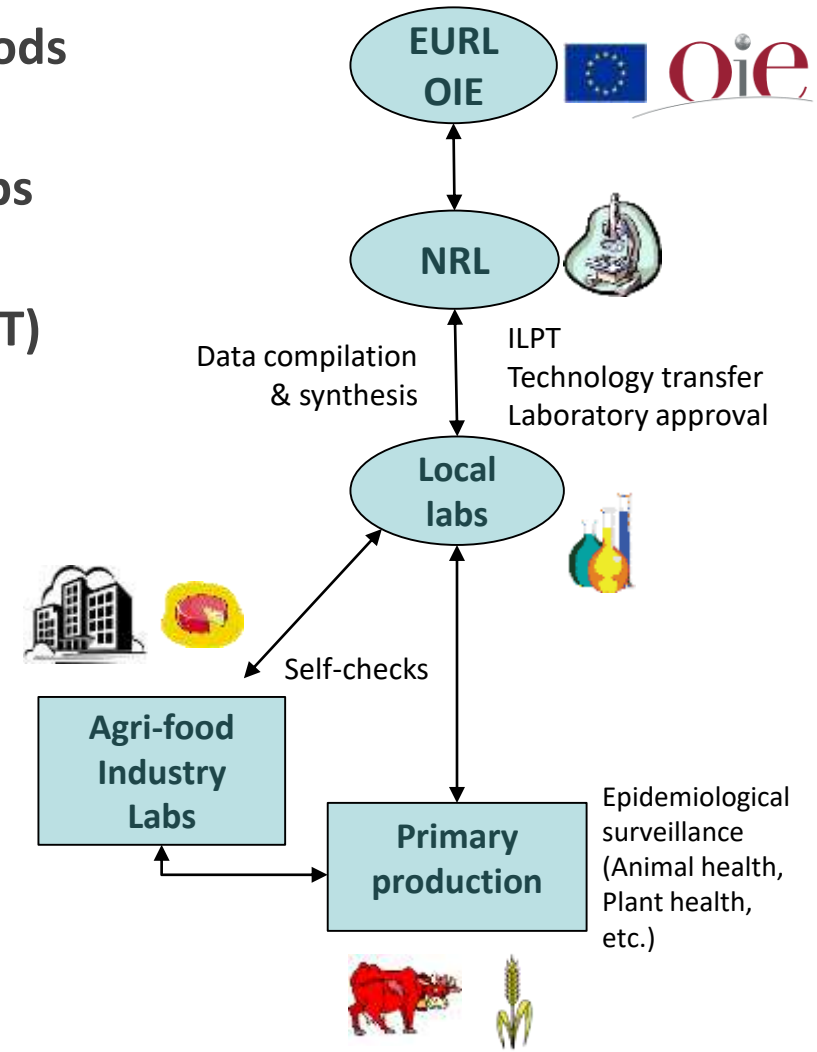
*Anses/Paris-Est University, EU/OIE/FAO & National Reference Laboratory for Brucellosis, Animal Health Laboratory,
Maisons-Alfort, France*

☐ ANSES / Animal Health Laboratory / Bacterial Zoonoses Unit (Maisons-Alfort)

- ✓ Development and validation of methods
- ✓ Networking and training of official labs
- ✓ Inter-laboratory proficiency tests (ILPT)
- ✓ Provide scientific & technical support
- ✓ Maintain a scientific watch



A fixed framework for the reliability of official controls



Diagnosis/Confirmation

Bacteriology

Serology

Molecular biology

Sequencing



Workshops/trainings

- Annual meeting (~30 countries)

Workshop LRUE Brucellosis

- >2 training sessions / year

Bacteriology, serology,



Control of biological products – production of reference materials

Controls

-RBT, CFT, SAT, MRT antigens; ELISA kits

Reference materials

-standards, panel of reference sera

-strains

-reagents: phages, mono-specific sera...

Annual proficiency tests

<https://leila.anses.fr/>

-Network of 130 labs (France+EU)

-serum / milk serology / CBP / bacteriology / molecular tests



- Brucellosis is a **worldwide zoonosis**
- **In animal**: reproductive troubles (abortions), arthritis...
- **In human**: undulant fever, chronic affections
- **6 classical species** with host preferences



B. abortus



B. melitensis



B. suis



B. neotomae



B. ovis

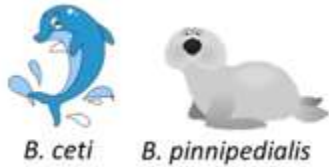


B. canis

Since 1994, 6 new species described !

