European Union Reference Laboratory for Bee Health

WORK PROGRAMME 2014

I. Legal duties

The functions and duties of the European Union Reference Laboratory (EURL) for bee health are described in Commission Regulation (EU) No 415/2013 of 6 May 2013 laying down additional responsibilities and tasks for that laboratory and amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council and repealing Regulation (EU) No 87/2011.

II. Objectives for 2014

Summary:
As a priority mission for 2014, the EURL for honeybee health will pursue the coordination and the organisation of the pan European epidemiological study on honeybee colony losses (EPILOBEE) programmed and co-financed by the European Commission (EC). The field phase of the first year of the study, involving 17 Member States (MS), has begun in 2012, and the second year should start in autumn 2014. The management and the analysis of the 2013-2014 program data should be done in 2014. Moreover the set of data being important and complex, the data analysis from the first year started in 2013 require to be continued in 2014. The specific work dedicated to EPILOBEE is detailed in Chapter 2 (Epidemiology).
In keeping with the work began in 2011, In 2014 the EURL for honey bee health will also continue the work that started in 2011 by providing scientific and technical assistance to national reference laboratories on analytical methods, biological material and information on the area of honey bee health. More specifically, the EURL will continue the development and the validation of relevant analytical methods on the main honey bee diseases and pathogens. The EURL will organise a comparative testing and a training of the National Reference Laboratories (NRLs) experts with a view to the harmonisation of diagnosis methods in Europe.
Moreover, the EURL for honeybee health also works on plant protection products, due to their possible impact on honey bee health. The laboratory will continue the development and validation of relevant assays for measuring insecticide residues at low concentration levels. These processes will complete the set of methods already available and validated by the EURL for honeybee health.

Depending upon the needs, the EURL for honeybee health should provide scientific and technical support to the EC, the MS and the NRLs, specifically on the EPILOBEE survey or on any other subjects.

The large number of scientific areas that has to be covered by the EURL work justifies the costs of the 2014 budget.

Activity 1. Coordination of the network of NRLs for honeybee health

Description:

The coordination of the NRL network for honeybee health is an important task for the EURL. In addition to the European NRLs, other NRLs from third countries are involved in this network: Kosovo, Norway, Switzerland, and Kenya (African reference laboratory for honey bee health co-financed by the EC). The EURL animates the network by organising an annual workshop to inform the NRLs on the achievement of the work program for the different activities and on the news related to honey bee health. This annual workshop is the opportunity to exchange information with the NRLs, to receive news from the partners (e.g.: needs of new pathogen leaflets, technical support, protocols, and/or reference material - see activity 3 on various honeybee diseases) and to harmonise the methods (a ring test and a training meeting are planned in 2014 for the first time). In 2014, the EURL will submit a questionnaire to the network to record updated data about NRL activities in 2013. The design of a collaborative website was implemented in 2013. The EURL will feed the website with documents (leaflets, protocols, material reference requests, proficiency test reports) and with information useful for the NRL network. This website will be used to submit to the NRL network satisfactory questionnaires and collect the answers. In addition to this website, the animation of the network will be continued through the dedicated email address.

Objectives:

- **Coordination of the NRL network.** It will rely on the animation of the network via the collaborative website, the organisation of the **annual workshop** and the dedicated email box.
- **A questionnaire** filled by each NRL should record updated data for 2013 **concerning the ring tests organised, and the activities of the NRLs concerning the diagnosis of the various diseases**. These results should help to follow and harmonise the activities of the network.
- **A summary of the results** should be produced by the end of 2014.

In the light of the previous years experience some meetings between EURL staff (head - Magali Chabert-Ribière, deputy head - Marie-Pierre Chauzat, the epidemiologist in charge of the epidemiological study) and the EC officers are needed. Consequently the cost of 2 travels for x persons to Brussels has been included in the 2014 budget.

Expected outputs:

- Organisation of the annual workshop
- Production of the annual workshop minutes
- Production of supporting documents and presentations for the meeting
- Production of a report on the updated data from the NRL activities

Activity 2. Epidemiology

Description:

The priority mission of the EURL for honeybee health is to provide support to the EC with the development of the epidemiological study on honeybee colony losses in Europe (EPILOBEE).

