

Honey Bee Diagnostics at Fera: Part 2

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Concluding our look at the way the NBU helps to protect the health of our bees

PART 1 of this article was published in July 2012, page 7. It covered field diagnostics, including the use of the lateral flow device, and a description of laboratory diagnostic techniques and details of their use for foul brood diseases.

Laboratory diagnostics of exotic pests

Samples of mites, beetles, other insects and hive floor debris are regularly received into the laboratory. These may come from Inspectors who have collected them during routine Exotic Pest Surveillance that forms part of the AIP (see June 2012, page 8). Otherwise they may be submitted by concerned beekeepers or Sentinel Apiary holders (who monitor their colonies for exotic pest threats on behalf of the National Bee Unit (NBU)). We also monitor honey bee imports for exotic pest threats (see below).

Recently, we have been receiving reports of suspect Asian hornet sightings through the information portal of the Non-Native Species Secretariat (alert_nonnative@ceh.ac.uk). In this case, evidence is usually in the form of photographs, but we have also received a few insect specimens for identification.

Whatever the case, it is generally a straight forward matter for diagnosis to be achieved by microscopy in the NBU's own laboratory. Our technicians are trained in the recognition of the statutory notifiable pests small hive beetle (SHB), *Tropilaelaps* mites, and other similar species (Figure 1). However, on the occasions where a sample cannot be identified, it is passed on to Fera's specialist entomologist team for identification.

We can also turn to molecular diagnostic tools, for example, if sample fragments are too tiny to allow certain identification. In a typical year, we receive several hundred exotic samples, all of which to date have proved negative.

Laboratory Checks of Honey Bee Imports from 'Third Countries'

Figure 2. Workers escorting imported queens undergo diagnostic tests



In 2011, over one and a half thousand honey bee queens were imported into the UK from outside the EU (and many more from within



Figure 1. Three mites and a wingless fly, all of which will be recognised by the NBU laboratory technicians

the EU). The NBU laboratory receives all of the attendant workers and cages from such 'third country' imports for adult bee diagnostic checks (Figure 2).

Once the samples arrive, each cage is checked visually for signs of exotic pests. The attendant workers are washed to remove any mites on the bees' surfaces for subsequent identification via floatation in alcohol. An adult bee disease diagnosis is also performed on a sub-sample of the attendant workers, typically looking for Acarine, Amoeba and Nosema (both *N. ceranae* and *N. apis*).

In addition to these microscopic checks, attendant worker samples can also be screened for the NBU research projects using molecular diagnosis techniques. These allow us to look for viruses, including Kashmir bee virus (KBV), deformed wing virus (DWW), black queen cell virus (BQCV), sacbrood virus (SBV), chronic bee paralysis virus (CBPV), acute bee paralysis virus (ABPV) and Israeli acute paralysis virus (IAPV) (Figure 3).

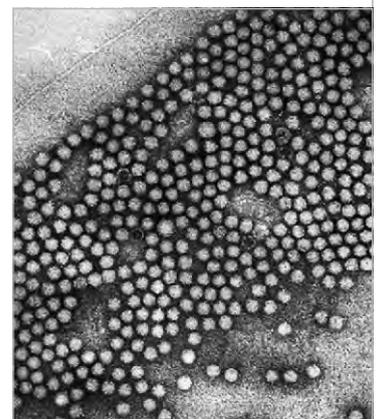


Figure 3. Molecular diagnostic techniques enable the detection and identification of viruses

Adult Bee Disease Diagnoses

Adult bees are dissected by securing a bee on its dorsal side with a double pin on a piece of cork board. Using a

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Figure 4 (left). Dissection of bees for tracheal mites and other diagnostic tests

Figure 5 (above) Dissected bee to show a healthy trachea (left) and one infected with acarine mites (right)



dissection microscope, the head and forelegs are removed with a pair of forceps before removing the chitinous collar to expose the trachea (Figure 4). Infested trachea will show a darkening pattern where melanisation has occurred due to the wounding action of the mite whilst

healthy trachea will appear pearly white in colour (Figures 5 and 6).

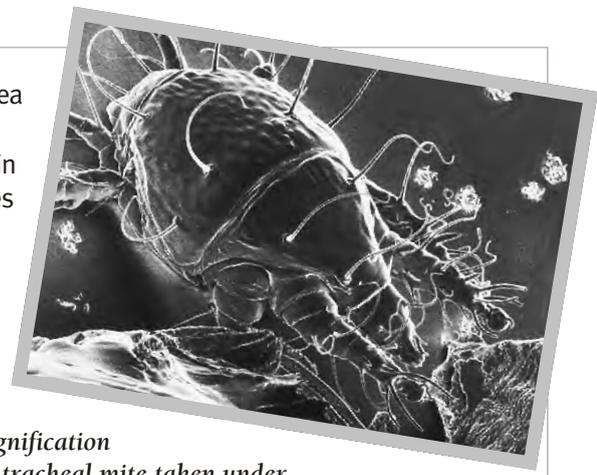


Figure 6. A high magnification image of a tracheal mite taken under the electron microscope

The abdomen is then removed for a *Nosema* diagnosis. In the case of a full adult bee diagnosis, every abdomen is analysed individually. If only a rapid screening is required, 30 bees can be ground together in distilled water using a mortar and pestle.

