



# **7<sup>th</sup> Workshop of the EU Brucellosis National Reference Laboratories**

## **Technical Report**

Sept. 8-9, 2014  
Berlin, Germany



## 1. Organisation of the Workshop

The 2014 annual workshop was first announced on January 17, 2014. The EURL sent the official invitation letter to NRLs of all Member States, Norway, Switzerland<sup>1</sup>, Candidate Countries and West Balkan Countries on May 7, 2014.

Amongst the NRLs from Member States, 4 NRL replied that their attendance was not possible (Greece, Cyprus, Bulgaria and Malta). The representatives from Montenegro and from Serbia both needed to cancel their trip and apologized for not attending the Workshop.

Namely, participants to this workshop were:

Country	Participant
AUSTRIA	Romana POSCH
BELGIUM	David FRETIN
BELGIUM	Marcella MORI
CZECH REPUBLIC	Stefania SANOVA
GERMANY	Falk MELZER
DENMARK	Lars Ole ADRESEN
DENMARK	Christian GRÄS
ESTONIA	Age KARSSIN
SPAIN	Lorena ARROGANTE PENUELAS
FINLAND	Teresa SKRZYPCZAK
FRANCE/EURL	Claire PONSART
FRANCE/EURL	Maryne JAY
FRANCE/EURL	CORDE Yannick
FRANCE/EURL	Moulay Ali CHERFA
CROATIA	Silvio ŠPIČIĆ
HUNGARY	Béla DENES
IRELAND	Karina WRIGLEY
ITALY	Manuela TITTARELLI
LITHUANIA	Jurate BUITKUVIENE
LATVIA	Dita KRASTINA
THE NETHERLANDS	Hendrik-Jan ROEST
POLAND	Krzysztof SZULOWSKI
PORTUGAL	Regina CARDOSO
ROMANIA	Ruxandra Alina RADULESCU
SWEDEN	Therese RASBACK
SLOVENIA	Brane KRT

<sup>1</sup> No contact person identified in Iceland



SLOVAKIA	Eva CECHOVA
UNITED KINGDOM	John MC GIVEN
UNITED KINGDOM Northern Ireland	Mary O'CONNOR
SWITZERLAND	Sabrina RODRIGUEZ
NORWAY	Tone BJORDAL-JOHANSEN
F.Y.R.O.M.	Kiril KRSTEVSKI
TURKEY	Mustafa Sencer KARAGÜL
ALBANIA	Arla SHEHU
KOSOVO	Xhemajl DERVISHI
DG SANCO	Nicolas KRIEGER
BfR	Sacha AL-DAHOUK
BfR	Bernd APPEL

*(The signed attendance lists are attached to this report)*

This 2014 annual workshop was held in Berlin, Germany on the premises of Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung BfR), on September 8 and September 9, during two days. The workshop was organized before the 2014 International Research Conference for Brucellosis that was held at the Federal Institute for Risk Assessment in Berlin from 9 to 12 September 2014. Dr Nicolas Krieger, Project Officer for Brucellosis in the DG SANCO joined the workshop on September, 8.

Four persons from the EURL staff attended the workshop: Dr. Claire Ponsart recently nominated as head of the EURL, Yannick Corde, Moulay-Ali Cherfa (technical experts) and Dr. Maryne Jaÿ (EURL coordinator).

## 2. Welcome address and opening of the Workshop

Dr Claire Ponsart, recently nominated as head of the EURL after the recent departure of Dr Garin-Bastuji, welcomed all the participants and opened the meeting. She introduced herself and the EURL team to the NRLs participants and thanked the Federal Institute for Risk Assessment (BfR) for welcoming this annual workshop. She briefly presented the agenda of the meeting (*see agenda in Annex 1*).

Dr Nicolas Krieger, representative of the DG SANCO, thanked the EURL for organizing this workshop and welcomed all the participants. He underlined the main responsibilities for European and National reference laboratories, and more generally for laboratories involved in



official controls. As for European reference laboratories, he insisted on the importance of developing validated methods and organizing training, inter-laboratory trials, and workshops. He reminded the EURL should coordinate the different methods employed, bring active assistance to NRLs, favour exchanges on best practices and assist the Commission on the implementation of new regulations. As for National reference laboratories, he highlighted that they should coordinate the activities of Official labs within their countries and organize PT with a deep follow-up.