... 1994 – 2019: 25-years of new *Brucella* hosts and species

→ First isolate from an aborted fetus



1994

J Vet Diagn Invest 6:448-452 (1994)

Characteristics of a *Brucella* species from a bottlenose dolphin (*Tursiops truncatus*)

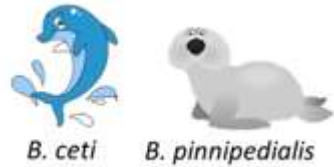
Darla R. Ewalt, Janet B. Payeur, Barbara M. Martin,
Donna R. Cummins, W. George Miller

→ Description of human infections (1999, 2003, 2006)

→ Whatmore et al. 2008 = **ST27**, genotype associated with zoonotic infection



... 1994 – 2019: 25-years of new *Brucella* hosts and species



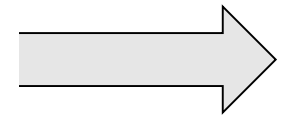
→ systemic disease in a wild population of the common vole *Microtus arvalis* in South Moravia (Czech Republic)

1994

2007

→ 1999–2003.

→ **Acute infections:** edema of extremities, occasionally with colliquating abscesses, arthritis, lymphadenitis, perforations of the skin resulting from colliquated abscesses, orchitis, and peritoneal granulomas



Vector-Borne and Zoonotic Diseases, VOL. 7, NO. 4 |

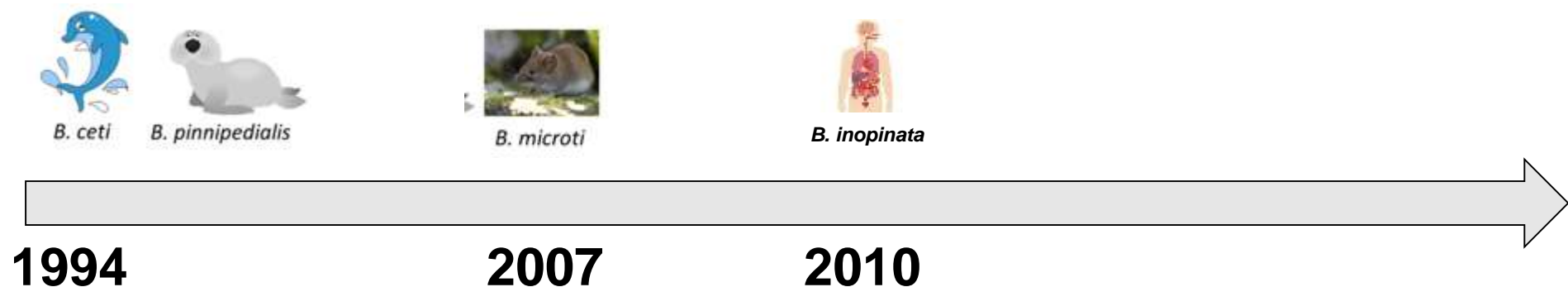
Brucellosis of the Common Vole (*Microtus arvalis*)

Z. Hubálek, H.C. Scholz, I. Sedláček, F. Melzer, Y.O. Sanogo, and J. Neovadbová

Published Online: 2 Jan 2008 | <https://doi.org/10.1089/vbz.2007.0143>



... 1994 – 2019: 25-years of new *Brucella* hosts and species



International Journal of Systematic and Evolutionary Microbiology (2010), **60**, 801–808

DOI 10.1099/ijse.0.011148-0



Brucella inopinata sp. nov., isolated from a breast implant infection

Holger C. Scholz,¹ Karsten Nöckler,² Cornelia Göllner,² Peter Bahn,² Gilles Vergnaud,^{3,4} Herbert Tomaso,¹ Sascha Al Dahouk,⁵ Peter Kämpfer,⁶ Axel Cloeckert,⁷ Marianne Maquart,⁷ Michel S. Zygmunt,⁷ Adrian M. Whatmore,⁸ Martin Pfeffer,¹ Birgit Huber,⁹ Hans-Jürgen Busse⁹ and Barun Kumar De¹⁰

→strain BO1 isolated from a breast implant infection of a 71-year-old female patient with clinical signs of brucellosis.



... 1994 – 2019: 25-years of new *Brucella* hosts and species



B. ceti



B. pinnipedialis



B. microti



B. inopinata

1994

2007

2010

Tiller et al. *BMC Microbiology* 2010, 10:23
<http://www.biomedcentral.com/1471-2180/10/23>



RESEARCH ARTICLE

Open Access

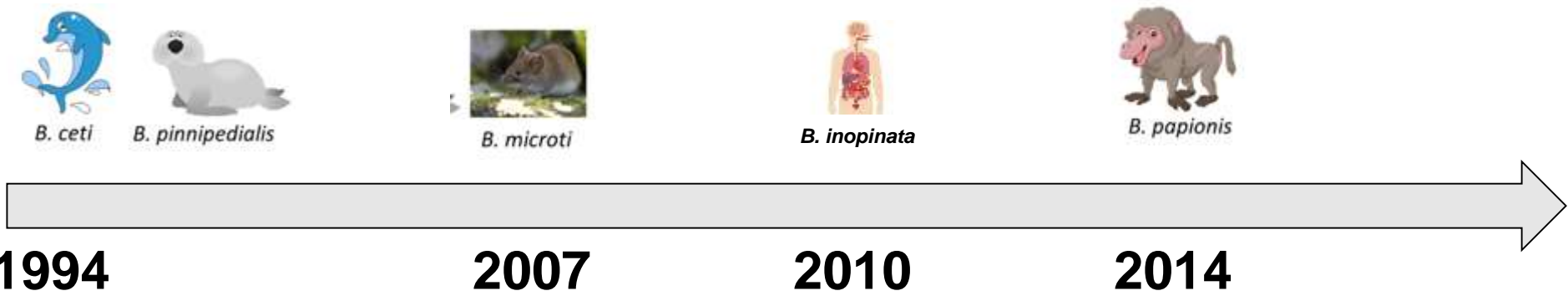
Identification of an unusual *Brucella* strain (BO2) from a lung biopsy in a 52 year-old patient with chronic destructive pneumonia

