



*French Agency for Food,
Environmental &
Occupational Health
Safety*

Maisons-Alfort

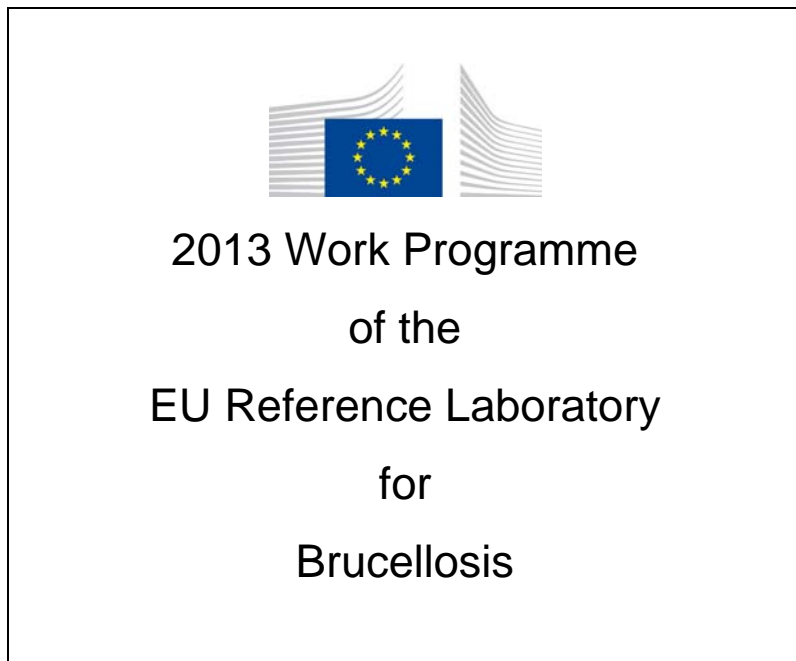
LABORATOIRE DE SANTE
ANIMALE

ANIMAL HEALTH LABORATORY

Unité Zoonoses Bactériennes
Bacterial Zoonoses Unit

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Centre National de
Référence des *Brucella*
*National Reference Centre
for Human Brucellosis*
Laboratoire National de
Référence pour la
Brucellose, la Chlamydie, la
Fièvre Charbonneuse, la
Mélioïdose, la Morve, les
Mycobactérioses & la
Tularémie animales
*Animal Anthrax, Brucellosis,
Chlamydiosis, Glanders,
Melioidosis, Mycobacterioses
& Tularaemia*
*National Reference
Laboratory*
Laboratoire de Référence
O.I.E. / FAO
pour la Brucellose,
la Paratuberculose et
la Tuberculose bovine
OIE /FAO
*Reference Laboratory
for Brucellosis,
Bovine Paratuberculosis
and Tuberculosis*
Laboratoire de Référence de
l'UE pour la Brucellose
*EU Reference Laboratory for
Brucellosis*
Laboratoire de Référence de
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Equines (Morse)
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Centre National de Référence
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*National Reference Laboratory
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for Brucellosis*
Laboratoire de Référence de l'UE
pour la Brucellose


OIE/FAO Brucellosis Reference Laboratory
Laboratoire de Référence OIE/FAO
pour la Brucellose




Introduction

The **Laboratoire de Santé Animale de Maisons-Alfort** (*Animal Health Laboratory*) of **ANSES** (*French Agency for Food, Environmental and Occupational Health Safety*), formerly the LERPAZ laboratory (*Animal Diseases & Zoonoses Research Laboratory*) of AFSSA (*French Food Safety Agency*) was designated by the Commission Regulation (EC) No 776/2006 of 23 May 2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community Reference Laboratories, as the Community Reference Laboratory (CRL) for Brucellosis (*and late 2009, the name CRL became EURL*).