This study involves a relatively high number of MSs (17). For the first year the field phase began in September 2012 and ended in September 2013. The field phase of the second year should begin in September 2013 and finish in September 2014.

Apart from the surveillance of colony losses (overwintering colony mortality, season colony mortality), the major honeybee diseases should also be surveyed during this study. The clinical prevalence of varroosis, American Foulbrood (AFB), European Foulbrood (EFB), Nosemosis and Chronic paralysis (CBPV) should be estimated. The *Varroa destructor* infestation rate before winter should be assessed. Finally, this project should ensure an early alert in case of the detection of the two exotic arthropods *Aethina tumida* and Tropilaelaps spp.

The high number of MSs participating to the study, the large amount of data to be processed and analysed and the need for day-to-day communication with the network, request appropriate staff allocation to the study which justifies the budget required for the staff effort.
Depending upon the necessity of the project and the requests of the EC, the objectives should be:

- **scientific and technical support to the EC**:
  - Provide advices and guidance on the coordination of the epidemiological study (amended guidelines, evaluation of follow-up projects for each MS).
  - Production of the models of the intermediate and the final technical reports for the visits related to EPILOBEE.

- **scientific and technical support to the MSs for the coordination of EPILOBEE** :
  - Technical support: improvement of a European database online, models of forms for visit 2 and visit 3,
  - Advices and guidance on the setting up of the epidemiological protocols (explanation of the project protocol, advice on data entry in the European database, management and processing of data).

- **scientific and technical support to the NRLs**:
  - Information on surveyed pathogens and diseases (leaflets on American foulbrood and varroosis),
  - Advice on analytical methods, supply of protocols developed and used by the EURL
  - Supply of biological material (positive and negative analytical controls),
  - Confirmatory diagnosis.

- **management of the data for EPILOBEE:**
  - Supply of automatic processes to extract data from the database and data cleaning steps,
  - Management and processing of data (mathematical formulation for the calculation of the mortality rates, calculation of disease prevalence),
  - Presentation of the results to the MSs and to the EC,
  - Writing a technical report.

- **organisation of a training meeting:**

- With the MSs in order to exchange on EPILOBEE results and practical aspects. The training date will be planned to enable the best possible organization.

In 2012, it has been shown that the development and the management of the Web-based data base needs updates and improvements to fit to the specificity of the national programmes conducted by the participating MS and to answer their needs of organisation. Some of these tasks were subcontracted in 2013. It is expected that new updates will be needed also for the second year of EPILOBEE. Consequently the cost of subcontracting has been included in the 2014 budget.

The experience from 2012 and 2013 has shown that the coordination of the epidemiological study and the animation of the NRL network induced a lot of mail exchanges with the NRLs. The transmission of methods and biological materials induces some scientific secretary work (management of the requests, of the answers, post sending). Moreover the EU trainings and missions require a precise and complex budgetary control and organisation. For the EURL to be fully operational, a scientific secretary is necessary (part time on the EURL, 6 months).

Expected outputs:

- Update and improvement of the Web database for the management of EPILOBEE data
- Production of a template for the scientific and technical reports (intermediate and final) to the MSs
- Production of a report on the data from the different MSs
- Production of the training meeting minutes
- Production of a table for the evaluation of the follow up studies (second year of EPILOBEE)
- Supply biological materials and confirmatory diagnoses to the NRLs for the disease study

Activity 3. Work programme on the various honeybee diseases

Description:

In 2014, the EURL work program will focus on the major honey bee diseases and pathogens:

- the two main bacteria responsible of the American and the European foulbrood (*Paenibacillus larvae, Melissococcus plutonius*, respectively*);*
- the main parasites present in Europe: the parasitic mites *Varroa destructor* and *Acarapis woodi* and *Nosema* spp.
- the exotic parasites *Tropilaelaps* mites and *Aethina tumida* ;
- the five major viruses (CBPV, SBV, ABPV, DWV and BQCV).