### 3. Presentation of the BfR (Prof. Bernd Appel, Head of biological, Head of the Department of Biological Safety, BfR)

Prof. Bernd Appel from BfR presented the institute and an overview of its activities. BfR is the scientific body of the Federal Republic of Germany that prepares expert reports and opinions on questions of food and feed safety and the safety of substances and products. Its tasks include the assessment of existing and the identification of new health risks (chemical and microbiological), the drawing up of recommendations on risk reduction, and the communication of this process. In doing so, the Institute assumes an important task in improving consumer health protection and food safety. The activities of the BfR are conducted under the responsibility of the Federal Ministry of Food, Agriculture and Consumer Protection. At the three BfR locations in Berlin, a staff of about 750, among them 300 scientists, is being employed to work in the field of consumer health protection.

### 4. Brucellosis – What's up in Berlin (Prof. Sascha Al Dahouk, Scientific Director, BfR)

Prof Sascha Al Dahouk presented BfR activities regarding Brucellosis. He briefly described the epidemiological situation of animal and human brucellosis in Germany (mostly imported cases). He detailed different areas of research his team is involved in:

- Development of biochemical typing (substrate metabolism) for rapid identification of *Brucella* species and biovars,
- Constitution of spectra database for MALDI TOF identification of *Brucella*,
- Development of genus specific qPCR,
- MLVA and its use in epidemiology
- Study of *Brucella* metabolic adaptations during the intracellular trafficking (effect of low oxygen/starvation on proteins expression)



## 5. EURL activity report and 2015 workprogramme (Dr. C. Ponsart, Head of EURL)

Dr Claire Ponsart presented the different activities the EURL performed during 2013-2014. In particular:

- The support reference activity of the EURL in 2013-2014 regarding serology and bacteriology (secondary analyses, strain or reagent supply, antigens, kit and vaccine control, establishment of standardised technical procedures),
- The feedback of the 2013 annual meeting in Zagreb, Croatia
- the organisation of three 2-day training sessions in 2013-2014 (since the last workshop), (the last one concerning serology was to take place in November, 2014 and information regarding registration will be soon sent to NRLs )
- The report of two ring-trials conducted in 2013-2014 (2013 iELISA in bovine milk & 2014 serological tests in bovine serum).
- The results of a validation trial of the European standard sera for *B. ovis* and the European standard sera for porcine brucellosis in 2013.
- The organisation, at the end of 2014, of another ring trial concerning the performance of reagent batch control (RBT antigen).

She also presented the activities planned for 2015:

- One ring trial that is to be organized in 2015 concerning the performance of iELISA in bovine milk.
- Two training sessions that are to be organized in 2015, one dedicated to *Brucella* bacteriology/molecular methods and the other one dedicated to serology. Expectations of NRLs regarding the topics of these trainings were actively discussed during this workshop (See 12).
- The 2015 annual workshop that is to be held in fall 2015 in Maisons-Alfort, France.
- The EURL website that is now structured and will be enriched with useful documents and links. It is to be launched at the end of 2014.

She reported on the progress of serological studies to be achieved during 2015 by in order to address EFSA's recommendations on serological tests (sheep and goats).

She reminded that the EURL planned to organize the shipment of some particular strains (*B. melitensis* bv. 3, bv. 1 and bv. 2 for 2014) from some particular NRLs, *i.e.*, of the South-



European area (Croatia, Spain, Greece, Portugal and Italy, as well as if possible from other Balkan countries included in the EU Brucellosis Network).

#### **6. 2014 serum ILPT results** (Dr. M. Jaÿ, EURL Coordinator)

The objective of this ring-trial was to assess the proficiency of the NRLs in performing serological tests (RBT, CFT, SAT, iELISA, cELISA). It was a first presentation of the results since the final report has not been completed yet. The overall results were very satisfactory for the 36 participants, compared to last ring-trial. Only one laboratory faced critical problem in RBT, other failures observed were minor. A clear improvement was observed compared to last ILPT (2012), during which critical failures were faced by several laboratories in RBT, CFT and iELISA.

#### **7. 2013 milk ILPT results** (Dr. M. Jaÿ, EURL Coordinator)

The objective of this RT was to assess the proficiency of milk iELISA in every NRL. The overall results were really satisfactory for the 22 participants, as shown in the report sent in March, 2014. The failures identified were minor except for 1 laboratory. This laboratory found the origin of the troubles faced and obtained satisfactory results on second panel sent. Important progresses were made in milk iELISA compared to last ILPT (2011).