Viewed at a magnification of x400, *Nosema* spp. appears as green spores approximately 4–5 µm in length. With experience, it is possible to identify pure *N. apis* infections visually. *N. apis* spores tend to appear very regular and are wider than the narrower, more irregular *N. ceranae* spores (Figure 7). However, a molecular analysis is required for an accurate diagnosis to species level.

Recording and Reporting Results

The laboratory diagnostics services is supported by BeeBase, our secure online database. Once a the sample has been screened and the diagnosis is ready (ie, the result can be issued by the laboratory), the NBU office staff then log the sample onto BeeBase.

BeeBase stores the diagnosis with the beekeeper's inspection and sample history. For the routine testing of statutory samples, BeeBase will generate a results letter for the beekeeper. Beekeepers can also view their own results online via a secure login to their BeeBase record.

Chemical Screening in Honey Bee Health and Honey Bee Products

As well as the detection of honey bee pathogens, Fera affords the NBU with access to highly skilled and well-equipped analytical chemists and entomologists who also provide valuable support to our work. For example, for the purposes of the United Kingdom Accreditation Service

BeeBase

Figure 7. Spores of *Nosema apis* (left) and *Nosema ceranae* (right)

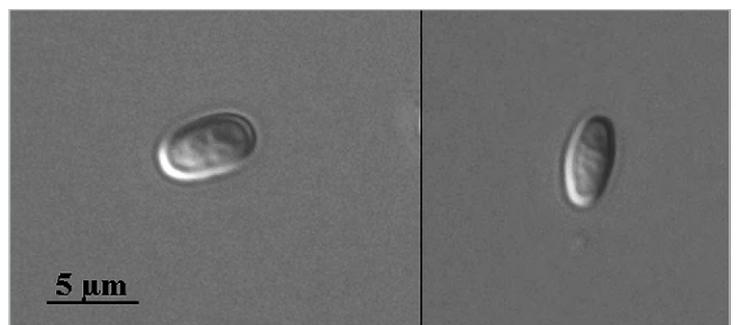




Figure 8. Taking samples of honey for the Veterinary Medicines Directorate

(UKAS) residue monitoring programme, Fera screens honey samples for chemical residues on behalf of the Veterinary Medicines Directorate (VMD) (in a typical year, approximately 120 samples (Figure 8)).

Under the Wildlife Incident investigations Scheme (WIIS), honey bee samples suspected of spray poisoning are also analysed for pesticide residues (In a typical year, around 40 samples).

Quality Assurance in Diagnostics

All of the work undertaken at the NBU is compliant with the quality standard Good Laboratory Practice (GLP). GLP is regulated by the Medicines and Healthcare products Regulatory Agency (MHRA) which is an executive agency of the Department of Health.

Our diagnostics tests are carried out in accordance with the World Organisation for Animal Health (OIE) standards (more specifically, the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2011) and with methods developed in-house at Fera.

All tests are fully validated, and use standardised methodologies. NBU diagnostics staff work to more than 60 Standard Operating Procedures which are updated regularly and subject to audit (every two years they are reviewed and updated as necessary).

Chargeable Adult Bee Disease Diagnostic Services at the NBU

The diagnosis of any notifiable pests and diseases is a statutory service which the NBU provides at no charge to the beekeeper. It is important to emphasise here that detection of foul broods (EFB and AFB), and suspected exotic pest species are thus 'free'. Any beekeeper who suspects that his/ her apiary may be affected by any of these organisms is obliged to report this to the NBU and we then provide all confirmatory testing, as well as assist in any subsequent treatment required.

However, the NBU also provides a chargeable adult bee disease diagnosis service for beekeepers who suspect other diseases in their colonies.

If you are interested in using this service, you will need to submit 30 adult bees to the NBU.

Full details and price lists are available from the BeeBase website

(www.nationalbeeunit.com). Our standard test uses microscopic techniques for the presence of Acarine, *Nosema* spp and Amoeba; Molecular pathogen screening (using TaqMan® PCR) can test for viruses (KBV, DWV, BQCV, SBV, CPV, ABPV and IAPV), and/or *N. apis* and *N. ceranae*

Prices vary according to the number of samples submitted and turnaround time varies from 10 working days to longer for larger batches. For over 100 samples, please contact us in advance. Urgent samples can be accommodated with prior agreement. *



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