Rebekah V Tiller¹, Jay E Gee¹, David R Lonsway¹, Sonali Gribble^{2,3}, Scott C Bell², Amy V Jennison⁴, John Bates⁴, Chris Coulter^{2,3}, Alex R Hoffmaster¹, Barun K De^{1*}

→ strain BO2 isolated from a lung biopsy in a 52-year-old patient with chronic destructive pneumonia



... 1994 – 2019: 25-years of new *Brucella* hosts and species



International Journal of Systematic and Evolutionary Microbiology (2014), 64, 4120–4128

DOI 10.1099/ijs.0.065482-0

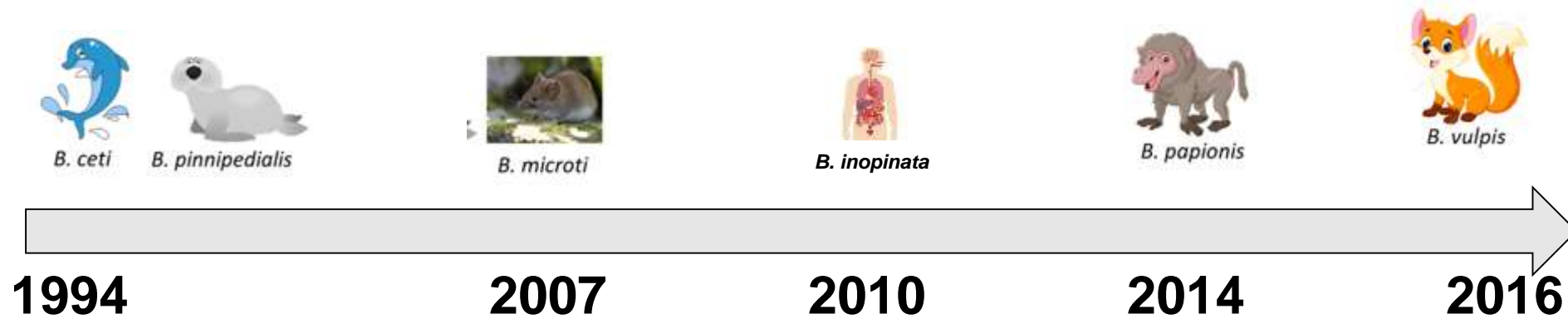
Brucella papionis sp. nov., isolated from baboons (*Papio* spp.)

Adrian M. Whatmore,¹ Nicholas Davison,^{2†} Axel Cloeckaert,^{3,4} Sascha Al Dahouk,⁵ Michel S. Zygmunt,^{3,4} Simon D. Brew,¹ Lorraine L. Perrett,¹ Mark S. Koylass,¹ Gilles Vergnaud,^{6,7,8} Christine Quance,⁹ Holger C. Scholz,¹⁰ Edward J. Dick, Jr.,¹¹ Gene Hubbard¹² and Natalia E. Schlabritz-Loutsevitch^{13‡}

→2 strains obtained from baboons (*Papio* spp.) that had delivered stillborn offspring



... 1994 – 2019: 25-years of new *Brucella* hosts and species



International Journal of Systematic and Evolutionary Microbiology (2016), 00, 1–9

DOI 10.1099/ijsem.0.000998



Brucella vulpis sp. nov., isolated from mandibular lymph nodes of red foxes (*Vulpes vulpes*)

→2 strains

Holger C. Scholz,¹ Sandra Revilla-Fernández,² Sascha Al Dahouk,³ Jens A. Hammer,³ Michel S. Zygmunt,^{4,5} Axel Cloeckaert,^{4,5} Mark Koylass,⁶ Adrian M. Whatmore,⁶ Jochen Blom,⁷ Gilles Vergnaud,^{8,9} Angela Witte,¹⁰ Karin Aistleitner¹ and Erwin Hofer²



... 25-years of new *Brucella* hosts and species

- Atypical hosts & new reservoirs



ORIGINAL RESEARCH ARTICLE

Front. Microbiol., 28 May 2018 | <https://doi.org/10.3389/fmicb.2018.01065>



High Shedding Potential and Significant Individual Heterogeneity in Naturally-Infected Alpine ibex (*Capra ibex*) With *Brucella melitensis*