The EURL will provide assistance to the NRL network by supplying protocols and reference materials and should initiate the harmonisation of the analytical techniques by organising:
- A ring test on the molecular identification of the American foulbrood bacterial agent,
- The first training on the identification of the exotic parasites.

Activity 3.1. Bacteriology programme

Description:

There is a wide range of methods currently used by the NRLs for diagnosing American foulbrood (AFB) and European foulbrood (EFB) and for detecting and identifying the primary causal agents *P. larvae* and

M. plutonius. Among the set of methods already available, the EURL will validate real-time PCR quantification assays for identification and quantification of these bacterial agents and will produce positive-control samples. These reference methods and reference materials will allow to compare and to evaluate the sensitivity and specificity of other methods used by the NRLs in the purpose of harmonisation of the diagnostic techniques throughout the European Community.

Objectives:

- **Finalisation of the real-time PCR quantification assays for identification and quantification of *P. larvae* (AFB)**

The validation of the real-time PCR assays for identifying and quantifying *P. larvae* performed in 2013 will be implemented. This method should be submitted for publication. This publication step will allow the production of a validated method to the NRLs and to the international scientific community.

- **Validation of the real-time PCR quantification assays for identification and quantification of *M. plutonius* (EFB)**

The validation of the real-time PCR assays developed in 2013, for identifying and quantifying *M. plutonius* should be pursued. The real-time PCR assay performances should be assessed by determining the specific characteristics, such as analytical specificity, limit of detection (LOD_{PCR}), limit of quantification (LOQ_{PCR}) and linearity range of the real-time PCR technique. Moreover, specific characteristics of the method should be assessed in the larvae matrix. The assays should be performed in accordance with the criteria of the French Standard XPNF U47-600 for animal health analysis methods - PCR. The results will allow the validation of the method.

- **Comparative laboratory test/ Ring test on molecular identification of *P. larvae* (AFB)**

In 2013, a first comparative laboratory test on the detection in larva and honey samples of *P. larvae* was prepared in collaboration with Dr. W. Ritter (OIE laboratory of Freiburg). The results provided by the 11 participating laboratories showed a weak sensitivity and specificity for the detection of *P. larvae* in honey. Moreover, a wide diversity of techniques was used by the NRLs.
Another ring test will be organised for the identification of *P. larvae* with molecular techniques (PCR, real time PCR). All the NRLs will be invited to participate to this ring test. The specificity of the molecular techniques used by the NRLs will be tested on a panel of samples of *P. larvae* and of related bacteria.
The objective will be to assess the capacity of the NRLs laboratories to specifically identify the etiological agent of AFB by molecular techniques. These results will allow estimating the need for harmonisation in Europe for these molecular methods.

- **Internal stock-culture collection**

The EURL maintains and updates an internal stock-culture collection with bacterial isolates and strains from different geographical origins to have an overview on the epidemiology of the bacterial situation in Europe. NRLs should be requested to contribute to supply the collection.

- **Supply of advices and biological materials to the NRLs and confirmatory diagnosis (within EPILOBEE)**

In 2012 and 2013, the EURL provided biological materials (e.g. bacterial isolates or plasmid constructs) and scientific support to NRLs. This routine activity should be carried out continuously and more specifically in the EPILOBEE framework. The EURL will pursue to supply scientific and technical support to the NRLs for AFB and EFB prevalence estimation, advice on analytical methods, supply of biological material and confirmatory diagnosis.

Expected outputs:

- Submission of a paper on the real-time PCR method for the detection and quantification of *P. larvae* to a scientific journal
- Writing up of the validation steps for the real-time PCR method for *M. plutonius*
- Production of a report from the comparative laboratory test on the capacity of the NRLs to identify *P. Larvae by molecular-based methods*
- Production of an updated reference list with strains and isolates available in the stock culture collection (number of strains and isolates in the stock culture collection).
- Production of specific chapters on AFB and EFB diseases in the EPILOBEE report
- Number of reference material and protocols provided to the NRLs

Activity 3.2. Parasitology programme

Description:

The EURL work program will focus on three parasitic diseases:
- The major bee parasitosis varroosis,
- Nosemosis for which the involvement of two protozoan fungal agents *Nosema apis* and *Nosema ceranae* is intensely debated.
- Acariosis caused by the tracheal mite *Acarapis woodi*.