#### **8. 2013 Interlaboratory validation trial EUPBSS & EUBoSS** (Dr. M. Jaÿ, EURL Coordinator)

The objective of this trial was to set conditions of standardization in iELISA of both European candidate standards in comparison to international standards. The analysis took into consideration results obtained with international standards but also CV% between duplicates, control results to validate results on candidate sera. Few punctual results have not been considered because of too high CV% between duplicates or due to important discrepancies with participants overall results.

Concerning the validation of EUPBSS, the protocol has been adapted according to recent changes (ISaBsS is no longer recognized as an OIE standard and is no more available). Thus, a limited number of laboratories tested both sera in parallel and other NRLs tested only EUPBSS. Nevertheless, results obtained by the 23 participants with the kit provided (Ingenasa) allowed proposing a positive interpretation cut-off at 1/64 and a negative interpretation cut-off at 1/512.



Concerning the validation of EUBoSS, results obtained by the 21 participants with the kit provided (Idexx with adapted cut-off according to EURL's recommendations at 30-60%) allowed to propose positive interpretation cut-off at 1/16 and negative interpretation cut-off at 1/256. These two sera are now available with these corresponding characteristics for NRLs upon request.

### 9. Protocol of the ILPT biological controls (Dr. M. Jaÿ, EURL Coordinator)

An inter-laboratory trial regarding official batch to batch control of Rose-Bengale diagnostic antigens is to be organised by the end of 2014. General guidelines of its organisation were presented: participants will be asked to control 3-4 different RB antigens and evaluation will focus on sensitivity assessment against OIEISS ((+) at 1/45 & (-) at 1/55).

### 10. LFIA validation study on small ruminants (Y. Corde, EURL technical expert)

In the French Alps, a high prevalence of *B. melitensis* biovar 3 was observed in wild Alpine ibex (*Capra ibex*). In order to control the disease in the Alpine ibex local population, a test-and-slaughter programme has been implemented since fall 2012. Animals are first captured for blood sampling (laboratory tests required) and identified (Ear-tags and GPS/VHF collar). Animals with positive RBT/CFT results are then tracked (collars), and killed.

This study aimed to assess, a commercial LFIA designed for sheep and goat brucellosis diagnosis in Alpine ibex, in comparison with the OIE prescribed tests for small ruminant brucellosis, *i.e.* RBT and CFT. The results showed a very good correlation between the LFIA and the other tests. On the 172 sera, the consistency of LFIA with other tests exceeded 97.6% no matter which test was applied. Moreover, the ISaBmS and EUGBSS standards, directly diluted in whole blood, were positive in LFIA. These results showed that LFIA can be an interesting alternative for serological surveillance as regards wildlife.

### 11. Study on Veronal Buffer replacement (CFT) (Dr. M. Jaÿ, EURL Coordinator)

Several countries faced a recent ban on the use of barbital due to its toxicity and its drug properties. This compromises the use of VB in CFT. The EURL studied an alternative buffer which is recommended by Alton G.G *et al.* (Techniques for the brucellosis laboratory, 1988): MgCa Buffer. A comparative study was conducted in 2013-2014: sera from - bovine, porcine, sheep and goat-infected and free population, were tested using both buffers. Results obtained with both buffers are mostly identical or rarely showing a difference not exceeding one dilution (not significative). Results on European and international standard sera are also identical. Both buffered were also compared in *B. ovis* CFT, and were satisfactory. In conclusion, no significant difference was observed in CFT by



using either one of the buffers for international or European standard sera as well as for field serum samples, whatever the host species (cattle, small ruminants or pigs).

These results highlight the non-toxic, easy to produce and cheap MgCa buffer is a suitable alternative to the barbital VB in the OIE approved CFT. The possible use of the MgCa buffer will be therefore proposed for inclusion in the next revision of the OIE Terrestrial Manual.

## 12. Discussion and survey on EURL training sessions (Dr. M. Jaÿ, EURL Coordinator)

The prospects of training topics were actively discussed after a brief presentation of current training sessions. There is still a need to maintain existing training session on basic knowledge in bacteriology as well as in serology. However participants expressed a need for other kinds of training sessions regarding several topics:

- building a routine strategy of identification for *Brucella* strains (combining molecular and phenotypical methods)
- Identification and typing by molecular methods (Real Time PCR, MLVA),
- Reagent control (esp. CF antigen control).

One participant suggested associating other NRLs in the organization of training sessions to enrich the practice overview allowed by the training.