Sébastien Lambert¹, Emmanuelle Gilot-Fromont¹, Pauline Freycon¹, Anne Thébault¹, Yvette Game¹, Carole Toigo¹, Elodie Petit¹, Marie-Noëlle Barthe¹, Gaël Reynaud¹, Maryne Jay¹, Bruno Garin-Bastuji¹, Claire Ponsart¹, Jean Hars¹ and Sophie Rossi¹



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EMERGING INFECTIOUS DISEASES®

EID Journal - Volume 26 - Number 4—April 2020 - Main Article

Volume 26, Number 4—April 2020

Research Letter

Brucella melitensis in Asian Badgers, Northwestern China

Xiafei Liu¹, Meihua Yang¹, Shengnan Song¹, Gang Liu, Shanshan Zhao, Guangyuan Liu, Sándor Hornok, Yuanzhi Wang¹, and Hai Jiang

Author affiliations: Shihezi University, Shihezi, China (X. Liu, M. Yang, S. Song, G. Liu, S. Zhao, Y. Wang); Lanzhou Veterinary Research Institute, Lanzhou, China (G. Liu); University of Veterinary Medicine, Budapest, Hungary (S. Hornok); Chinese Center for Disease Control and Prevention, Beijing, China (H. Jiang)

[Cite This Article](#)

PLOS ONE

RESEARCH ARTICLE

Molecular Survey of Bacterial Zoonotic Agents in Bats from the Country of Georgia (Caucasus)

Ying Bai¹*, Lela Urushadze^{2,3}*, Lynn Osikowicz¹, Clifton McKee^{1,4}, Ivan Kuzmin⁵, Andrei Kandaurov⁶, Giorgi Babuadze^{2,3}, Ioseb Natradze⁶, Paata Imnadze², Michael Kosoy¹



Only DNA



... 25-years of new *Brucella* hosts and species

- Exotic FROGS



A *Brucella* spp. Isolate from a Pac-Man Frog (*Ceratophrys ornata*) Reveals Characteristics Departing from Classical *Brucellae*

Pedro F. Soler-Lloréns^{1,2}, Chris R. Quance³, Sara D. Lawhon⁴, Tod P. Stuber², John F. Edwards⁴, Thomas A. Ficht⁴, Susiee Robbe-Austerman⁵, David O'Callaghan^{1,2} and Anne Kariel^{1,2*}

frontiers
in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 26 September 2016
doi: 10.3389/fcimb.2016.00116



Isolation of Potentially Novel *Brucella* spp. from Frogs

Tobias Eisenberg,^a Hans-Peter Hamann,^a Ute Kalm,^a Karen Schlez,^a Helga Seeger,^a Nicole Schauerte,^b Falk Melzer,^c Herbert Tomaso,^c Holger C. Scholz,^d Mark S. Koylass,^e Adrian M. Whatmore,^a and Michael Zschöck^a



SCIENTIFIC REPORTS

OPEN

Brucella spp. of amphibians comprise genomically diverse motile strains competent for replication in macrophages and survival in mammalian hosts

Received: 11 October 2016
Accepted: 07 February 2017
Published: 16 March 2017

Sescha Al Dahook^{1,2}, Stephan Köhler^{1,2}, Alessandra Occhialini^{1,2}, María Pilar Jiménez de Bagüés³, Jens Andre Hammer⁴, Tobias Eisenberg⁵, Gilles Vergnaud⁶, Axel Cloeckaert⁷, Michel S. Zygmunt⁸, Adrian M. Whatmore⁹, Falk Melzer¹⁰, Kevin P. Drees¹¹



2017 – two case reports, *B. neotomae*

Villalobos-Vindas et al. *Journal of Medical Case Reports*. (2017) 11:202
DOI: 10.1186/s12229-017-1466-8

Journal of
Medical Case Reports

CASE REPORT

Open Access

Brucellosis caused by the wood rat pathogen *Brucella neotomae*: two case reports

Juan M. Villalobos-Vindas¹, Ernesto Amuy¹, Elias Barquero-Calvo², Norman Rojas³, Carlos Chacón-Eraz⁴, Edeban Chaves-Olarte⁵, Caterina Guzmán-Vent³ and Edgardo Moreno^{1,6*}



→64-year-old Costa Rican white Hispanic man (Central Valley of Costa Rica), hypertensive, a lung infection and a stroke with an intraparenchymal hemorrhage

→51-year-old Costa Rican white Hispanic man from Puntarenas (East Pacific coast of Costa Rica), 4 years after case 1. Recurrent headache, disorientation, general muscle and joint pain, weight loss, cough, anorexia, and intermittent nocturnal fever (3 weeks)

2019 – First case of *B. suis* biovar 1, Germany




Infection

October 2019, Volume 47, Issue 5, pp 863–868 | [Cite as](#)

A headache with surprising outcome: first case of brucellosis caused by *Brucella suis* biovar 1 in Germany

Authors

Authors and affiliations

Sabine Zange , Kim Schneider, Enrico Georgi, Holger C. Scholz, Markus H. Antwerpen, Mathias C. Walter, Lothar Zoeller,

Heiner von Buttlar, Johannes P. Borde

→56-year-old male patient presented in June 2018 to a family practice with a 3-day history of headache, minimal photophobia, malaise, and febrile temperatures.

