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EUROPEAN REFERENCE LABORATORY (EU-RL)

FOR BOVINE TUBERCULOSIS

WORK-PROGRAMME 2012

PROPOSAL – Version 2

VISAVET

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Table of contents

Preamble

The work-programme of the European Union Reference Laboratory for bovine tuberculosis during 2012 is based on the actions planned for five years that were designed to fulfill the responsibilities for Community Reference Laboratories described in the Article 32 of Regulation (EC) No 882/2004 and the additional responsibilities and tasks laid down in Annex II to Commission Regulation (EC) No 737/2008 of 28 July 2008.

This current programme follows the one designed for 2011 in both the outline and description of tasks including new activities suggested by the Commission (ie. diagnosis of tuberculosis in camelids). Tasks described in this programme will be performed in consultation and collaboration with the Commission and Member States. A degree of flexibility should be understood to suit the needs of the Commission and the Member States arising during this period.

The objective of the work-programme is to cover the five points of Annex II to Commission Regulation (EC) No 737/2008:

1. To coordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing bovine tuberculosis; and

2. To facilitate the harmonization of techniques throughout the Community, in particular specifying standard test methodologies.

3. To organize workshops for the benefit of national reference laboratories as agreed in the work-programme and annual budget referred to in Articles 2 to 4 of Regulation (EC) No 156/2004, including training of experts from the Member States and, as appropriate, from third countries, in new analytical methodologies.

4. To provide technical assistance to the Commission and, upon its request, to participate in international for a relating to the diagnostic of bovine tuberculosis, concerning in particular the standarisation of analytical methods and their implementation.

5. To perform research activities and, whenever possible, co-ordinate research activities directed towards the improved control and eradication of bovine tuberculosis.

A. MAIN ACTIVITIES OF THE EURL FOR BOVINE TUBERCULOSIS FOR 2012

1. Potency test of tuberculins

A key task of the EURL are the potency testing studies of all tuberculins (Purified Protein Derivative, PPD) and antigens submitted by National Reference Laboratories. These reagents are essential for *in vivo* and *in vitro* diagnostic assays based on cell-mediated immune response. Single intradermal tuberculin (SIT) or single intradermal cervical comparative tuberculin (SICCT) test, are the main techniques used worldwide as diagnostic tests in the tuberculosis eradication campaigns and large differences among potencies depending on manufacturers and batches could affect to the detection of reactor animals.

The potency of a tuberculin will estimated by comparing the size of the reaction elicited by an intradermal inoculation and comparison to the size of the reactions of a "standard" tuberculin of known potency (CVI, Lelystad). To meet the requirements of statistical analysis each tuberculin is used at two dilutions, usually, at normal strength (1mg/ml) and 20% of normal strength.

The PPDs and antigens will be evaluated by using the interferon-gamma assay (IFN-γ). This *in vitro* test is based on the detection of IFN-γ in plasma supernatants from tuberculin-stimulated whole blood culture. Comparative evaluation of different PPDs and antigens will be carried out using blood from infected cattle and goats housed at the research farm and/or field trials. The true infection status to determine sensitivity and specificity will be been determined by post-mortem studies (presence/absence of macroscopic lesions and samples will be collected for culture of mycobacteria).

Several potency testing has been carried out in 2010-2011 by the EURL. The results showed that some tuberculins (from at least five manufacturers) need to be re-evaluated in 2012. Therefore, field trials will be done to test these tuberculins. In addition, the EURL is open to the Commission's suggestions.

The duration of this task will be yearly.

1.1. Factors affecting the intradermal skin test

Apart from the potency of the tuberculin, other factors such as keep syringes in working order, specific training of the veterinarians, or inoculation site, can affect to the correct detection of reactors by skin testing. During 2012, the EURL will evaluate the equipment of skin testing used in the National Reference Laboratories (callipers, syringes, etc.). Skin testing is considered relatively a subjective technique. To minimize the effect on the results due to human factors, training courses about skin testing are required. EURL has

previously organized a training course for veterinarians involved in the eradication campaigns in Madrid and the objective is to arrange similar courses for 2012 for the countries that request them. Other factors considered by the EURL that can affect the results of the skin test are the correct maintenance of the tuberculins (storage conditions) or management related to the productive type of the animals (i.e. in dairy cows the development of the SIT test is easier than bullfighting animals)

In summary, a main task for the EURL will be the study of all these factors separately and determine how they affect to the outcome of the test. Moreover, it is mandatory to unify the procedures to perform the skin test.

The duration of this task will be yearly.