Work programme 2013

As requested by letter SANCO/G5/SG/ck (2012), the following activities are foreseen for 2013

Activity 1. *Support to DG Sanco and to EU Brucellosis NRLs*

Activity 2. *Training activities*

Activity 3. *Inter-laboratory ring-trials*

Activity 4. *Meetings/Workshops*

Activity 5. *Website management*

Activity 6. *Specific studies*

The details of the estimated budget per activity are provided in the attached table. The objectives and expected outputs per activity are as follows:

Activity 1. *Support to DG Sanco and to EU Brucellosis NRLs*

The activity includes in particular the following permanent activities:

- 1.1. Studies on sera presenting unexpected or doubtful results;
- 1.2. Identification and biotyping of *Brucella* strains (when the NRL is unable to fully identify/biotype the strain or in case of atypical strains);
- 1.3. Supplying available field or reference *Brucella* strains and standardised reagents for *Brucella* typing;
- 1.4. Supplying available standardised reagents for brucellosis immunological diagnosis;
- 1.5. Control of diagnostic antigens or kits (EU official tests only) according to EU or OIE standards;
- 1.6. Control of national secondary standards as adequately standardised against international primary standards;

Sub-activities (1.1) to (1.6) cannot be planned in advance since it depends on the NRLs and DG-Sanco requests.

- 1.7. Establishment of Standardised Technical procedures at the EU level;

No work planned in 2013 within this sub-activity

- 1.8. Collection of representative samples of *Brucella* strains isolated in the EU and maintenance of the collection;





Due to the very low number of strains spontaneously sent by the NRLs in the past, the EURL foresees to request a number of strains per member state based on the frequency of isolation in the corresponding member state. For 2013, the species concerned will be *B. melitensis* biovar 3 and the EURL will organise one shipment per concerned NRL, i.e., of the South-European area (Spain, Greece, Portugal and Italy, as well as if possible from Croatia, FYROM and Turkey). Due to the recent outbreaks in Belgium, *B. abortus* biovar 3 will be also collected from this country.

1.9. Report of the Brucellosis EU NRLs' annual activity.

A questionnaire will be sent early 2013 to all EU NRLs in order to get a detailed report of their activities in 2011 and 2012. The information data will be gathered with those already collected from the years 2008-2010 to prepare a 5-year activity report.

All activities will be reported in the EURL annual technical report and presented to the NRLs at the latest during the next EURL 2013 workshop. The way of optimising these activities for the benefit of each NRL (especially the way of collecting and shipping strains) will be discussed during this workshop as well. The EURL will also continue to provide full assistance to the services of DG SANCO in charge of animal and public health as regards Brucellosis in man and animals.

Activity 2. Training activities

Taking into account the too high number of applications received from the NRLs for attending the 2 training sessions organised in 2012, the EURL plans to organise again the same two sessions in 2013, i.e.:

- A 2-day training session on "Bacteriological isolation, identification and typing of *Brucella* spp. According to OIE and EU standards". For bio-safety and bio-security reasons (work in BSL3 facilities) this session will be limited to 8 NRLs (one participant per NRL);
- A 2-day training session on "EU standardised Complement Fixation test in Brucellosis serological diagnosis". This session will be open to 8 NRLs (one participant per NRL) with a priority given to NRLs having faced difficulties in implementing the EU standardised CFT during the last inter-laboratory ring-trials;

The objective is to evaluate the technical skills of the EU NRLs on the direct diagnosis of *Brucella*, and to help some NRLs in implementing the EU CFT Standard Operating procedure for indirect diagnosis of brucellosis.

Activity 3. Inter-laboratory ring-trials

Two inter-laboratory ring-trials are foreseen for 2013:

- A first inter-laboratory ring-trial is planned for the 1st semester of 2013. This trial aims at the inter-laboratory validation of the newly established EU Porcine brucellosis (EUPBSS) and EU *Brucella ovis* (EUBoSS) Standard Sera in comparison with the OIE corresponding standards (ISaBsS and ISaBoS). The objective of this 1st ring-trial is to establish the standardisation criteria for indirect ELISA with regards