Source of infection ? Wild animals ? Imported meat ?

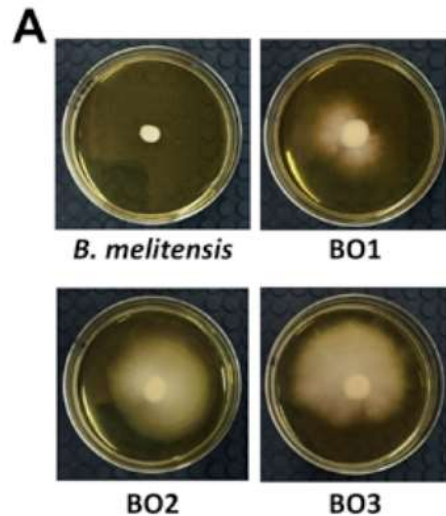
CORRECTED PROOF

First Case of Brucellosis Caused by an Amphibian-type *Brucella*

Nicolas Rouzic, Ludovic Desmier, Marie-Estelle Cariou, Eugénie Gay, Jeffrey T Foster, Charles H D Williamson, François Schmitt, Mikael Le Henaff, Alain Le Coz, Aurélien Lorréac'h... [Show more](#)

Clinical Infectious Diseases, ciaa1082, <https://doi.org/10.1093/cid/ciaa1082>

Published: 28 July 2020 [Article history](#) ▼



- Mid-January 2019 (day 0), a French 28-years-old patient hospitalized, exploration of polyadenopathies associated with multiple pulmonary condensations
- **animal keeper working with exotic animals** (contacts with reptiles, amphibians, rodents or birds, several imported from abroad).
- day 6, a left cervical lymph node surgically removed, which triggered fever in the patient
→ Aerobic cultures positive after 48h incubation
 - **isolates cannot be identified using specific typing serum ; flagellated and motile**
- isolate most similar to the ***B. inopinata* -like** isolates BO2 and B13-0095
 - isolated from a patient with severe pulmonary infection [Tiller et al. 2010] and from a Pac-man frog [Soler-Llens et al., 2016],
- After 6 weeks of antibiotic therapy : left cervical abscess under a surgical scar → *Brucella* DNA detected by PCR amplification of the *Brucella* -specific sequence IS711
- **Not detected using commonly available serological tests**

First isolation of a *Brucella* in a domestic frog farm

- Frog breeding in France for human consumption
→ Pan-pathogen examination
- A suspect strain has been isolated
(API20-NE: *O. anthropi*, MALDI-TOF assay: *Brucella* spp.)
 - Internal organs
 - Cutaneous lesions



