European Union Reference Laboratory for Bee Health

WORK PROGRAMME 2013

I. Legal duties

The functions and duties of the European Union Reference Laboratory (EU RL) for Bee Health are described in Commission Regulation (EU) No 87/2011 of 2 February 2011 designating the EU reference laboratory for bee health, 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.

II. Objectives for 2013

The EU RL for honeybee health will work on an extensive number of scientific subjects related to honeybee health including 3 bacteria (2 main pathogens: *Paenibacillus larvae, Melissococcus plutonius* and one secondary *Paenibacillus alvei*), 3 species of exotic parasites (2 *Tropilaelaps* mite species and one coleopteran, *Aethina tumida*), 3 parasites (the *Varroa destructor* mite and 2 species of fungi, *Nosema apis* and *Nosema ceranae*) and 5 viruses (CBPV, ABPV, SBV, DWV, BQCV). The EU RL for honeybee health aims at working on plant protection products by developing and validating assays for measuring insecticide residues. In addition, the EU RL for honeybee health scientifically coordinates and organises the pan European epidemiological study on honeybee colony losses run by the European Commission (EC).

The large number of scientific areas that has to be covered by the EU RL work justifies the costs of the 2013 budget.

The priority mission of the EU RL for honeybee health in 2013 will coordinate and organise the pan European epidemiological study on honeybee colony losses programmed and co-financed by the Commission. The field phase of this study, involving 17 Member States (MS), has begun in 2012, and should end during the summer 2013. The management of the data and the analytical phase should be performed in 2013.

Depending upon the needs, the EU RL for honeybee health should provide scientific and technical support to the EC, the MS and the National Reference Laboratories (NRLs). The specific work dedicated to the study is detailed in chapter 2 Epidemiology.

1. Coordination of the network of NRLs for honeybee health

Objectives:

- **Coordination of the NRLnetwork.** It will rely on the animation of the network via the organisation of the **annual meeting**, sending emails and documents. The development of a collaborative website should be implemented.
- **A questionnaire** filled by each NRL should record updated data for 2012 **concerning the analytical activities of the NRLs for the diagnosis of the various diseases**. These results should help to get updated data on the activities of the network.
- **A summary of the results** collected should be produced by the end of 2013.

The experience of 2011 and 2012 has shown that some hot events required inevitable meetings between EU RL staff (director - Magali Chabert-Ribière, deputy head - Marie-Pierre Chauzat, the epidemiologist in charge of the epidemiological study) and the EC. Consequently the cost of 2 travels to Brussels has been included in the 2013 budget.

Outputs:

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

2. Epidemiology

The priority mission of the EU RL for honeybee health is to provide support to the EC with the development of the epidemiological study on honeybee colony losses in Europe.
This study involves a relatively high number of MSs (17). The field phase begins in September 2012 and finishes in June 2013 with a possible extended deadline to autumn 2013.

This epidemiological studyis taking a lot of our resources in time.

During this study apart from the surveillance of colony losses (overwintering colony mortality rate, season colony mortality rate), the major honeybee diseases should be surveyed. The clinical prevalence of varroosis, American Foulbrood (AFB), European Foulbrood (EFB) and Nosemosis should be estimated. The prevalence of the Chronic paralysis (CBPV) should possibly be considered with the number of the cases of typical symptoms of trembling bees observed. . The *Varroa destructor* infestation rate before winter should be assessed. For ABPV and DWV (viruses related to *V. destructor*), MSs can choose between two protocols: infection rate assessment or a case control study to verify if these viruses can be risk factors inducing winter mortality or winter disorder. Finally, this project should ensure an early alert in case of the detection of the two exotic arthropods *Aethina tumida* and *Tropilaelaps* spp.

Depending upon the requests of the EC, the objectives should be:

- **scientific and technical support to the EC**: by offering advice and guidance on the coordination of the epidemiological study (amended guidelines, evaluation of follow-up projects for each MS).
- **scientific and technical support to the MSs for the coordination of the project** :

- technical support (development and improvement of a European IT database online, model of forms for visit 2 and visit 3, model of technical report…),
- advice and guidance on the setting up of the epidemiological study (explanation of the project protocol, advice about data entry in the European IT database, management and processing of data …).

