European Union Reference Laboratory for Bee Health

WORK PROGRAMME 2012

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 2012

It is necessary to remind that the EU RL Honeybee health will work on an extensive number of scientific subjects related to honeybee health. This includes the study of 6 viruses, 3 bacteria (2 main pathogens and one secondary), 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*). In addition, the EU RL Honeybee health aims at working on pesticides by developing and validating assays for measuring insecticide residues in honeybees and in pollen. Part of these topics will be integrated in a pan European Pilot Project on the surveillance of honeybee mortality run by the Commission.

1. Coordination of the network of NRLs for bee health

The coordination of the National Reference Laboratory (NRL) network will rely on the animation of the network via sending emails and documents.

- The data from a detailed questionnaire filled by each LNR will be collected. The questionnaire was presented and explained to representatives of MSs during the Kick off workshop (6-7 June 2011, Brussels). These results will help to define the sphere of activity and expertise available in each NRL and the expectations of each laboratory. Dedicated parts of the questionnaire will shed more light on data on occurrence and diagnostic of the various diseases. During the Kick-off meeting, it has already been observed a certain degree of diversity between NRLs. The results from the questionnaire will help to estimate the degree of harmonisation that already exists in diagnostic techniques between MS, hence the lacks and further needs.

- Depending on the data collected, a new questionnaire specifically dedicated to major diseases may be proposed to MSs. This dedicate questionnaire should identify any additional data needed and enable the recommended measures to be planned.

The various activities to take place in 2012 will therefore be:

- finalisation of the report summarising the answers to the questionnaire from the MSs

- diffusion of a simplified version of the 2012 questionnaire in order to get up-dated data from each MS

- collection of the data related to 2012 to update the data base on analytical capacities of NLRs and beekeeping activities in each MS

- coordination of the NRL network and implementation of shared tools for information exchange and remote working (including an assessment of the feasibility of a collaborative website).

In 2012, the EU RL will organise one training session with the MS involved in the Pilot project co-financed by the EU. This session will be organised in order to work on the Pilot project that should be implemented during the autumn (September to October 2012).

2. Epidemiology

One of the priority missions of the EU RL Honeybee health will be to provide scientific support to the Commission with a view of improving the surveillance of bee colony mortalities in Europe.
The Commission already asked the EU RL Honeybee health in 2011 for technical assistance in laying down guidelines for honeybee colony mortality surveillance in MSs. Depending upon the needs that are requested by the Commission, the laboratory will:

- offer advice and guidance on the implementation and coordination of the epidemiological Pilot project,

- help with the training for sampling, drafting of questionnaires, collection, management and processing of data and writing of reports.

The group of experts related to the Pilot project (Epi team) will meet in Paris in early 2012 in order to discuss and finalise the protocol in a training session. This group will finalize the protocol of the Pilot Project on honeybee mortality surveillance by harmonizing the protocols proposed by the selected MSs.

3. Work programme on the various bee diseases

All pathogens and pests that are not currently found in France or Europe will be dispatched by suppliers to the EU RL after being inactivated. When necessary, however, specimens are to be sent according to the rules for transporting infectious material and, at a minimum, handled in a type-2 microbiological safety cabinet, or even housed in a bio-safety level 3 laboratory. This implementation will be prioritised and performed over the 5 year of the programme of the EURL depending of the importance of the diseases/ pathogens and the difficulties to produce the agents/ reference materials.

1 Bacteriology programme

The two major brood diseases are bacterial: American and European foulbrood.

The 2012 programme is as follows:

- Depending on the bibliographical studies performed on the primary bacterial agents of each of these diseases (*Paenibacillus larvae, A*merican foulbrood, *Melissococcus plutonius* and *Paenibacillus alvei,* European foulbrood), tests will be implemented on different real-time PCR quantification techniques described in the literature. These methods will be initially assessed in order to provide tools for identifying and quantifying these bacterial agents. Depending on their suitability, it is expected that methods will be developed for the primary bacterial agents of each of these diseases (*Paenibacillus larvae,* American foulbrood) (*Melissococcus plutonius* and *Paenibacillus alvei,* European foulbrood). In future years, they will be developed and validated at EU RL level, in accordance with the criteria of the French Draft Standard PR NF U47-600 'Animal health analysis methods - PCR – Good practices guide' for the development and implementation of PCR in veterinary biology.

- Tests will be conducted in order to establish controlled production of appropriate biological material to conduct ILPTs for diagnosing these brood diseases (production of sick brood, control of the infection).

2 Parasitology programme

Varroosis:
Validation and publication of the method: diagnosis of varroasis, the major bee parasitosis (infestation by the mite *Varroa destructor*). The varroasis diagnostic method recommended by the OIE takes into consideration parasitic infestation in the debris stemming from the colonies, the brood or adult bees in the samples examined. However, no threshold is given for assessing the infestation. OIE method relates to the detection of the parasites. The laboratory has performed experiments to provide additional data by observing symptoms on brood and on adult bee, to achieve a more complete diagnosis.

Nosemosis:
Nosemosis will also be studied by focusing essentially on the production of reference material.

- Tests will be conducted in order to establish controlled production of sufficient biological material to perform the ILPT for diagnosing this disease in adult bees.

