Honey Bee Diagnostics at Fera: Part 1

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Information about how the NBU seeks to protect the health of our bees

THE NATIONAL BEE UNIT (NBU) has been involved in the diagnosis of bee pests and diseases for over 60 years. Most readers will know about our work on behalf of Defra and the Welsh Government's Bee Health Programme for beekeepers in England and Wales, which we deliver through an integrated programme of apiary inspections, laboratory diagnosis, research and development and extensive training and advice.

Our current team of 80 people thus comprises programme support, research personnel and bee inspectors and, at the heart of much of what we do, a busy group of diagnosticians. Our statutory Apiary Inspection Programme (AIP) focuses on diagnosis (and of course treatment) of American foul brood (AFB) and European foul brood (EFB). These notifiable diseases are both present in the UK and are regarded as posing the greatest immediate risk to honey bee health. Exotic pest species, the small hive beetle (SHB) and *Tropilaelaps* mites, are not present in the UK, but monitoring for their arrival (and other threats from overseas) and examining suspect samples to check identity are also important elements of our work.

Apart from addressing foul broods and exotic pest threats, the AIP encompasses a range of other diagnostics activities that impact on improving bee health. These include import and export examinations of bees under veterinary checks directives, assistance with suspect pesticide damage to honey bee colonies (through the Wildlife Incident Investigations Scheme (WIIS)) and honey sampling for chemical residues analysis on behalf of the Veterinary Medicines Directorate. Every year we receive many thousands of samples for NBU research projects and routine diagnostics from NBU inspectors, scientists and beekeepers.

Figure 1. An Inspector at work

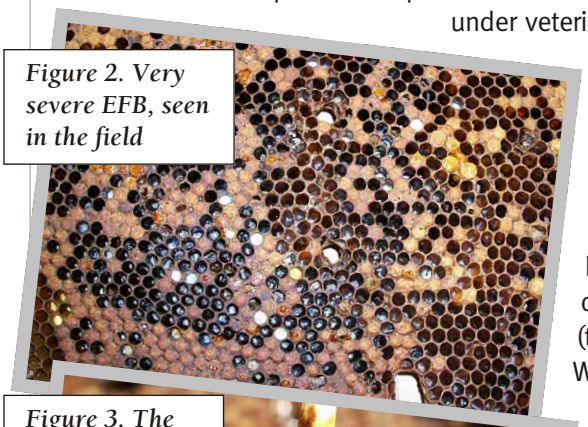


Figure 2. Very severe EFB, seen in the field

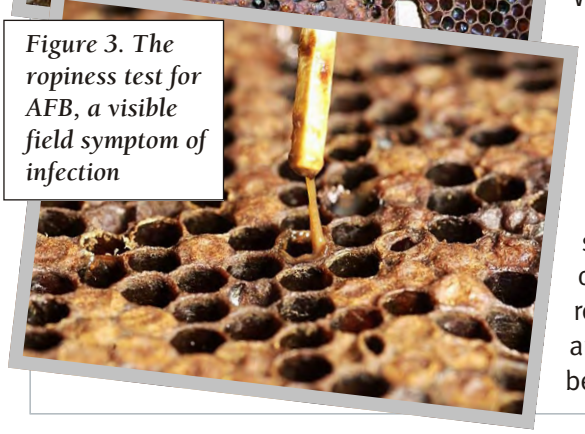
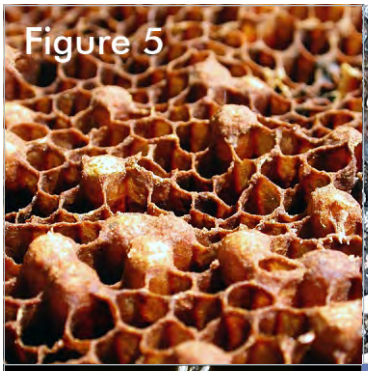


Figure 3. The ropiness test for AFB, a visible field symptom of infection

Field Diagnostics

Often the first diagnosis of any problem in a honey bee colony does not take place in the laboratory but is, in fact, carried out by an NBU Inspector in the field (Figure 1). All our Inspectors are trained in the recognition of visual symptoms of the bacterial diseases EFB and AFB (Figures 2 and 3), and the exotic pest species, SHB (see June, page 8) and *Tropilaelaps* mites. In addition, colonies can be field-checked for the presence of *Varroa destructor* (Figure 4), Nosema, Tracheal mites, viral symptoms and any other colony abnormalities such as failing queens (Figure 5), chalk brood (Figure 6) or sacbrood (Figure 7), as well as general the colony condition.