## 13. Discussion on NRL activity survey (Dr. C. Ponsart, Head of EURL)

Dr Claire Ponsart presented the draft prepared for 2014 NRL activity survey. The objective of the survey is to have an overview of NRLs activities regarding their national network (role and methods performed, scientific activities, PT organization and follow-up, training organization) and to collect their expectations toward the EURL. The participants discussed the need of exchange between NRLs around good practices and on other technical topics in dedicated working groups.

## 14. Free communications

Participants to the Workshop had been invited to present free communications.

Dr. Falk Melzer from German NRL started with a presentation of surveillance and control plan of brucellosis in Germany and of its activities as Brucellosis NRL. The surveillance strategy presented were actively discussed which underlined the need of exchange on this kind of topics among the network.

Dr. Hendrik-Jan Roest from the Dutch NRL presented results of a study concerning *Brucella* MALDI-TOF identification and the topic was actively addressed by the NRLs that has already





work with this tool. Inactivation procedure and accuracy of identification were especially discussed.

Dr. Teresa Skrzypczak from Finnish NRL described a *B. canis* outbreak occurred in Finland. Then, Dr. Therese Rasback, from the Swedish NRL, shared their experience regarding suspicions of *B. canis* infections from imported dogs. This presentation was the opportunity to exchange on the diagnostic strategy of canine brucellosis among the network.

Finally, Dr Mary O'Connor presented the outcomes of an audit mission dedicated to CFT conducted in South Africa and raised the issues faced during this evaluation.

Their participation was well appreciated by all participants and contributed to the exchanges amongst the network.

## 15. Round Table and Closing

After all the presentations had taken place, a general discussion was conducted by the EURL in order to draw the conclusions of this two-day workshop of exchange of experience and knowledge. Each NRLs representative had the opportunity to give a few words about their expectations regarding EURL meetings and areas of work. The main points emphasized by the participants were the need of exchange regarding techniques and practices, the importance of EURL website development, the problem of False Positive Serological reaction (FPSR) detection in pigs, the need of training sessions and PTs and the lack of harmonization in EU surveillance plan/trade control requirements.

Dr. Claire Ponsart closed the workshop with the highlights of this meeting. She underlined that excellent results were obtained by the participants during last two ILPTs. As regards training expectations, she reminded that NRLs wish to include new topics such as molecular tools but maintain sessions on basic diagnostic knowledge such as CFT. She proposed to reinforce the networking by implementing small working groups to exchange on technical practices (Rt PCR, biosecurity, surveillance plan, MALDI-TOF...) and eventually build new projects with diagnostic applications. Dr. C. Ponsart thanked the BfR for their warm welcome and their remarkable organization of this 2014 annual workshop. Finally, she thanked all the participants for attending this meeting and the EURL team for the preparation of the meeting.

*Note: A CD with all presentations made during this workshop and a scanned copy of the different documents distributed during the workshop will be sent to all participants as well as to those NRLs that could not attend and to the Commission as soon as possible. Certificates of attendance were distributed to all participants before the closing of the workshop.*





## Annexes

### Annex 1: Final Agenda



European Union Reference Laboratory  
for Brucellosis



7<sup>th</sup> Workshop of the EU Brucellosis National Reference Laboratories

Berlin, Germany – September 8-9, 2014

*Draft Agenda*

#### Sept. 8, 2014

9h00	9h30	Registration
9h30	10h15	Welcome address : EURL DG Sanco Opening of the Workshop
10h15	10h45	Brucellosis _ What's up in Berlin? Prof. Al Dahouk, BfR
10h45	11h00	Presentation of the BfR Prof. Bernd Appel, BfR
COFFEE BREAK		
11h30	12h00	Activities of the EURL during 2013-2014 2015 EURL expected activities
12h00	12h45	ILPT session - 2014 serum ILPT results
LUNCH		
14h15	15h30	ILPT session - 2013 milk ILPT results and follow-up - Inter laboratory validation trial EUPBSS & EUBoSS results
15h30	15h50	Protocol of the ILPT biologicals control (RBT antigen)
COFFEE BREAK		
16h20	17h30	LFIA validation study (wild ruminants) Discussion on NRLs activity survey



French Agency for Food, Environmental and Occupational Health Safety – Animal Health Laboratory – Bacterial Zoonoses Unit  
23, avenue du Général-de-Gaulle – F-94706 Maisons-Alfort Cedex - Tel : + 33 (0)1 49 77 13 00 - Fax: + 33 (0)1 49 77 13 44 -  
[www.anses.fr](http://www.anses.fr)



