French Agency for Food, Environmental & Occupational Health Safety

Maisons-Alfort

LABORATOIRE DE SANTE ANIMALE

ANIMAL HEALTH LABORATORY

Unité Zoonoses Bactériennes

Bacterial Zoonoses Unit

Centre National de Référence des *Brucella*

National Reference Centre for Human Brucellosis

Laboratoire Associé au CNR du Charbon

National Reference Centre for Anthrax Associated Laboratory

Laboratoire National de Référence pour la Brucellose, la Chlamydiose, 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 l'UE pour les Maladies Equines (Morve)

EU Reference Laboratory for Equine Diseases (Glanders)

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2012 Work Programme of the EU Reference Laboratory for Brucellosis

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Introduction

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 EU-RL)*.

As of July 1st 2010, AFSSA has become **ANSES** (*French Agency for Food, Environmental and Occupational Health Safety*) and LERPAZ has become the **Laboratoire de Santé Animale de Maisons-Alfort** (*Animal Health Laboratory*).

Work programme 2012

As requested by letter SANCO/G5/SG/aal Ares(2011) 768299, the following activities are foreseen for 2012

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 is given 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:

- *(i)* Studies on sera presenting unexpected or doubtful results;
- *(ii)* Collection of representative samples of *Brucella* strains isolated in the EU and maintenance of the collection;
- *(iii)* Identification and biotyping of *Brucella* strains (when the NRL is unable to fully identify/biotype the strain or in case of atypical strains);
- *(iv)* Supplying available field or reference *Brucella* strains and standardised reagents for Brucella typing;
- *(v)* Supplying available standardised reagents for brucellosis immunological diagnosis;
- *(vi)* Control of diagnostic antigens or kits (EU official tests only) according to the EU or OIE standards;

(vii) Control of vaccines final batches (to be put on the EU market) and original seed-lots according to the EU or OIE standards.

(viii) Establishment of Standardised Technical procedures at the EU level.

All these activities will be reported to the NRLs at the latest during the next NRLs' 2012 meeting. The way of optimising these activities for the benefit of the NRLs (especially the way of collecting and shipping strains) will be discussed during this workshop as well. The EU-RL will also go on providing 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*

Two training sessions are planned in 2012:

- A training session of 5 days as regards the "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 5 NRL's participants (one participant per NRL);
- A training session of 2 days as regards the "EU standardised Complement Fixation test in Brucellosis serological diagnosis". This session will be open to 5 NRL's participants (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 improve the technical skills of the EU NRLs in two important and complicate methods of brucellosis diagnosis in animals.

Activity 3. *Inter-laboratory ring-trials*

Two inter-laboratory ring-trials are foreseen for 2012:

- A first ring-trial planned for the 1st semester of 2012 and regarding the performance of serological tests in brucellosis (RBT, CFT, SAT and iELISA). The objective of this 1st ring-trial is to evaluate the progress made by the EU NRLs for performing the respective tests, which are the one prescribed for the control of animal movements at EU and international levels and/or recommended for the control/eradication programmes in the EU.
- A second ring-trial planned for the 2nd semester of 2012 and regarding the isolation, identification and typing of *Brucella* strains. This trial will also include the VNTR analysis (MLVA). This ring-trial will be the first one regarding such a topic ever organised in the whole EU. It will need an important work of preparation, due, in particular, to the international and intra-community rules in force as regards the movement of strains potentially usable as biological warfare. This trial aims at evaluating the capacity of the NRLs in identifying the *Brucella* strains.

Activity 4. *Meetings/Workshops*

A one-day meeting will be organised at the end of 2012 to present, share and discuss with all EU NRLs:

- the 2011 activity report and the 2012 work programme of the EU-RL

- the results and the analysis of the 3 ring-trials organised by the EU-RL since the 4th workshop held in Malta in May 2011, *i.e.* the ring-trial on milk tests planned to be launched in October 2011 and the two ring-trials planned in 2012 as mentioned above.

A technical report of this workshop will be prepared by the EU-RL with a CD including all presentations made during the workshop. As previously, the report will be sent to DG-Sanco within one month after the meeting.

Activity 5. *Website management*

A Brucellosis EU-RL website will be developed to facilitate NRLs access to information and exchanges between NRLs and the EU-RL. The architecture of the site, the design and the selection of the different items included, concerning brucellosis, in the EU in particular, will be achieved during the second semester 2011. The site should be available in 2012. This website will be regularly managed and updated.

Activity 6. Specific studies

- **Future EU standard sera for porcine brucellosis and *B. ovis* contagious epididymitis:**

The batches of future EU standard sera for porcine brucellosis and B. ovis contagious epididymitis that are to be freeze-dried by the end of 2011 will be checked for homogeneity and stability during 2012. An interlaboratory ring-trial aiming at final validation of the standards is planned to be held in 2013.

- **To address the recommendations of EFSA for bovine brucellosis:**

In order to address the recommendations (http://www.efsa.europa.eu/en/scdocs/doc/432.pdf) made by EFSA "on a request from the Commission concerning Brucellosis Diagnostic Methods for Bovines, Sheep, and Goats", 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 reactions;
2. The collection of 300-500 serum samples from infected herds (in 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 will be completed by the end of 2011. Step 4 will be implemented in 2012

- **To address the recommendations of EFSA for sheep and goat brucellosis:**

In order to address the recommendations (http://www.efsa.europa.eu/en/scdocs/doc/432.pdf) made by EFSA "on a request from the Commission concerning Brucellosis Diagnostic Methods for Bovines, Sheep, and Goats", an additional activity is proposed for 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 EURL study project will try to address these recommendations. The objective would be similar as the one under progress in 2011 as Bovine brucellosis is concerned.

The project could include:

1. The collection of 5 000 serum samples from OBmF flocks;
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 and if possible step 3 will be implemented in 2012. Then end of step 3 as well as step 4 are foreseen to be completed in 2013