➢ **scientific and technical support to the NRLs** of the MSs:
- information about surveyed pathogens and diseases,
- advice on analytical methods,
- supply of biological material (positive and negative analytical controls),
- confirmatory diagnosis.

➢ **management of the data and the results of analyses:**
- collect data in the database (management and exchange with the MSs),
- management and processing of data,
- data analysis,
- presentation of results to the MSs and to the EC,
- writing technical reports,

➢ **organisation of training meetings:**
- with the MSs in June 2013 in order to exchange on the first results and the practical aspects,
- at the end of the year or in early 2014 to present the results of this epidemiological study.

The experience from 2012 has shown that the coordination of the epidemiological study and of the NRL network induces a lot of exchanges with the NRLs. The transmission of methods and biological materials induce 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 study to be fully operational in 2013, the hiring of a part time (6 months) scientific secretary is necessary.

Outputs:
- Web based data base operational for MSs
- Production of a template for the scientific and technical report (intermediate and final) to the MSs
- Production of a report on the data from the different MSs
- Production of the minutes of the two training meetings
- Production of a table for the evaluation of the follow up studies
- Supply biological materials and confirmatory diagnoses to the NRLs for the diseases

3. Work programme on the various honeybee diseases

1 Bacteriology programme

The two major brood diseases are bacterial: American foulbrood (AFB) and European foulbrood (EFB).

Objectives:

➢ **Real-time PCR quantification assays : determination of specific characteristics**

The development of the real-time PCR assays, which started in 2012, for identifying and quantifying the primary bacterial agents of AFB (*Paenibacillus larvae*) and EFB (*Melissococcus plutonius*) should be pursued. The real-time PCR assay performances should be assessed by determining its specific characteristics, such as analytical specificity, limit of detection (LOD_{PCR}), limit of quantification

(LOQ_{PCR}) and linearity range. The assays should be performed in accordance with the criteria of the French Standard XPNF U47-600 Animal health analysis methods - PCR - Good practices guide for the development and implementation of PCR in veterinary biology. These results should allow the future validation of the method.

- **Implementation of methods to isolate and culture secondary agents of EFB**

A method to isolate, on a specific culture medium, the etiological agent of European foulbrood, *Melissococcus plutonius*, was implemented in the EU RL in 2012. This work should be pursued in 2013, with the implementation of methods to isolate and culture the secondary agent of EFB, *Paenibacillus alvei*.

- **Comparative Laboratory Test on American foulbrood search**

A Comparative Laboratory Test will be organised, related to the search of American foulbrood in two matrices (brood smear and honey). The test is organised by the European Union Reference Laboratory for honey bee health of ANSES-Sophia Antipolis in collaboration with the OIE Laboratory - CVUA Freiburg Germany Institute for Animal Health and Food control.
About twenty laboratories will be contacted, among them a dozen of European NRLs. The choice of the NRLs contacted by the EU RL was done as follow: the NRLs had previously expressed their wish to participate to a laboratory test concerning AFB, or the NRLs use a validated and/or an accredited method for the detection of AFB.
The objective is to assess the capacity of the laboratories to detect the etiological agent of AFB, the bacteria *Paenibacillus larvae*, from brood smear and/or from honey matrices.

- **Internal stock-culture collection**

The EU RL began the elaboration of an internal stock-culture collection with bacterial isolates and strains from various part of France. To have an overview of the bacterial epidemiological status in Europe, the collection has to be supplied with isolates and strains from different geographical origins in the continent. NRLs of Member States have been contacted, and should continue to be asked for their contribution to supply the collection.