- ***Brucella-microti* like confirmed**

PCR

Typing





MLVA-16, RFLP

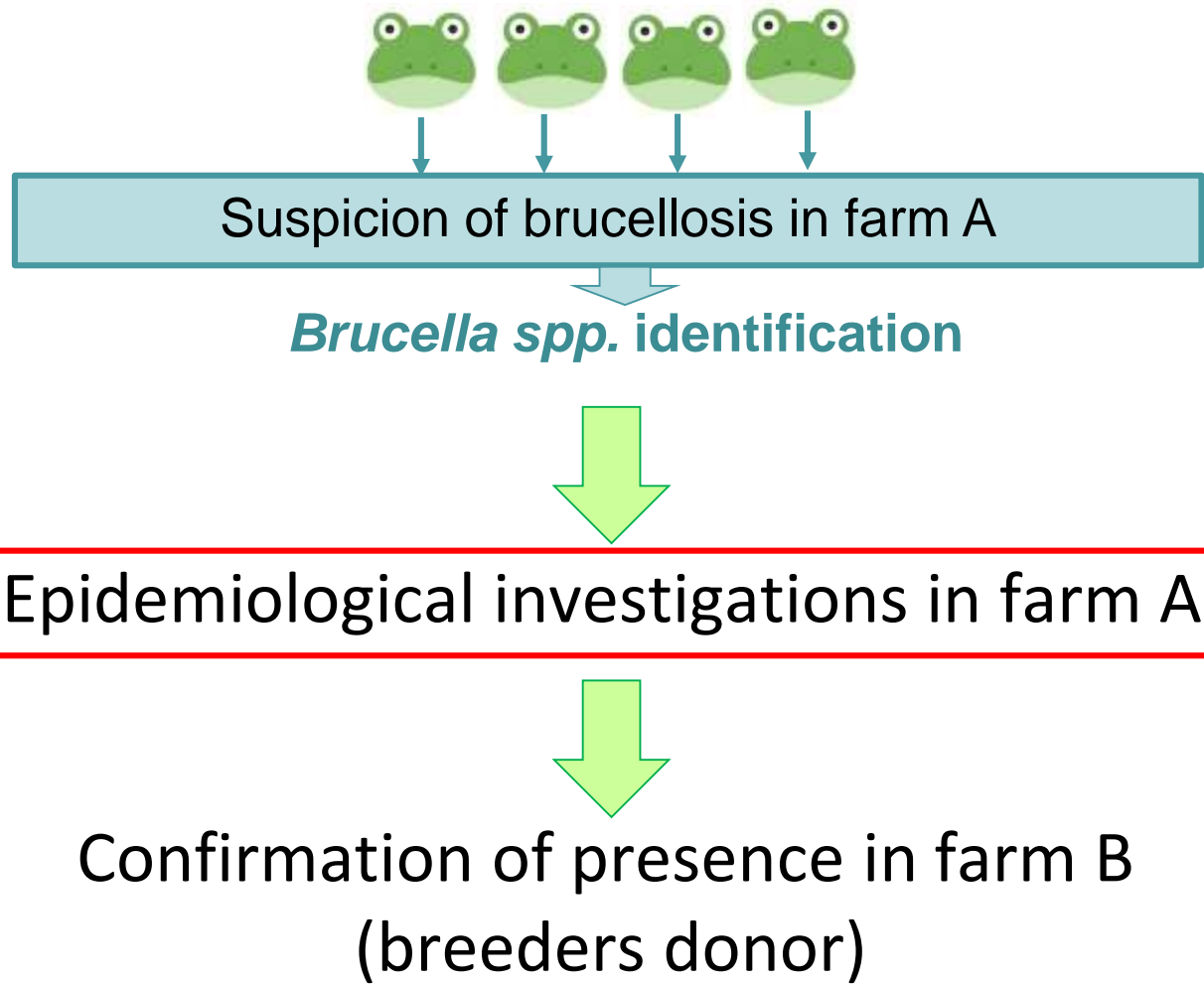
Whole genome sequencing

ORIGINAL RESEARCH ARTICLE Provisionally accepted The full-text will be published soon. [Notify me](#)

Front. Vet. Sci. | doi: 10.3389/fvets.2018.00283

Phenotypic and molecular characterization of *Brucella microti*-like bacteria from a domestic marsh frog (*Pelophylax ridibundus*)

 Maryne JAY¹,  Guillaume GIRAULT², Ludivine PERROT², Benoit TAUNAY², Thomas VUILMET², Frédérique ROSSIGNOL², Pierre-Hugues PITEL², Elodie PICARD²,  Claire PONSART² and  Virginie MICK²



*June - Septembre
2017*

*September -
December 2017*

*April -June
2018*



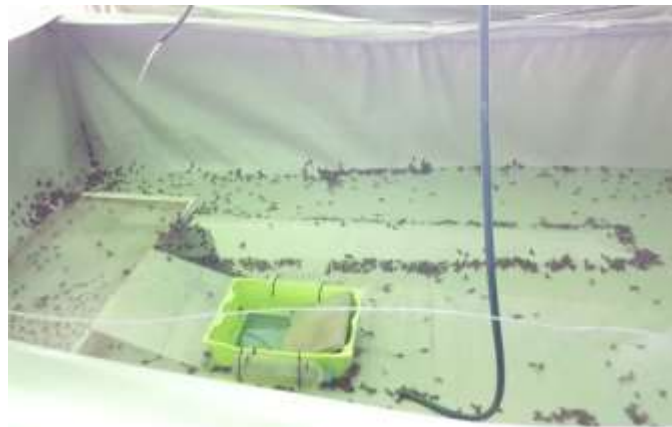
- Greenhouses, with many basins...