Figure 4. Varroa and bee deformities



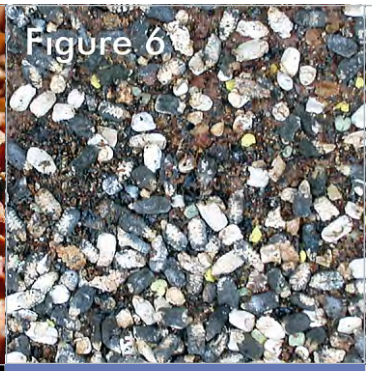


Figure 5. Failing queen
Figure 6. Chalk brood
Figure 7. Sacbrood

Lateral Flow Device

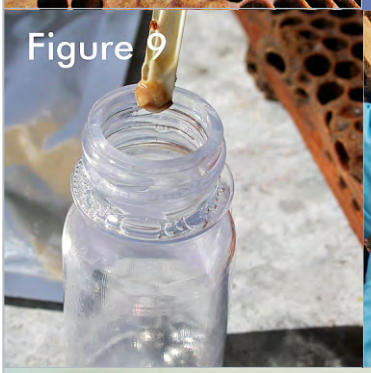
When diagnosing AFB or EFB in the field, Inspectors use a lateral flow device, commonly referred to as an 'LFD' (Figures 8–11). For those unfamiliar with LFDs, these are pocket-sized diagnostic kits that work in a similar way to a pregnancy test. They can be can be used to confirm foul brood disease by detecting the causative organisms *Mellissococcus plutonius* (EFB) and *Paenibacillus larvae* (AFB) in symptomatic larvae. LFDs are very useful tools because they provide a rapid (almost instantaneous) result for the beekeeper and can even allow appropriate colony treatments to begin at once, on the same day as diagnosis, depending on what action is required. However, if there is ever any doubt about field symptoms, the Inspector will send samples to the laboratory for a confirmatory diagnosis.

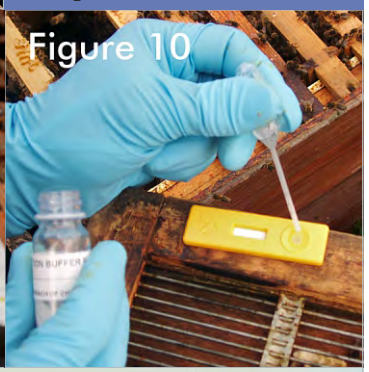
If an Inspector ever suspects that he or she had has found either SHB or *Tropilaelaps*, then samples would always be sent for confirmatory diagnoses at the Food and Environment Agency's (Fera's) various laboratory facilities.

Laboratory Diagnostics at Fera

The NBU has its own dedicated diagnostics laboratory, staffed by a team comprising a manager and two highly experienced technicians. It is here that many (thousands) of honey bee samples are first screened for suspect pests and diseases. However, as part of Fera, we also benefit from unrivalled access to an exceptional range of scientific expertise and resources across the site. Consequently, we are able to use a comprehensive array of diagnostic techniques, from traditional microscopy, that offers beekeepers the advantages of a rapid and reliable result

Figure 8. Selecting a suspect larva for testing with an LFD
Figure 9. Placing the suspect larva in buffer solution
Figure 10. Drops of buffer placed into the well of the LFD
Figure 11. Negative (above) and positive (below) LFDs





Figures 12–14. Honey bee diagnostics in the Molecular Technology Unit

Figure 15. Pyrosequencer

(typical turnaround time 24 hours), to state-of-the-art, molecular biology techniques (Figures 12–14). The latter enable a large number of samples to be analysed simultaneously and are most often used in support of the NBU's research projects (eg, during the recently completed Random Apiary Survey (November 2011, page 32).

Both 'targeted' and 'non-targeted' diagnostic approaches can be adopted, depending on what is required.

Targeted techniques are used to confirm or rule out the presence of a specific pest or disease, ie, you know what you are looking for in the sample and either find that it is indeed present, or that it is not.

Non-targeted diagnostics are used to ascertain the range of organisms that may be present in a suspect honey bee sample, rather than specific causative agents. For example, the powerful technology called Pyrosequencing can now be used by Fera scientists to identify an enormous range of unknown organisms within a honey bee sample, to support our bee health programme (Figure 15).

In parallel, targeted molecular techniques can be employed to detect known honey bee pathogens and viral infections such as *Nosema* spp. and chronic bee paralysis virus (CBPV). Whilst these techniques are generally not economically viable or appropriate for day-to-day honey bee diagnostics, the combination of targeted and non-targeted molecular detection tools provides us with an unprecedented depth of understanding of samples for the NBU's research projects.

Laboratory Diagnostics of Foul Brood Diseases

For routine foul brood diagnosis, samples are analysed via microscopy in the NBU's own laboratory (Figure 16). A slide is made from a symptomatic larva. For EFB, the larval gut is dissected out and then used to make the slide as it is the digestive tracts that will contain the most bacterial cells. The slide is then heat-fixed and, to help expose any signs of bacteria in the preparation, it is stained to improve the contrast in the microscopic field of view.

Viewed at a magnification of x1000 with an oil emersion lens to further increase the resolution of the microscope, *M. plutonius* appears as white, diamond-shaped cells that can often form chains (upper inset, Figure 16), whereas *P. larvae* spores (not shown) appear the shape of solitary white 'fly eggs'. In cases where colonies are showing very heavy EFB symptoms, a secondary infection can occur. In these cases, the bacterium, *Paenibacillus alvei* can also populate infected larvae and be observed using a microscope (lower inset, Figure 16).

Our technicians are trained in the microscopic recognition of different bacteria in order to ensure accurate diagnoses. You can read more about diagnosis and treatment of foul brood diseases in our leaflet *Foul Brood Disease of Honey Bees*. This is available, at no charge, to download from the foul brood pages of our BeeBase website (www.nationalbeeunit.com), or you can request a copy from the NBU office (contact details below). ✱

[To be continued.]

Figure 16. Light microscopy for honey bee diusease diagnosis (upper inset, **Melissococcus plutoneus***, lower inset,* **Paenibacillus alvei***, a secondary infection that can occur with a heavy case of European foul brood*

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