- Since evidence of *N. ceranae* was discovered in honey bees in Europe, diagnosing nosemosis in colonies has become more complex. The symptoms of the disease have changed to the absence of diarrhoea or systematic bee mortalities at the entrance of the hives. The EU-RL will begin the study with a survey in the different member countries on field observations of colonies (disease history) related to the quantification of the parasite in house bees and foraging bees, and its typology (*N. apis* or *N. ceranae*). This work will be done by sending out questionnaires once the laboratories have been identified (NRLs supplemented by research laboratories conducting studies on this disease). The goal is to offer a harmonised field diagnostic protocol, in future years.

- In terms of quantitative molecular diagnostics, various real-time PCR techniques are described in the literature but have not yet been validated in comparison with the detection of spores. A review of the literature and the genetic studies will be conducted to assess the feasibility of diagnosing the disease by these molecular techniques. Depending of the results of this study, the use of real-time PCR techniques will be evaluated as a reference method for the diagnosis of the disease.

3 Entomology programme

The 2012 work programme will focus primarily on the exotic diseases not yet introduced into Europe, *Aethina tumida* and *Tropilaelaps* spp.
To accomplish this, the EU RL plans to:

- obtain reference materials (adults, larvae, eggs, but also specimens obtained from infested colonies showing symptoms of these infestations) that have been inactivated and certified by laboratories in the countries where these pests originate (South Africa, Asia) and countries where they have been introduced (USA, Canada). These samples will feed a reference collection held by the EU RL Honeybee health.

- obtain 'negative control' reference materials, such as coleoptera and mites that may be confused with the pests in the colonies. To accomplish this, the EU RL will contact agencies that can advise on and supply this biological material.

Concerning the identification methods (morphological and by PCR):

- the EU RL Honeybee health will ask the relevant NRLs for a collaboration in writing up a common and subsequently shared method for morphological identification of adult *Aethina tumida*.

- for *Aethina tumida* the PCR identification used in the EU RL was adapted from the method indicated in the OIE Manual. This method was validated according to COFRAC Standard XP N47-600 during the 2011 program. This method will be disseminated among the NRL network in order to implement harmonisation and data exchange at EU level.

- for *Tropilaelaps* spp. the method indicated in the OIE Manual involves morphological identification and description of the symptoms in colonies. In order to prevent the spread of the two main species of parasitic mites harmful to *Apis mellifera* - *Tropilaelaps clarae* and *Tropilaelaps mercedesae* - a diagnostic procedure confirming identification by PCR seems appropriate and will be evaluated.

4 Virology programme

Developing diagnostic methods and preparing ILTs
For CBPV, the laboratory has developed and validated a quantitative PCR method with defined thresholds, making it possible to reach conclusions about the viral carriage or the disease. Based on its contacts with the other OIE Laboratory in Freiburg and on the answers to the questionnaire by the NRLs, an initial proficiency ILT will be prepared late 2012 to be run in 2013. The analytical method will be finalised by defining its inter-laboratory reproducibility characteristics.

During the year 2012, the EU RL Honeybee health will concentrate the work on finalising the publication of real time PCR methods initiated in 2011 on SBV and BQCV viruses. These methods will also be validated according to the *had hoc* norms.

The EU RL Honeybee will also start the development of a quantitative PCR method for the diagnosis of viruses that threaten the health of the colonies, specialty in synergy with the mite *V. destructor*. Indeed, the Acute bee paralysis virus (ABPV) and the Deformed wing virus (DWV) seem to be implicated in bee losses linked with *V. destructor*. Regarding especially ABPV, the work programme for 2012 will concentrate on developing a real-time PCR for its detection and quantification, in accordance with the COFRAC Draft Standard NF U47-600.

Collection of viral isolates from diverse geographical origins and 'new viruses', monitoring viral variability

Since bee viruses are mostly RNA viruses, they are genetically variable. It is necessary to monitor this variability and ascertain the detection capability of the tools used by the EU RL Honeybee and the NRL network. This work on genetics has already been done for CBPV.

For this purpose, the laboratory will:

- continue to develop contacts in various countries in order to start collecting isolates of different geographic origins for the main viruses, which are ABPV, IAPV, KBV, SBV and BQCV. In subsequent years, this collection will make possible to assess this variability as well as the sensitivity and specificity of the detection tools implemented by the EU RL Honeybee and the NRL network. The collection will also be used as a supplement for comparing techniques using the ILTs.

- sequence viral isolates obtained by the EU RL Honeybees from the EU and third countries, and from isolates detected in France. This work will be done for ABPV, DWV, SBV or BQCV depending of the number of isolates collected.

4. Pesticide programme

Bees are in direct contact with pesticides, especially through the pollen they collect. Pollen stored in the hive to feed the colony can play a role in colony health. The 2012 work programme will focus on developing and validating assays for measuring organochlorines, organophosphorus and synthetic pyrethroid residues in bees and in pollen. The insecticides chosen are those that are most toxic to bees. The objective will be to develop a multi-residue method covering 22 compounds. The critical aspects of specimen preparation (homogeneity of the sample, stability of the molecules, etc.) will be taken into account during development of the analytical methods.