Tadpoles, young frogs, breeding / adult frogs







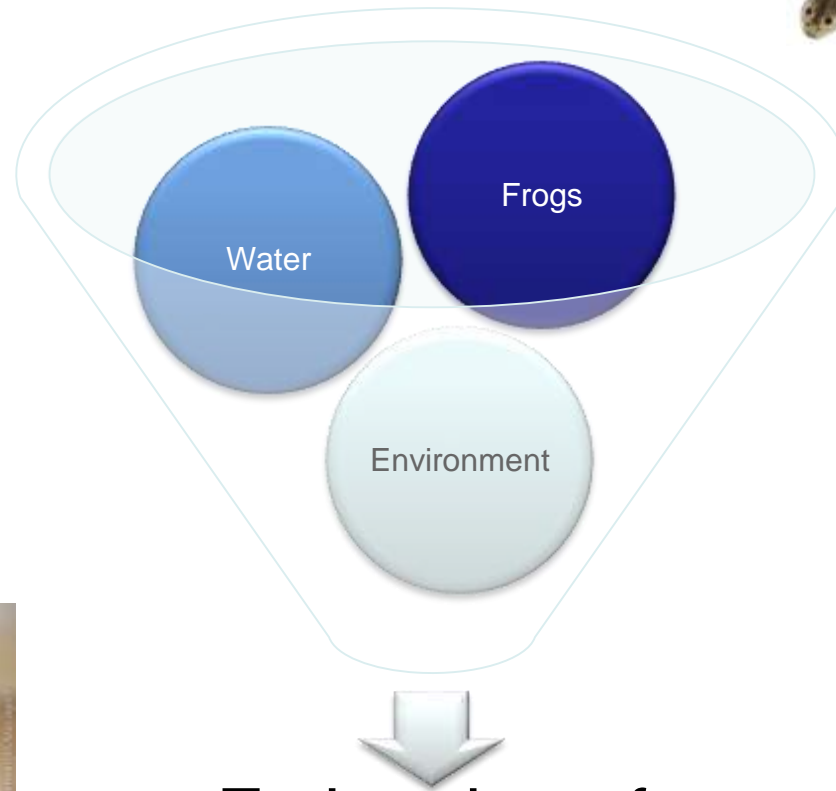
Soil

Basins water

Food



**10 pools
of 20 tadpoles**



**+ 10 pools
of 10 frogs**

Animal:



Stage	Live animals		Dead animals	
	Nb of basins	Nb of frogs	Nb of basins	Nb of frogs
Adults (large)	5	57	4	16
Adults (young)	5	55	2	15
Froglets	2	27	1	10
Tadpoles	6	113	2	20
Total	18	252	9	61



1-2 L water from 16 basins

Surface swabs: 22 basins



2L entry water (Groundwater)

14 soil samples

3 food samples (adults, froglets, tadpoles)

Detection

Bacteriology



- muscles (legs)
- internal organs
- lesions



Swabs

Bacterial counts
☞ Contamination level

PCR *Brucella* IS711

Tadpoles and froglets



8 pools of 20 tadpoles

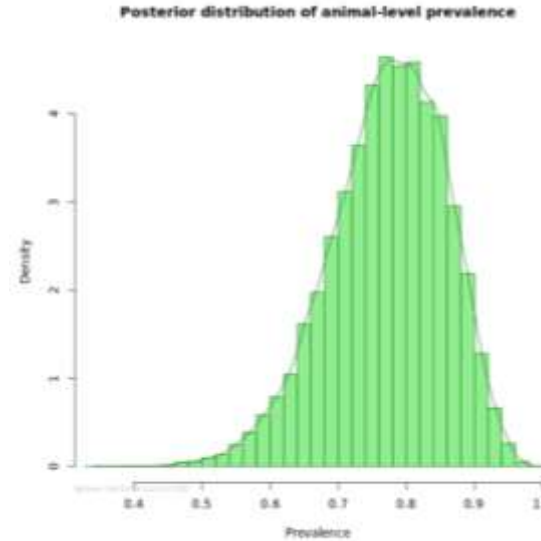
**Median value
of true prevalence: 84%**
CI: 57,4%-97,5%



Adults

10 pools of 10 adults

**Median value
of true prevalence : 85,1%**
CI: 59,3%-97,7%



Baysian approach
Se=95% ; Sp= 100% ;
Beta distribution



ORIGINAL ARTICLE

Brucella microti-like prevalence in French farms producing frogs

Maryne Jay, Luca Freddi, Virginie Mick, Benoit Durand, Guillaume Girault, Ludivine Perrot, Benoit Taunay, Thomas Vullmet, Didier Azam, Claire Ponsart, Gina Zanella

First published: 01 October 2019 | <https://doi.org/10.1111/tbed.13377> | Citations: 1

Maryne Jay and Luca Freddi are contributed equally to this work.

WATER

- Basins (16) : **6 positive results**
- Surface swabs (22) : **all positive**
- Biofilters (2) : **all positive**
- Groundwaters (2) : **all negative**



SOIL

- Sand or gravel samples (14) : **5 positive results**



FOOD

- Samples (3) : **all negative**

Identification of emerging *Brucella* species : IDEMBRU project



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<https://onehealthjep.eu/jrp-idembru/>



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 OneHealthEJP.eu

IDEMBRU: Identification of emerging *Brucella* species: new threats for human and animals

Start: 1 January 2020
Duration: 2.5 Years
Domain: Emerging Threats
Key Words: emerging *Brucella*, reservoir, virulence, whole genome sequencing, RNA detection
Contact: [Claire Ponsart \(ANSES\)](#)



GRENOUILLES
PARIS

The Project #IDEMBRU

Brucellosis is a highly contagious zoonosis usually caused by ingestion of unpasteurised milk or undercooked meat from infected animals, or close contact with their secretions. Brucellosis is one of the most widespread zoonotic diseases globally, with 500,000 new human cases estimated each year. For many years six 'classical' *Brucella* species were identified but, since the late 1990's, several new *Brucella* species (including *B. inopinata*, *B. microti* and *B. vulpina*) were isolated from humans, wild animals and/or environmental sources demonstrating a wider range of hosts and new potential zoonotic threats. Some of these species are genetically and/or phenotypically atypical in comparison to the 'classical' species.