1.2. Desensitization of infected animals

In the potency testing studies performed in the last years a desensitization effect in cattle that have reacted previously has been detected. These naturally-tuberculosis infected animals have not reacted to the skin test after several potency trials. This is a phenomenon has been already described in some scientific studies in cattle and it is an urgent task to study for the EURL, not only for the consequences in the diagnosis but also for the limitations in the number of trials to perform each year in the same infected animals.

The duration of this task will be yearly.

2. Titration of tuberculins

The aim of this study is to find a future alternative to the *in vivo* potency testing trials, reducing animal experiments and improving animal welfare. The EURL will determine the effect of different tuberculin concentrations on the IFN-γ assay production and also correlate the biological potency of PPDs in intradermal tests with the production of IFN-γ in tuberculosis infected animals. To achieve this goal it will be necessary to adjust the working concentration in relation to the International Standard tuberculin.

Several concentrations had already been tested, lower and higher concentrations than the standard one (20µg/ml) although no conclusive results were found in the preliminary data. The new studies will be performed in cattle with natural tuberculosis infection (experimental model) and then it will be performed in goats.

The duration of this task will be yearly.

3. Diagnosis of tuberculosis in camelids

The EURL has as a priority to continue with the studies about sensitivity and specificity of diagnostic tests in camelids. During 2011, several studies focused mainly in the specificity of skin tests and serology has been carried out. The objective for 2012 is to infect around 30 alpacas (aerogenous route of infection) and to evaluate the current diagnostic test. We will perform studies about sensitivity of skin test, IFN-γ assay, and serological tests based on the detection of specific antibodies that has been developed in recent times. The objective is to find a suitable test for the camelids' trade. Animal infection will be confirmed by bacteriology.

The duration of this task will be yearly.

4. Cut-off point in the IFN-γ test

The effect of applying different cut-off points on the sensitivity and specificity of the IFN-γ assay has been previously studied by the VISAVET Centre, mainly in goats. In this regard, it is necessary to establish the most adequate cut-off point depending on the species in which the assay is applied and the epidemiological conditions of the region or country. In this sense, more studies will be perform mainly in cattle but also in camelids to evaluate several cut-off points used in different countries and to find new ones that could be more suitable.

The duration of this task will be yearly.

5. Development of an European Standard

The European Pharmacopoeia recognized several years ago the WHO international standard for bovine tuberculin. The stock of the actual international standard is limited and long-standing and it could be desirable to produce another standard to replace it. The objective for 2012 is to start the studies that are necessary to find another tuberculin with similar potency to the current standard. Moreover, the best preservation system of the tuberculin has to be evaluated with the aim of keeping the quality of the tuberculin throughout the years.

The duration of this task will be yearly to be continued during the following years.

6. Reference reagents

The EURL will prepare and control the reference reagents in order to standardise the protocols used in the different countries and to validate the ring trials. Also, reagents submitted by National Reference Laboratories will be evaluated. During 2012, the EURL priorities will be:

- positive and negative serum and/or plasma from different animal species infected with bovine tuberculosis or other mycobacteria that could interfere in the diagnostic test (i.e. cattle, goat, pig, wild boar, etc.);
- positive and negative lymph nodes for the organization of ring trials.

The duration of this task will be yearly to be continued during the following years.

7. World Wide Web page

The roles and tasks of the EURL for Bovine Tuberculosis will be disseminated via national and international routes to several levels. The best way to report on news regarding the EURL activities is a Word Wide Web page. This will contain:

- addresses of the NRLs for bovine tuberculosis;
- protocols submitted by the NRLs related to the microbiological culture, identification, molecular characterization of mycobacteria, and IFN-γ diagnosis;
- pictures related to the disease (i.e. tuberculosis lesions, tuberculosis skin test reactions);
- control and surveillance programmes for the tuberculosis eradication in the MSs;
- conferences and activities carried out by the EURL;
- comparative tests;
- working programme of the EURL and suggestions.

All information included in this page will require previous authorization from the Commission.

The duration of this task will be yearly to be continued during the following years.

8. Harmonisation of protocols

The EURL for bovine tuberculosis will prepare a protocol to improve the MIRU-VNTR analysis. The protocol will include the allele calling table with the correspondence between PCR amplicons and numbers of whole repeat units for each MIRU–VNTR locus.

Depending on the results of the comparative test for the direct extraction from tissue samples organized at eh end of 2011, the protocol will be optimized for all NRLs.

The duration of this task will be yearly.

