Action FA0803

COLOSS Work Shop

Working Group 2

EFB – Presence, Prevalence and Monitoring

13-14th March 2012

Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK

The Food and Environment Research Agency

Contents

Agenda

TIME	DETAILS
12/03/2012 – Arrival	
20.00-20.30 - open	WG Dinner La Piazza York **(Self funded)**
13/03/2012 (Monday) – Room 12F53 Fera, Sand Hutton, York	
08:30- 09:20	Registration and Coffee
09:20 – 09:35	Welcome and organizational matters, introductions etc
09:35 -11.00	Individual Presentations of Abstracts
11:00 – 11:30	Coffee break
11:30 – 12:30	Individual Presentations of Abstracts Continued
12:30 – 13:30	Lunch
13:30 – 15:00	Individual Presentations of Abstracts Continued
15:00 – 15:30	Coffee Break
15:30 – 17:00	Open Discussion – Areas of Interest/Collaboration
20:00 – open	**Social dinner in York at Ate O Clock**
07/02/2012 (Tuesday) - Room 12F53 Fera, Sand Hutton, York	
09:00 – 11:00	Open discussion 1
11:00 – 11:30	Coffee break
11:30 – 12:30	Open discussion 2
12.30-13.30	Lunch
13:30 – 15:00	Tour of Fera/NBU Facilities – Labs, BeeBase etc
15:00 – 15:30	Coffee, Close and - Depart

LOCATION AND INFORMATION	
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Abstracts

The Random Apiary Survey – England and Wales 2009-2011

Giles Budge*, Ben Jones, Ilex Whiting, Ruth Grant, Michelle Powell, Gay Marris, Andy Wattam, Mike Brown.

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One of the primary remits of the NBU has always been the prevention and control of notifiable honey bee diseases. The NBU currently uses a targeted, risk-based method for its inspection programme. In 2009 the Department for Environment, Food and Rural Affairs (Defra) and the Welsh Government commissioned a two year survey of apiaries across England and Wales. The survey was based collecting samples from colonies in approximately 4600 apiary sites. These samples were analysed for a wide range of honey bee pests and pathogens using real-time PCR (TaqMan®) plus visual screening for Varroa and both foulbrood diseases. The aim of the study was to obtain unbias estimates of prevalence and distribution of bee pathogens, pests and diseases in England and Wales. The evidence gathered and a robust analysis of the risks will be used to direct the future of honey bee health policy in England and Wales. The results of the survey indicate that current inspection strategies employed in England and Wales for monitoring honey bee colonies for the presence of European foul brood and American foul brood are efficient. The findings of this large project will be discussed.

Early detection of European Foulbrood using real-time PCR

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A real-time PCR test was developed for the detection of *M. plutonius* residing on adult honey bees, with the goal of identifying reliably and at an early stage diseased apiaries, therefore possibly reducing the work of the bee inspectors. For this purpose samples (adult bees) from 88 apiaries were collected and assayed for the presence and quantification of *M. plutonius* DNA by real-time PCR. Results were compared to the subjective classification of the apiaries (presence or absence of clinical symptoms) done by the bee inspectors at the time of sampling. In 93.5 % of the cases with clinical symptoms real-time PCR gave positive results, but 41.2 % of the samples with positive results came from colonies without clinical symptoms, indicating either a poor specificity of the PCR, a lack of accuracy of the visual inspection for clinical symptoms or the development of the infection in a pre-symptomatic stage.

An evaluation of the costs of the introduction of this method and delegation of sample collection to the beekeeper was also assessed, and shows that it is a very expensive way to detect the responsible agent.

In conclusion, our data show that at the moment, the costs for the real-time PCR analyses make this technique less attractive to replace the routine visual control performed by the bee inspectors.

Characterization of honeybee colony tolerance against EFB / identification of *Melissococcus plutonius* virulence genes

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We are about to launch a project in order to identify honeybee colony tolerance against EFB in Switzerland and to characterize the basis of this tolerance. In parallel we aim at identifying candidate virulence genes on Melissococcus plutonius genome.

Honeybee tolerance. The first part of the project will consist in collecting queens from colonies displaying EFB symptoms and to maintain them in minicolonies. In parallel queens from the same apiaries but from colonies without clinical signs will be collected and used as control group. Larval resistance will be assessed from these colonies using an *in vitro* larval rearing assay. The expression of several genes linked with the bee immune system will be monitored from larvae collected in the control and in the EFB sensitive groups. The aim of this experiment is to provide data showing the possibility to fight against EFB using queen selection.

M. plutonius virulence genes. EFB strains displaying different virulence levels have been identified in Switzerland and one can hypothesize that some highly virulent EFB strains can predominantly affect certain areas of the territory for still unknown reasons. The genome comparison of these different strains might allow the identification of virulence genes and help to understand the dynamics of the bacterium with its host.