## 6<sup>th</sup> Workshop of the EU Brucellosis National Reference Laboratories

Berlin, Germany – September 8-9, 2014  
*Draft Agenda*

### Sept. 9, 2014

9h30	9h45	Workshop Administrative and financial issues (EURL)
9h45	10h15	Comparison study between an alternative buffer with veronal buffer
10h15	10h45	Discussion and survey on EURLs training sessions : expectations and perspectives

COFFEE BREAK

11h30	13h00	Free communications: - NRL GERMANY - NRL SWEDEN - NRL NETHERLANDS - NRL UK NORTHERN IRELAND
13h00	13h30	Round table Closing-up of the workshop

LUNCH – Registration to Brucellosis 2014



French Agency for Food, Environmental and Occupational Health Safety – Animal Health Laboratory – Bacterial Zoonoses Unit  
23, avenue du Général-de-Gaule – F-94706 Maisons-Alfort Cedex - Tel : + 33 (0)1 49 77 13 00 - Fax : + 33 (0)1 49 77 13 44 -  
[www.anses.fr](http://www.anses.fr)



Annex 1: Attendance sheets



European Union Reference Laboratory for Brucellosis



7<sup>th</sup> Workshop of the EU Brucellosis National Reference Laboratories

Berlin, Germany – September 8-9, 2014

	Country	Participant	Sept. 8, 2013	Sept. 9, 2013
R	AUSTRIA	Romana POSCH		
R	BELGIUM	David FRETIN		
NR	BELGIUM	Marcella MORI		
R	CZECH REPUBLIC	Stefania SANOVA		
R	GERMANY	Falk MELZER		
R	DENMARK	Lars Ole ADRESEN		
NR	DENMARK	Christian GRÄS		
R	ESTONIA	Age KARSSIN		
R	SPAIN	Lorena ARROGANTE PENUELAS		
R	FINLAND	Teresa SKRZYPCZAK		



7<sup>th</sup> Workshop of the EU Brucellosis National Reference  
Laboratories

Berlin, Germany – September 8-9, 2014

	Country	Participant	Sept. 8, 2013	Sept. 9, 2013
R	FRANCE/EURL	Claire PONSART		
R	FRANCE/EURL	Maryne JAY		
NR	FRANCE/EURL	CORDE Yannick		
NR	FRANCE/EURL	Moulay Ali CHERFA		
R	FRANCE/EURL	Gabriela VECCHIO		
R	CROATIA	Silvio ŠPIČIĆ		
R	HUNGARY	Béla DENES		
R	IRELAND	Karina WRIGLEY		
R	ITALY	Manuela TITTARELLI		
R	LITHUANIA	Jurate BUITKUVIENE		

2



7<sup>th</sup> Workshop of the EU Brucellosis National Reference  
Laboratories

Berlin, Germany – September 8-9, 2014

	Country	Participant	Sept. 8, 2013	Sept. 9, 2013
R	LATVIA	Dita KRASINA		
R	THE NETHERLANDS	Hendrik-Jan ROEST		
R	POLAND	Krzysztof SZULOWSKI		
R	PORTUGAL	Regina CARDOSO		
R	ROMANIA	Ruxandra Alina RADULESCU		
R	SWEDEN	Therese RASBACK		
R	SLOVENIA	Brane KRT		
R	SLOVAKIA	Eva CECHOVA		
R	UNITED KINGDOM	John MC GIVEN		
R	UNITED KINGDOM Northern Ireland	Mary O'CONNOR		





European Union Reference Laboratory  
for Brucellosis



7<sup>th</sup> Workshop of the EU Brucellosis National Reference  
Laboratories

Berlin, Germany – September 8-9, 2014

	Country	Participant	Sept. 8, 2013	Sept. 9, 2013
NR	SWITZERLAND	Sabrina RODRIGUEZ		
NR	NORWAY	Tone BJORDAL-JOHANSEN		
NR	NORWAY	Siv KLEVAR		
I	F.Y.R.O.M.	Kiril KRSTEVSKI		
I	TURKEY	Mustafa Sencer KARAGÜL		
I	ALBANIA	Arla SHEHU		
I	SERBIA	Jadranka ZUTIC		
I	KOSOVO	Xhemajl DERVISHI		
NR	DG SANCO	Nicolas KRIEGER		
NR	BfR	Sascha AL- DAHOUK		

4