Objectives:

Varroosis:

- **Publication of a method assessing the impact of varroosis on honeybee colonies:**

The EURL will finalise the publication of a method assessing the impact of varroosis on honeybee colonies.

- **Additional data on the description of varroosis disease**

Over the last years the EURL has performed experiments to provide additional data on the infestation with *V. destructor* correlated to the survival of colonies. In 2014, the first results obtained should be published (see above). However the experiments previously conducted by the EURL did not allow an accurate description of the disease symptoms because of the early death of colonies. The EURL should provide a new experimental protocol for the description of the symptoms of varroosis on honey bee colonies.

The infestation loads by varroa recorded through EPILOBEE should be analysed to complete the description of the disease and estimate thresholds to predict *V. destructor* impact on the colonies survival.

Nosemosis:

- **Reference biological material**

Tests on stored *N. apis* and *N. ceranae* spores produced in 2013 should be conducted to ensure the stability of the biological material.

- **Supply of advice and biological materials to the NRLs and confirmatory diagnosis (within EPILOBEE)**

In 2012 and 2013, the EURL provided the NRLs with biological materials (e.g. plasmid constructs) and scientific support. This routine activity should be carried out within the EPILOBEE framework. The EURL will pursue to supply scientific and technical support to the NRLs for the estimation of *Nosema* prevalence and for the identification of *Nosema* species.

Acariosis:

- **Stock-collection of mite**

A stock-collection of *A. woodi* is available at the EURL. This stock-collection should be enlarged with positive (*A. woodi*) and negative (*A. externus* / *A. dorsalis* / other mites) controls in order to test PCR methods. However, other species of Acarapis might be difficult to obtain as there are quite difficult to collect in the wild.

- **Tests of molecular identification methods**

Acariosis is caused by the tracheal mite *A. woodi* which parasitises the respiratory system, living and reproducing mainly in the large pro-thoracic trachea of the honeybees. The detection of these very small mite performed by dissection of the adult honeybee trachea is highly time consuming. To date, different molecular methods are described to identify the *A. woodi* species (Evans et al 2007, Kojima et al 2011, Garrido-Bailon et al, 2012). However, the specificity of these methods is questioned. Further tests should be performed on a stock collection of positive and negative controls to verify the performance (discrimination, sensitivity) of these methods for *A. woodi* identification and detection. The achievement of this work depends on the species added to the stock-collection. Depending on the results of these tests the development of a new molecular detection and identification technique will be considered.

Expected outputs:

- Submission of the publication on the impact of varroosis to a scientific journal
- Production of reference *N. apis* and *N. ceranae* spores
- Production of specific chapters on varroosis and *Nosema* diseases in the report of EPILOBEE
- Stock-collection with various species of mites and especially *Acarapis* spp..
- Reference materials and protocols provided to the NRLs

Activity 3.3. Entomology and exotic arthropod programme

Description:

The work programme for 2014 plans to continue working on molecular identification methods for the exotic diseases *Aethina tumida* and *Tropilaelaps* spp.. It is also planned to organise a training meeting on the identification of the exotic parasite for the NRLs.

Objectives:

- **Internal stock-collection**

A collection of reference materials (adults, larvae and eggs) was initiated in 2012 for both parasites and enlarged in 2013 with genetically similar parasites, or parasites that could be found in honeybee hives in the purpose of discrimination. This collection should be enlarged in 2014 with a specific attention to parasites closely related to *Tropilaelaps* mites.

- **Supply of advice and confirmatory diagnosis to the NRLs**

The EURL should supply scientific and technical support to the NRLs for the identification of the two exotic arthropods in case of suspicion of introduction and specifically confirmation. In the framework of EPILOBEE, the identification of these parasites must be performed at national level by the NRLs and the confirmation is required by the EURL.

- **Dissemination of expertise on the identification of the exotic parasites:**
    - **Organisation of a training meeting on the identification of the exotic parasites**

The EURL will organise a training meeting for all NRLs on the identification of the exotic parasite *Aethina tumida, Tropilaelaps* spp. and of *Vespa velutina* to transfer the EURL's morphological methods of identification to European laboratories. The works on the molecular techniques of identification should also be presented during this training meeting.