- respectively to Porcine brucellosis and *B. ovis* infection serological diagnosis. ELISA has been recently introduced as an official test for Porcine brucellosis in the EU, applicable to intra-Community trade in and imports of semen of domestic animals of the porcine species (*Commission Implementing Regulation (EU) no 176/2012 of 1/03/12*) and criteria for the standardisation of ELISA have also been recently established at OIE level with the respective international standard. As far as *B. ovis* infection is concerned, there are no criteria at OIE level for the standardisation of ELISA while this test is not yet an official test for trade in the EU for this particular disease. But the EURL has recently published a study validation of the ELISA in comparison with the CFT for the control of *B. ovis* infection and aims at proposing the ELISA as an official test for trade in the next future based on this validation and once the standardisation criteria will be established.
- A second ring-trial (ILPT) regarding the performance of milk ELISA in bovine brucellosis (Milk- iELISA) is planned for the 2nd semester of 2013. The objective of this 2nd (proficiency) ring-trial is to evaluate the progress made by EU NRLs in performing the test, prescribed in dairy cattle the control/surveillance/eradication programmes in the EU. A similar proficiency ring-trial was organised in 2008-2009 and in 2011-2012. This ILPT will give the EURL the opportunity to evaluate the improvement of the indirect diagnostic performance through the EU NRL network, in particular for NRLs who showed some difficulties during the last ILPTs.

Activity 4. *Meetings/Workshops*

A two-day workshop will be organised during fall 2013, in order to present, share and discuss with all EU NRLs:

- the 2012 activity report and the 2013 work programme of the EURL
- the results and the analysis of the ring-trials organised by the EURL since the 5th workshop planned to be held in Maisons-Alfort in December 2012, *i.e.* the ILPT on direct diagnosis planned to be launched in October 2012 and the first ring-trial planned in 2013 as mentioned above. The second day will be dedicated to discussions and free-presentations (topic to be discussed and decided during the next workshop). This workshop is planned to be held, if possible, in Zagreb, Croatia.

Added to the Member states' NRLs (including 2013 new member state Croatia), the Norwegian and the Swiss NRLs, one representative per NRL of the candidate countries (FYROM, Iceland, and Turkey), as well as one contact per West Balkan country (Albania, Kosovo, Bosnia-Herzegovina, Montenegro and Serbia), will be invited to attend this workshop. A technical report of this workshop will be prepared by the EURL with a CD including all presentations made during the workshop. As previously, the report of this workshop will be sent to DG-Sanco within two months after the meeting.

Activity 5. *Website management*

A Brucellosis EURL website is being developed to facilitate NRLs access to information and exchanges between NRLs and the EURL. The architecture of the site, the design and the selection of the different items included,





concerning brucellosis, in the EU in particular, were delayed due to the complete change of the Anses Website in 2011-2012 that has taken a longer time than initially foreseen. The specific EURL website is now planned to be available in 2013. This website will be then regularly managed and updated.

Activity 6. Specific studies

6.1. To address the recommendations of EFSA for bovine brucellosis:

In order to address the recommendations made by EFSA “on a request from the Commission concerning Brucellosis Diagnostic Methods for Bovines, Sheep, and Goats” (<http://www.efsa.europa.eu/en/scdocs/doc/432.pdf>), a study was implemented in 2011 as regards the main recommendation concerning the Competition Enzyme Linked Immunosorbent Assay (cELISA) in bovine brucellosis diagnosis, i.e.: “ *this type of test should remain in the EU legislation on intra-Community trade, where it is currently included i.e. as a complementary test, pending the conduct of further studies*”. /...“*aiming at providing further data generated through studies conducted in accordance with the OIE procedure and consistent with the fitness for purpose*”.

The study included:

1. The collection of 5 000 serum samples from OBF herds, including samples evidencing false positive serological reactions (FPSR);
2. The collection of 300-500 serum samples from infected herds (from MS still infected with bovine brucellosis);
3. The comparative analysis of this collection in EU approved tests (RBT, CFT, SAT, FPA, and iELISA) and cELISA of different formats (i.e. the 3 different commercial kits available in the EU);
4. The analysis of test results of cELISA in terms of (i) sensitivity and specificity in comparison with other EU approved tests and (ii) efficiency as a confirmatory test in relation with the test format and the respective standardization with the international standard sera.

