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 National de Référence pour la Brucellose, la Chlamydiose, la Fièvre Charbonneuse, la Morve, la tuberculose & la Tularémie animales

Animal Anthrax, Brucellosis, Chlamydiosis, Glanders, Tuberculosis & Tularaemia National Reference Laboratory

Laboratoire de Référence O.I.E. / FAO pour la Brucellose, et la Tuberculose bovine

OIE /FAO Reference Laboratory for Brucellosis, and Bovine 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)

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2014 Work Programme of the EU Reference Laboratory for Brucellosis

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 2014

The following activities are foreseen for 2014

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 2014 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 decided in 2012 to request a number of strains per member state based on the frequency of isolation in the corresponding member state. For 2014, the species concerned will be B. melitensis biovar 1 and 2 (biovar 3 collected in 2013) and the EURL will organise one shipment per concerned NRL, *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). These strains will be biotyped in details before inclusion in the EURL Brucella collection.

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

A questionnaire will be sent to all EU NRLs in order to get a detailed report of their activities in 2013. The information data will be summarised and communicated to DG Sanco and the whole network.

All activities will be reported in the EURL annual technical report and presented to the NRLs at the latest during the next EURL 2014 workshop. The way of optimising these activities for the benefit of each NRL 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 2013, the EURL plans to organise again the same two sessions in 2014, *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) with a priority given to NRLs having faced difficulties during the last corresponding inter-laboratory ring-trial;
- 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.

While priority will be given to EU MSNRLs, these training sessions will be open to EEA countries having faced difficulties in previous corresponding ring-trials, and training/accommodation fees will be taken care of by the corresponding EEA NRL.

Activity 3. *Inter-laboratory ring-trials*

Two inter-laboratory ring-trials are foreseen for 2014:

- A first inter-laboratory proficiency ring-trial (ILPT) regarding the performance of serological tests in brucellosis (RBT, CFT, SAT and iELISA) is planned for the 1st semester of 2014. The objective of this 1st ring-

trial is to evaluate the progress made by EU NRLs in performing the respective tests, prescribed for the control of animal movements at EU and international levels and/or recommended for the control/eradication programmes in the EU. Similar proficiency ring-trials were organised in 2007-2008 and in 2012. This third session 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.

- A second ILPT regarding the performance of the official batch to batch control of Rose-Bengale diagnostic antigens is planned for the 2nd semester of 2014. The objective of this 2nd ILPT is to evaluate the performance of such controls in the EU and their conformity to the Annex C of EC 64/432 directive requirements.

Activity 4. *Meetings/Workshops*

A one and a half-day workshop will be organised during fall 2014, in order to present, share and discuss with all EU NRLs:

- the 2013 activity report and the 2014 work programme of the EURL
- the results and the analysis of the ring-trials organised by the EURL , as mentioned above, after the 6th workshop planned to be held in Zagreb in September 2013. The second day will be dedicated to discussions and free-presentations (topic to be discussed and decided during the 2013 workshop). The 2014 workshop is planned to be held, if possible, on 8-9 September 2014, in Berlin, Germany, just before the 2014 Brucellosis International Conference planned to be held on 10-12 September and organised by the BfR (German Federal Institute for Risk Assessment). This might encourage the European NRLs to attend this event and reinforce the European presence to this unique and well-known brucellosis biennial international event.

Added to the Member states' NRLs, the Norwegian and the Swiss NRLs, one representative per NRL of the candidate countries (FYROM, Iceland, Montenegro, Serbia and Turkey), as well as one contact per West Balkan country (Albania, Bosnia-Herzegovina, Kosovo), will be invited to attend this workshop. A summary of this workshop will be prepared by the EURL and information distributed to DG Sanco and all NRLs.

Activity 5. *Website management*

A Brucellosis EURL website will be operational by the end of 2013. It aims at facilitating NRLs access to information and exchanges between NRLs and the EURL. All NRLs will have access to a EURL private section and general information on Brucellosis and the missions of the EURL will be of public access. The content will be regularly managed and updated.

Activity 6. Specific studies

6.1. To address the recommendations of EFSA for ovine and caprine 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 on-going EURL study project aims at addressing these recommendations.

The project includes:

1. The collection of at least 4320 serum samples from OBmF flocks including, when possible, samples with FPSR;
2. The collection of at least 1040 serum samples from infected herds (in Southern Europe);
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 1-3 have been already completed. The step 4 is foreseen to be completed in 2014. If possible, the results of the analysis will be presented during the 2014 workshop.

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

Brucellosis, a worldwide zoonosis due to *Brucella* genus, has a serious public health impact and causes economic losses to animal sector. The remaining prevalence of the disease in several member states, as well as the recent outbreaks in cattle and wildlife in Belgium and France- two officially bovine brucellosis-free member states- and the high prevalence of *B. suis* bv 2 in European wildlife emphasise the need for improved surveillance tools.

Identifying genetic variants is important for the back-and-forward tracing of outbreaks (epidemiological links and sources) and is essential for surveillance programs in both human and animal health.

The current optimal tool for *Brucella* strains epidemiological genotyping is the MLVA-16 assay (Multiple-Locus-Variable-number-tandem-repeat-Analysis), which involves 16 genetic markers, organized in panels, according to their evolution speed: panel 1 (8 mini-satellites), more stable than the panel 2 micro-satellites (3 for panel 2A and 5 for panel 2B).

In other pathogens, closely related isolates could harbour some small VNTR pattern variations due to random genetic events (insertion, deletion, point mutation). These events may impact MLVA data interpretation in epidemiological investigations. In *Brucella* spp., no interpretation criteria of the similarity degree between two strains are available up to now; this complicates, and even leads 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 at each specific locus of the considered species.

Accordingly, we propose to investigate the genetic stability of MLVA-16 markers at species and biovar levels, and its consequences for *Brucella* MLVA-data interpretation.

We propose (*i*) to compare MLVA *in vivo* patterns, *i.e.* animal and human strains, clustered in epidemiologically-related groups (same outbreak, same contamination source, laboratory-acquired infection...) and (*ii*) to develop *in vitro* artificial systems miming the intracellular conditions (oxidative stress, acid stress, hypoxia, nutrient depletion, growth in antibiotic presence…) in order to test MLVA locus stability.

Our investigations started under the EURL 2013 work-programme and give priority to the most relevant *Brucella* species in the EU: *B. melitensis* and *B. suis* bv 2. Both *in vivo* and *in vitro* studies suggested that some pattern variations do exist between closely related *Brucella* strains which may alter data interpretation. The *in vivo* studies evidenced that 32 % of epidemiological groups showed at least one Single-Locus Variation, including 8 % with Double-Loci Variations and 12 % with multiple-loci variations (4 loci). In parallel, the *in vitro* studies suggested that the presence of antibiotics could affect the rate of genetic events, responsible for VNTR variations, in reference strains, as *B. melitensis* bv 1 strain 16M and *B. suis* bv 2 strain Thomsen.

The work planned in 2014 aims at completing the 2013 preliminary studies for an in-depth definition of the MLVA locus stability, in order to determine *Brucella* specific guidelines for MLVA data interpretation.