- **Supply of advices and biological materials to the NRLs and confirmatory diagnosis (epidemiological study)**

In 2012, a part of the activities consisted in providing NRLs with biological materials produced by the EU RL (e.g. bacterial isolates or plasmid constructs) as well as giving them scientific support. From now on this is a routine activity of the EU RL, which should be carried out continuously and more specifically in the epidemiological study framework. The EU RL should supply scientific and technical support to the NRLs for AFB and EFB prevalence estimation: information, advice about analytical methods, supply of biological material and confirmatory diagnosis.

Outputs:

- Writing up of the first steps for the real-time PCR method related to *P. larvae*
- Implementation of a method to culture *P. alvei*
- Production of a report from the Comparative Laboratory Test on the capacity of the laboratories to detect the etiological agent of AFB
- Production of a reference list with strains available in the stock culture
- Production of specific chapters on AFB and EFB diseases in the report of the epidemiological study

2 Parasitology programme

Objectives:

Varroosis:

- **Publication of the diagnosis method of varroosis**

The EU RL should finalise the publication of the diagnosis method of varroosis (infestation by the mite *Varroa destructor*).

Nosemosis:

- **Estimation of the prevalence and the geographical distribution of nosemosis in Europe:**

A questionnaire dedicated to the survey on *Nosema* species detection and *Nosema* disease in Europe filled by each NRL has been proposed and explained to representatives of MSs during the annual meeting 2012 (24 September in Brussels). The collection of the data should be finalised in 2013 and results expressed according to the prevalence and the geographical distribution of both *Nosema* species and the expression of *Nosema* disease in the MS.

- **Production of biological material**

First tests of production of reference material, *Nosema apis* and *Nosema ceranae* spores, conducted in 2012 should be pursued to establish production of biological material to perform the ILPT for diagnosing this disease in adult bees. Due to the difficulty of spore production in adult bees, a mission for two people is planned for 2013 in the team of Dr Higes specialized on Nosema study ("Centro Apícola Regional", Spain). Our laboratory already collaborates with the team of Dr Higes. This mission is crucial to gain the necessary expertise in spore production and laboratory experiments.

- **Supply of advice and biological materials to the NRLs and confirmatory diagnosis (epidemiological study)**

In 2012, a part of the activities consisted in providing NRLs with biological materials produced by the EU RL (e.g. plasmid constructs) as well as providing scientific support. This routine activity should be carried out continuously. In the epidemiological study framework, the EU RL should supply scientific and technical support to the NRLs for the estimation of *Nosema* prevalence.

Outputs:

- Submission of the method for the diagnostic of varroosis to a scientific journal
- Production of a report on the *Nosema* species (spores and diseases)
- Production of specific chapters on varroosis and *Nosema* diseases in the report of the epidemiological study

3 Entomology programme

The work programme for 2013 plans to continue with the work engaged on the exotic diseases *Aethina tumida* and *Tropilaelaps* spp.

Objectives:

- **Internal stock-collection**

A collection of reference materials (adults, larvae and eggs) has been started in 2012 for both parasites and for genetically similar parasites or parasites that could be found in honeybee hives in the purpose of discrimination with these two exotics. This collection should be enlarged in 2013.

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

The EU RL 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 the epidemiological study, the identification of these parasites must be done at national level and confirmation is required by the EU reference laboratory.

Aethina tumida

- **Validation of the specificity of the PCR method for *A. tumida* identification**

The EU RL developed and validated a conventional PCR method for *A. tumida* identification, which specificity was tested against two different beetle species to avoid false positives. Several beetles from various species were integrated in the collection in 2012. Assays should be performed to validate the specificity of this PCR on these new beetle species, genetically similar to *A. tumida*, or that can be found in beehives and morphologically mistaken for *A. tumida*.

- **Support in case of alert concerning introduction of *A. tumida***

The EU RL should contact suppliers providing devices for *A. tumida* detection (Australia and United-States for example) to ensure the rapid supply of the material in case of introduction of the pests in Europe.

Tropilaelaps spp.

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

To date, methods to identify the specific species of *Tropilaelaps* are scarce (Tangjingjai *et al.* 2003; Anderson & Morgan 2007). Further tests should be performed on the stock collection of positive and negative controls to verify the discrimination performance of the methods. The achievement of this work depends on the species added to stock-collection. Consequently the stock-collection should continue to be enlarged.