    - **Publication of the morphological methods of identification of the exotic parasites**

The morphological methods for the identification of the exotic parasites *Aethina tumida, Tropilaelaps* spp. and of *Vespa velutina* developed by the EURL should be submitted for publication to a scientific journal. This publication would allow the dissemination of expertise on these identifications.

- **Support to the NRL and the MS to limit the risk of introduction of *A. tumida* and *Tropilaelaps* spp.**

In 2012 two leaflets, written in English, on the exotic arthropods were produced by the EURL, the FLI (Germany) and DEFRA (the UK) and provided to the NRLs for translation and diffusion to the beekeepers. The goal of these leaflets was to ensure a good level of information in all the MSs concerning these exotic arthropods by increasing the surveillance, the prophylactic measures and the procedures of alert in case of suspicion. On the same basis, leaflets should be produced specifically to restrict the risk of introduction of the two exotic parasites in the EU during queen importations.

Aethina tumida

- **New validation of the PCR method for *A. tumida* identification**

Assays performed in 2013 to validate the sensitivity of the PCR on *Aethina tumida* specimens coming from various origins, collected in 2012 and 2013, revealed a lack of inclusivity on one positive sample of *A. tumida* coming from South Africa. The EURL is currently working to overcome this problem by testing the real-time PCR assay developed by Ward et al. (2007). New assays will be conducted in 2014 to validate a sensitive and specific molecular method for *A. tumida* identification. Several beetles from various species genetically similar to *A. tumida* and/or that can be found in beehives and morphologically mistaken with *A. tumida* were integrated in the collection in 2013. The sensitivity and the specificity of the assays will be tested against several beetle species and the *Aethina tumida* specimens available in the stock collection to avoid false positive and false negative results. NRLs, experts and reference laboratories from third countries should continue to be asked for their contribution to supply the stock collection.

Tropilaelaps spp.

- **Tests of molecular identification methods and stock-collection**

In 2012 and 2013, two methods available and described to identify the specific species of *Tropilaelaps* (one focusing the ITS1-5.8S-ITS2 genomic gene and the other one the CO-I mitochondrial gene) have been tested. To date, all these methods, including the one adapted in the laboratory, requires a sequencing step for the species identification. In order to have rapid and discriminating tests, new molecular techniques will be tested on the stock collection of positive (from Pakistan, Indonesia, Thailand, Philippines, Vietnam, and Sri Lanka) and negative controls. The achievement of this work depends on the species added to the stock-collection.

This work could require further prospecting to collect new specimens from various species genetically similar to *Tropilaelaps* spp. and/or that can be found in beehives and morphologically mistaken with*Tropilaelaps* spp.

Expected outputs:

- Production of the minutes of the training meeting on the identification of the exotic parasites
- Production of supporting documents and presentations for the training meeting
- Submission for publication of the reference protocol for morphological identification of SHB
- Production of the validation documents of the method for the molecular identification of SHB and preparation for publication
- Submission for publication of the reference protocol for morphological identification of *Tropilaelaps spp.*
- Development of a method for molecular identification of *Tropilaelaps* spp. mites
- Submission for publication of the protocol for morphological identification of *Vespa velutina*
- Production of a specific paragraph dedicated to the exotic arthropods in the EPILOBEE reports

Activity 3.4. Virology programme

Description:

The work programme for 2014 plans to continue with the ongoing work on the development and validation of reference methods for the detection and the quantification of the five major honey bee viruses that threaten the health of the colonies: the Chronic bee paralysis virus (CBPV), the Sacbrood virus (SBV), the Black queen cell virus (BQCV) and the two viruses acting in synergy with the mite *Varroa destructor,* the Acute bee paralysis virus (ABPV) and the Deformed wing virus (DWV).