9. Comparative tests

During 2012, the EURL will evaluate the performance of IFN-γ assay (BOVIGAM™) in the

National Reference Laboratories since this was a priority discussed in the first workshop (4th - 5th December 2008, Madrid). This assay complements the skin tests and it is capable to improve the overall probability of detecting infected cattle in regions and herds accounting for a high incidence of bovine tuberculosis.

Based on the results obtained in the ring trial of molecular characterization of the members of the *M. tuberculosis* complex (spoligotyping and MIRU-VNTR analysis), the EURL consider necessary to improve and standardize the MIRU-VNTR analysis. Therefore, an additional ring trial will be carried out in 2012.

The duration of this task will be yearly.

10. Missions

For 2012 there is one mission programmed although the EURL for bovine tuberculosis is available for any other missions proposed by the Commission.

A visit to several camelid infected herds is programmed to organize the field experience regarding the diagnosis of tuberculosis in camelids, mainly addressing study the study of the sensitivity of the skin test, IFN-γ detection, and serological tests in order to establish a tool for the camelids' trade.

The duration of this task will be yearly.

11. Meetings

The National Reference Laboratory for Bovine Tuberculosis in United Kingdom (Animal Health and Veterinary Laboratories Agency, AHVLA) has performed a protocol for diagnosis of bovine tuberculosis in camelids by the detection of IFN-γ. By this reason the EURL consider essential a meeting with the person in charge of the AHVLA immunology unit to know about this analysis.

The duration of this task will be yearly.

12. Workshop.

Unless the Commission or the NRLs would consider it essential, a workshop will not be organized in 2012.

B. OTHER ACTIVITIES OF THE EURL FOR BOVINE TUBERCULOSIS FOR 2012.

The following tasks will remain permanent activities of the EURL for 2012.

1. Preparation, control and supply of reference reagents, and protocols to NRL

2. Collection of representative samples of *Mycobacterium* spp.

3. Collection of representative serum/plasma recovered from infected animals

4. Isolation, identification and typing of *Mycobacterium* spp.

5. Supply of the spoligotyping membranes

For this period the EURL will supply the home-made spoligotyping membrane to all NRLs that would like to implement this technique in their own laboratories to make sure the high-quality of the membrane and that the results obtained are reliable.

The duration of this task will be yearly to be continued during the following years.

6. Technical assistance to the Commission and NRLs

The staff of the EURL for bovine tuberculosis will be accessible to provide technical assistance to the Commission and upon its request this will be extended also to its Institutions. The staff could also provide support to Member States on specific issues regarding eradication programs. A contact with Public Health Institutions would be established in order to increase awareness of the zoonoses. Moreover, the Director of the EURL will participate in the bovine tuberculosis subgroup of the Task Force.

The duration of this task will be yearly to be continued during the following years.

7. Training of personnel

Training of experts from the Member States will be performed by organisation of short courses or by individual training. These short visits will be open to all National Reference Laboratories to allow the establishment of new protocols and techniques in their laboratory of origin. Afterwards, the trainees will be requested to submit a brief report.

The duration of this task will be yearly to be continued during the following years.

8. Dissemination

Apart from the World Wide Web page which will contain basic updated information, the information will be disseminated mainly through presentations at international and national congresses or conferences, and publication in international and national journals.

The duration of this task will be yearly to be continued during the following years.

9. Keeping abreast of developments

Staff of the EURL for bovine tuberculosis will keep abreast of developments in

surveillance, epidemiology and prevention of tuberculosis throughout the world. To fulfill this commitment, the members of the Laboratory will get information through different ways (scientific papers in national or international journals, attendance to congresses, and workshops, specific training courses, reports from experts, legislation, etc.) and also through active participation in research projects.

The duration of this task will be yearly to be continued during the following years.

10. Research activities

The EURL for bovine tuberculosis will maintain its active research directed towards the improved control and eradication of bovine tuberculosis through: a) Collaboration with National Reference Laboratories, i.e. relevant problems associated to local farming practices on the epidemiology of the infection in the Member States (livestock breeding systems and specific role of wildlife) and impact on detection of infection in animals; and carrying out validation trials; and b) Analysis of the information collected and preparation of reports associated to the activities of the EURL.

The EURL for bovine tuberculosis will maintain research activities at international level participating in the EU project TB-STEP "Strategies for the eradication of bovine tuberculosis" FP7-KBBE-2007-1, co-ordinated by Dr. Lucas Domínguez. The consortium is made up of 12 partners from eight countries which research on eight workpackages devoted to improved tools and to develop strategies for the eradication of bovine tuberculosis in areas where the disease is present in both domestic and wildlife populations.

The duration of this task will be yearly to be continued during the following years.