- **Improvement of expertise for identification and discrimination between species of *Tropilaelaps***

Active research of new published studies describing methods for *Tropilaelaps* spp. detection, identification and discrimination between species should be performed. Additionally, specialists in acarology should be contacted in order to increase expertise with the aim of identifying the most relevant methods to be developed in the EU RL and to transfer to European laboratories.

Outputs:

- Production of a specific paragraph dedicated to the exotic arthropods in the reports concerning the epidemiological study
- Production of the validation documents of the method for the molecular identification of SHB
- Production of the protocol for morphological identification of *Aethina tumida* for NRLs
- Development of a method for morphological identification of *Tropilaelaps* spp. mites

4 Virology programme

Objectives:

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

The EU RL should finalise the publication of the method for quantification of SBV, the validation of real time PCR method of BQCV and should continue the implementation of real-time quantitative PCR methods started in 2012 for the detection and quantification of the Acute bee paralysis virus (ABPV) and the Deformed wing virus (DWV) in accordance with the COFRAC Standard NF U47-600. Depending on these results, the validation of the method should be performed and validation documents should be produced.

- **Supply of advice and biological materials to the NRLs and confirmatory diagnosis (**epidemiological study**)**

As a routine activity the EU RL 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 epidemiological study this work should specifically focus on CBPV (Chronic bee paralysis virus), DWV and ABPV.

- **Chronic bee paralysis analysis in the framework of the epidemiological study**

The molecular quantification of the CBPV on symptomatic honeybees is not funded in the epidemiological study. However, given the importance of this disease – the symptoms can easily be mislead with intoxication – the EU reference laboratory recommended to the MSs to sample symptomatic bees and to keep the samples. At the end of each visit and after the complete feedback from the MSs on the number of cases, the laboratory should subsequently evaluate the relevance of cases and which samples can be treated by the EU RL.

Outputs:

- Submission of the method for the quantification of SBV to a scientific journal
- Production of the validation document for the method of BQCV quantification
- Development of methods for quantification of ABPV and DWV (production of data for validation documents)
- Production of a specific chapter on CBPV disease in the report of the epidemiological study

4. Pesticide programme

Objectives:

- **Validation of a multi-residue method in honeybees and in pollen**

During 2013, the EU RL should finalise the validation of the multi-residue method developed in 2012 for measuring organochlorines, organophosphorus and synthetic pyrethroid residues in bees and in pollen, taking into account the critical aspects of specimen preparation (uniformity of the test specimen, variability of the sample, etc.).

- **Development of a method for detection of neonicotinoid insecticide residues at low concentration in nectar and honeybee larvae**

The 2013 work programme should focus on neonicotinoid insecticides: imidacloprid, clothianidin, acetamiprid, thiacloprid and thiamethoxam. These insecticides represent one of the fastest growing classes of insecticides introduced to the market since the launch of pyrethroids. Their physicochemical

properties make them useful for a wide range of application techniques including foliar, seed treatment, soil drench and stem application. Worker honeybees gathering nectar, water and pollen may be directly subjected to the action of pesticides or they may carry pesticide-contaminated pollen back to the hive and expose other honeybees. Our laboratory has developed and validated detection of neonicotinoid (imidacloprid, clothianidin, acetamiprid, thiacloprid and thiamethoxam) residues in honeybees, beebread and pollen.

To complete the set of methods already used by the EU RL for honeybee health, during the year 2013, the laboratory should develop a simple, sensitive and reliable method for determining neonicotinoid insecticide residues at low concentration levels in:

- nectar (indicating the contamination of the colony food)
- honeybee larvae (indicating the contamination of the brood and related to the health status of the colony).

Outputs:

- Production of the validation documents for the method of the detection and quantification of pesticides in pollen and honeybees
- Preparation of a paper on validation of pesticides in honeybees for submission in a scientific journal
- Development of a method for the detection and quantification of neonicotinoids in nectar and honeybee larvae