Steps 1-3 were completed in 2011. Step 4 is currently being completed (results presented at the next workshop in December 2012). A scientific article will be prepared and submitted in 2013.

6.2. To address the recommendations of EFSA for sheep and goat brucellosis:

In order to address the recommendations made by EFSA “on a request from the Commission concerning Brucellosis Diagnostic Methods for Bovines, Sheep, and Goats” (<http://www.efsa.europa.eu/en/scdocs/doc/432.pdf>), this activity was launched in 2012 as regards the main recommendation concerning the Indirect and Competition Enzyme Linked Immunosorbent Assay (iELISA and cELISA) in sheep and goat brucellosis diagnosis, i.e.: “ *It should be noted that, with the exception of RIDNH and BST, the new tests (cELISA1, cELISA3, FPA, iELISA1, iELISA3, and MRBT) have specificity lower compared to standard tests or not sufficiently documented (cELISA2 and iELISA2). When using Se and Sp as criteria for assessing the fitness for the purpose of intra-Community trade, it can be concluded that these new tests are not suitable for inclusion in Annex C unless new data demonstrate that these tests are at least as specific as the standard tests. Hence, studies may need to be conducted to evaluate whether changes in technical specifications may improve specificity of these new tests without compromising their sensitivity. For cELISA2 and iELISA2 it is recommended that the necessary specificity data be generated.*”





The already started EURL study project aims at addressing these recommendations. The objective is similar to the one mentioned above for Bovine brucellosis.

The project includes:

1. The collection of at least 5 000 serum samples from OBmF flocks including, when possible, samples with FPSR;
2. The collection of at least 300 serum samples from infected herds (in Southern MS);
3. The comparative analysis of this collection in EU approved tests (RBT, CFT) as well as in, FPA, iELISA (standardised according recently adopted criteria against the OIE ISaBmS international standard serum) and cELISA of different formats (*i.e.* at least 3 different commercial kits available in the EU);
4. The analysis of test results of cELISA in terms of (*i*) sensitivity and specificity in comparison with other EU approved tests and (*ii*) efficiency as a confirmatory test in relation with the test format and the respective standardization with the international standard sera.

Steps 1-2 have been already almost completed and step 3 will be implemented in 2012. The end of step 3 as well as step 4 is foreseen to be completed in 2013. If possible, the results of the analysis will be presented during the 2013 workshop).

6.3. Molecular typing as a tool for improving the investigation of Brucellosis outbreaks and human cases:

The recent outbreaks of *Brucella abortus* and *Brucella melitensis* in cattle farms, respectively in Belgium and in France, two officially bovine brucellosis member states, revealed the important need of providing a fast and reliable diagnosis, as well as an accurate epidemiological investigation for determining the origin and the potential extent of the infection. The fine identification of epidemiological links between strains isolated during the outbreak with strains isolated in the past and in other regions appears crucial for giving more accurate and rapid epidemiological answers..

Several molecular typing methods are currently available to identify specific genotypic markers in *Brucella* strains. So, the MLVA-16 (Multilocus VNTR analysis), based on the study of minisatellites and microsatellites repeats within 16 loci, has a high discrimination power according to the considered species. However, no interpretation criteria of the similarity degree between two strains are available up to now, what complicates, and even lead to erroneous interpretation of the results within the framework of epidemiological investigations. To establish such criteria, it is essential to estimate the probability of variability (mutation/addition/deletion) at each specific locus of the considered species. We propose to develop *in vitro* artificial systems miming the intracellular conditions (oxidative stress, acid stress, hypoxia, nutrient depletion...) to test the stability of MLVA markers. This approach will be completed by the optimization of the MLVA method (multiplex capillary-based MLVA assay development).

This work aims at the establishment of MLVA interpretation guidelines to *Brucella*, according to the existing criteria for pulsed field gel electrophoresis, allowing the definition of phylogenetic and epidemiological relationship without ambiguity. This preliminary work will be dedicated first to *Brucella melitensis*, which remains the most important species in the EU as well as in the rest of the world.