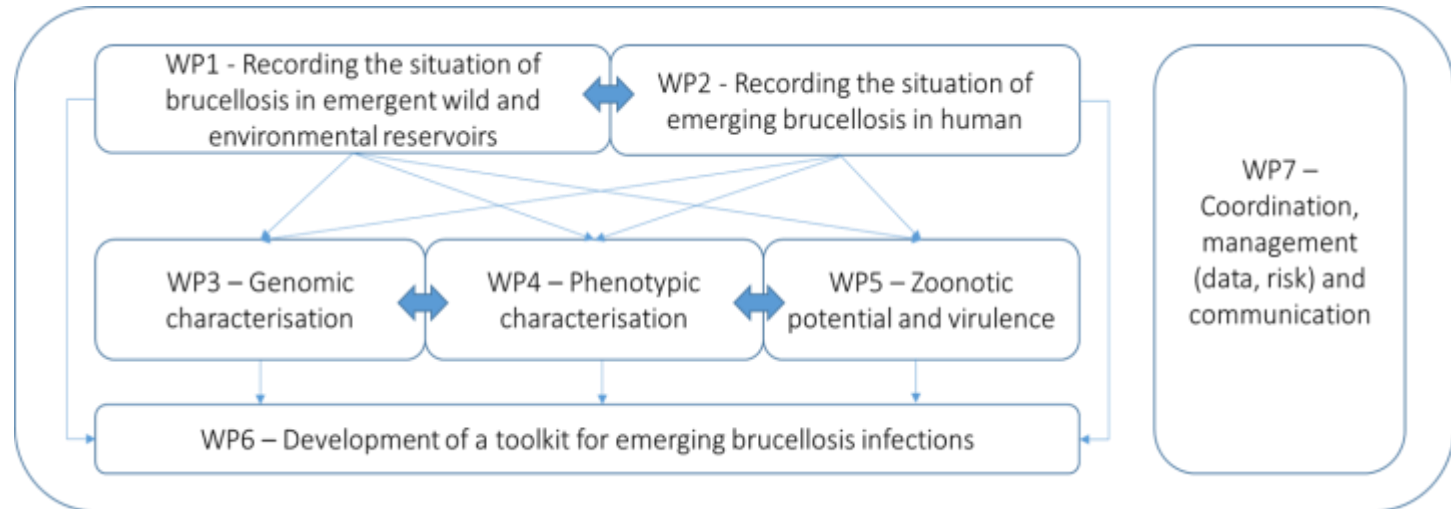




- East EU-partners:
- **NDRVMI – BFSA - National Centre of Food Safety:** Hristo Daskalov, Albena Dimitrova, Mihail Milanov;
- South EU-partners:
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- **INSA – Instituto Nacional de Saúde Doutor Ricardo Jorge:** Ana Pelerito, Isabel Carvalho, João Paulo Gomes, Sofia Núncio;
- **IZSAM - Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise:** Fabrizio de Massis, Giuliano Garofolo, Flavio Sacchini;
- West EU-partners:
- **APHA - Animal and Plant Health Agency:** Adrian Whatmore and Roland Ashford;
- **ANSES - French Agency for Food, Environmental and Occupational Health & Safety:** Vitomir Djokic, Luca Freddi, Acacia Ferreira Vicente, Guillaume Girault;
- **BfR - Bundesinstitut für Risikobewertung,** Sascha Al Dahouk;
- **FLI - Friedrich-Loeffler-Institut:** Falk Melzer;
- **WBVR –Wageningen Bioveterinary Research:** Alexander Umanets, Hendrik Jan Roest.



Objectives



- priority topic ET 2.1 → new brucellosis threats
- to develop a **toolkit** to detect and characterize emerging *Brucella* species and reservoirs.
- both emerging atypical *Brucella* strains, and classical species identified in atypical animal hosts, human populations, as well as re-emerging classical pathogenic *Brucella* linked with new consumption or movement patterns.

- **New species & reservoirs : more research is needed**
- **Atypical strains: difficulties to assess zoonotic potential / pathogenicity**
 - Limited numbers of reported human cases
 - “Classical” identification approaches are not appropriate for *B. inopinata* - like strains / frog isolates
 - Frog farms: no clinical signs in exposed workers
- **Confirmation of high prevalence of *Brucella* in a domestic frogs : origin of the contamination ?**
 - Normal bacterial flora ?
 - Presence in the environment?
 - future directions: **Environment / New host species (IDEMBRU project)**

Acknowledgments



The *Brucella* Team



<https://eurl-brucellosis.anses.fr/>

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INRAE
Didier Azam



APHA, BfR, FLI,
INIAV, INSA, ISZAM,
NDRVMI, WBVR