The Epidemiology of European Foulbrood of Honey bees

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My PhD has been based on using molecular methods to investigate the epidemiology of European foulbrood (EFB). I have developed a typing scheme to differentiate between isolates, analyzed *Melissococcus plutonius* genomes, and have investigated the presence of *M. plutonius* in apiaries to look for potential transmission routes. This work is still ongoing, but I will share some preliminary findings with the attendees.

The current status of Foul brood diseases throughout Europe – Coloss Questionnaire.

Jean-Daniel Charrière[1], Bjørn Dahl[2] and Selwyn Wilkins[3*]

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Throughout the European Union American foul brood is a statutory notifiable disease, European foul brood is notifiable in some member states only. Control methods throughout the member states differ and the numbers of outbreaks and cases are not readily available. With the benefits of membership the international Coloss Network of scientists it was decided to put together a questionnaire designed to collect information on the current status of these diseases, including legislative frameworks in place, monitoring and control systems employed, presence and distribution of the diseases and other information considered relevant. Once compiled the questionnaire was distributed to 1 Coloss MC member from each Member State to either complete the questionnaire or to forward to the relevant authority. There have been 14 positive responses so far, with18 null responses, including some of the larger beekeeping states. No data analysis has been carried out as yet, further direct contact will be made with the non-respondents to encourage participation.

Sampling and real-time PCR detection of *Melissococcus plutonius* in healthy carrier hives in an outbreak of European Foul Brood in Norway

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For 3 decades there had been no verified clinical occurrence of European Foul Brood (EFB) in Norway. In primo August 2010 it was verified by cultivation and standard PCR with detection of 16S rDNA that *Melissococcus plutonius* was occurring in diseased honey bee brood sampled in Aust Agder county in Southern Norway. Retrospectively, it was said that bee-keepers were suspecting EFB in the same area already in the summer of 2009. However, only one sample was submitted for diagnostic analyses in 2009 and *M. plutonius* was not detected by cultivation and standard PCR analyses. The Norwegian Food Safety Authority (NFSA) decided to stamp out diseased bee hives and reimburse the direct loss to the affected bee-keepers.

Mainly by standard-PCR and verification by cultivation 157 samples from 84 bee-keepers/apiaries were analyzed from August to mid-October 2010. Eleven of the samples were analyzed from hives in other districts without symptoms of EFB as a mean to map the outbreak. Of the samples from bee-hives with symptoms indicative of EFB, as evaluated by the bee-keepers, 84 samples (58 %) were found to carry *M. plutonius*. Suspected diseased brood frames were sent to the laboratory for sampling and in the laboratory there was found to be very good correspondence between diseased brood and positive standard-PCR results.

NFSA decided in the winter of 2011 to wipe out the EFB outbreak. The diagnostic laboratory therefore designed a sampling strategy based on real-time PCR detection that should end with a diagnostic screening of all bee hives within the outbreak area before 1. July 2011 ahead of development of clinical symptoms in the infected larvae brood of the hives. Samples representing all the hives in the outbreak area, close to 11.000 bee remaining hives, were analyzed and additional 47 apiaries were found to carry *M. plutonius*. In total 91 bee-keepers with a large fraction of all apiaries in the outbreak area were found to carry *M. plutonius* in their apiaries during 2010 and 2011 before the outbreak was stamped out and contained.

European Foulbrood in the Netherlands

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European Foulbrood is prevalent and an underestimated and therefore under-treated disease in Dutch honeybee colonies. This statement is based on the reporting of brood abnormalities by beekeepers to PRI bees@wur and on detection of EFB using the Vita diagnostic EFB test in brood samples sent to PRI bees@wur by beekeepers. The nationwide monitoring program in June 2008 showed the presence of *Melissococcus pluton* in 36% of the 170 apiaries where colonies were sampled. In the diagnosed brood samples sent by beekeepers to PRI bees@wur in 2008, 2009, 2010, and 2011 EFB was diagnosed respectively (year, n samples, n EFB): 2008, 3, 2; 2009, 19, 10; 2010. 14, 12; 2011. 3, 3.

In the Netherlands EFB is not a notifiable bee disease, this counts only for AFB. In case EFB is diagnosed, the beekeepers are advised to apply the shook swarm method; re-housing the colonies on new wax foundations and destroying all frames from the infested colonies.

There are no data how many beekeepers actually act upon an EFB infection. The incidence of EFB shows parallels with Varroa and with winter losses. There are indications that Varroa is a factor in disseminating the bacterium. The impact of a decreasing vitality (e.g. combination of factors bees, brood, beebread, vitellogenin) due to Varroa, lack of pollen (discontinuous flow and / or low diversity, pesticides, unknown factors), on the increasing prevalence of EFB is not yet studied but may reveal new insight on the increasing prevalence of EFB.

List of participants

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