Objectives:

- **Development and validation of real-time PCR quantification assays for quantification of BQCV, ABPV and DWV**

The EURL should finalise the validation of a real-time PCR method for BQCV quantification which, after a first development step in 2012, presented some lack of sensitivity due to the variability of this virus. A new step of development of this method was performed in 2013 and this new assay should be validated in 2014. The laboratory should also continue the validation of real-time quantitative PCR methods started for the detection and quantification of the ABPV and the DWV in accordance with the AFNOR Standard NF U47-600. The validation documents should be produced for ABPV and DWV and publication submitted to a scientific journal for BQCV quantification.

- **Reference biological materials and inter-laboratory test on CBPV quantification**

In 2012 and 2013, the EURL activity in virology consisted in producing reference materials and essentially plasmid constructs containing the genes targeted in the PCR methods developed by the EURL. However, some NRLs currently use molecular methods targeting other parts of the genome. In order to test, to compare and to harmonise molecular methods used for quantification and identification of viruses at the European level, the objective for 2014 will be to produce genomic materials corresponding to a wide part of the viral genome. The EURL will initiate the development of such reference materials for CBPV.

In addition, in 2013 the adoption of the CBPV quantitative PCR method, which is first step of inter-laboratory validation, has been conducted with several NRLs. In 2014 the inter-laboratory test should be conducted to define the inter-laboratory reproducibility characteristics of the CBPV quantitative PCR method. The validation document of this method should be finalised including the inter-laboratory reproducibility characteristics.

NRLs, experts and reference laboratories from third countries should continue to be asked for their contribution to supply the viral stock collection for the purpose of validation of the methods developed by the EURL.

- **Supply of advice and biological materials to the NRLs and confirmatory diagnosis** (within EPILOBEE)

As a routine activity the EURL should supply scientific and technical support to the NRLs (e.g. advice about analytical methods, supply of positive controls for PCR detection and confirmatory diagnosis). In the context of the second year of EPILOBEE, this work should specifically focus on CBPV.

Expected outputs:

- Submission of the method for the quantification of BQCV to a scientific journal
- Production of the validation document for the method of ABPV and DWV quantification
- Production of a specific chapter on CBPV disease in the report of EPILOBEE
- Production of reference material to test CBPV molecular detection methods
- Production of a report from the inter-laboratory validation of CBPV quantification method

Activity 4. Pesticide programme

Description:

The 2014 work programme should focus on the validation and the publication of the methods developed during the previous years. The multi-residue methods are focussing on more than twenty insecticides chosen within the most toxic to bees, and belonging to the organochlorines, organophosphorus and synthetic pyrethroid families. The methods have been developed in honeybees and pollen, and should be submitted for publication in a scientific journal. To complete the set of methods already used by the EURL for honeybee health, the laboratory should develop during the year 2014 a multi-residue method on the beebread matrix. This matrix is considered as a good indicator of the contamination of the honeybee food and is related to the health status of the colony. Concerning the neonicotinoid insecticides, the EURL has already developed and validated the detection of imidacloprid, clothianidin, acetamiprid, thiacloprid and thiamethoxam residues in honeybees, beebread and pollen. The method developed in 2013 in nectar (indicating the contamination of the colony food) and in honeybee larvae (indicating the contamination of the brood and related to the health status of the colony) should be validated.

Objectives:

- **Publication of the multi-residue method validated in honeybees and in pollen:**

During 2013, the EURL finalised the validation of the multi-residue method for measuring organochlorines, organophosphorus and synthetic pyrethroid residues in bees and in pollen. In 2014, this method will be submitted to a scientific journal for publication.

- **Development of a multi-residue method in beebread:**

The muliresidue method for detection of more than 20 compounds from the organochlorines, organophosphorus and synthetic pyrethroid residues should be adapted and developed on the beebread matrix.

- **Validation of the method developed for detection of neonicotinoid insecticide residues in nectar and honeybee larvae:**

The method developed for detection of neonicotinoid insecticide residues in nectar and honeybee larvae should be validated.

Expected outputs:

- Submission of the multi-residue method validated in honeybees and in pollen to a scientific journal,
- Development of the multiresidue method for the detection and quantification in beebread,
- Production of the validation documents for the method of the detection and quantification of neonicotinoid insecticide in nectar and honeybee larvae.