PEST AND DISEASE CONTROL/MPI

BEE PATHOGEN PROGRAMME AT MPI

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The MPI Bee Pathogen Programme is funded by MPI's Operational Research and Department funds through the Diagnostic and Surveillance Directorate. The programme is investigating the prevalence and incidence of bee pathogens in New Zealand, using new methods developed by MPI that are based on the National Apiculture Surveys in the USA and Canada.

The Bee Pathogen Programme has collected three national sample rounds since spring 2016, six months apart. We have two more sample rounds to go, ending in spring 2018.

Our measurements include:

1. microscopic counts of varroa mites and nosema spore loads
2. molecular tests for viruses, nosema, American foulbrood and trypanosomes
3. apiary productivity
4. colony losses at the apiary over time
5. apiary management practices.

Who are the apiary managers participating in the MPI Bee Pathogen Programme?

Sixty apiaries were selected based on location within the country from Northland to Southland, matching the density pattern of registered hives. Forty apiaries in the North Island and 20 in the South Island are participating. All participants have at least one apiary of eight hives or more (following the USA and Canadian National Apiculture Survey protocols). The 60 apiary managers participating in MPI's research represent 99,777 hives throughout New Zealand, or 13% of all registered New Zealand bee colonies.

Demographics from our beekeeper sample tell us that most apiary managers have 20 or more years of experience as beekeepers, they have employed staff with one to five years of experience, they extract their own honey (68%), and they chose a honey production apiary for us to sample (66%). Most of them produce their own queens (63%) to replace failed queens.

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The main benefit to participants in MPI's Bee Pathogen research is seeing how their bees benchmark against the national sample. We are the only country that reports bee weights against the national sample, and to apiary managers this appears to be one of the most important markers of apiary health.

Who visits the apiaries and collects the samples?

We employ nine trained Authorised Persons Level 2 (AP2s) to take bee samples, inspect hives and collect data from apiary managers. These AP2s are looking for exotic pathogens, reporting clinical signs of disease and making observations on queen health. Each AP2 collects two samples from each apiary and posts them to MPI labs. Our AP2s are experienced beekeepers who are competent at inspecting hives and using mobile devices (iPad) to collect data electronically. They send this data via a secure network to MPI laboratories where the samples are also received. The Bee Pathogen Programme would not be possible without the AP2s.

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How are data collected, and how are the tests done?

One of the most important factors in bee health is apiary management. We take composite samples from eight hives at each of 60 apiaries and ask the apiary managers about their practices. Even though some apiaries have been sold and/or moved to new overwintering locations, we still have all 60 apiaries enrolled.

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Crushing dead bees for a nosema spore count.

RESULTS SO FAR

Microscopy results

Number of apiaries in sample = 60	Round 1 Spring 2016	Round 2 Autumn 2017	Round 3 Spring 2017
Varroa			
% of apiaries with varroa detected	45	65	47
Average number of mites/100 bees among apiaries with varroa	1.98	3.68	0.72
Number of apiaries above the treatment threshold of 3 mites/100 bees	4	8	7
	Nosema[1]		
% of apiaries with nosema[1]	92	55	83
Average millions of spores per bee among apiaries with nosema[1]	1.33	0.49	1.71
Number of apiaries above the treatment threshold of 1 million spores/bee	26	5	21

[1]*Nosema apis* and *Nosema ceranae* are counted together in the microscopy data.

To determine the number of spores per bee, 100 dead bees are crushed and their gut contents examined under a microscope on a glass slide. Spores are counted by eye—the technical staff use a stock counter and a haemocytometer (grid) —in much the same way as blood cell counts were done in human medical laboratories in the past. It is important to note that this method cannot distinguish the spores of *Nosema ceranae* and *Nosema apis*.

New Zealand apiaries have higher nosema spore counts than those recorded in the USA. Our average nosema spore counts in spring samples (when brood rearing is more intense) exceed the treatment threshold of one million spores per bee—an uncommon occurrence in the USA.

In contrast, our varroa counts are lower than those seen in the USA. Our apiaries rarely exceed the treatment threshold of 3 mites/100 bees, even in the autumn when mite levels are high. It is worth noting that the infestation of USA apiaries with varroa mites is at least a decade ahead of New Zealand. We may see these numbers increase.

To check out the USA figures on seasonality of varroa and nosema infestation, look at this web page https://bip2.beeinformed.org/state_reports/

Link to US National Survey results: https://beeinformed.org/2016/05/04/the-national-honey-bee-disease-survey-Varroa-Nosema-in-the-us/

Molecular results

The detection of nucleic acid, deoxyribonucleic acid or ribonucleic acid (DNA or RNA) is often called molecular testing. We use a laboratory method called polymerase chain reaction (PCR). In our study, a sample of 50 bees are macerated in a solvent to release DNA and RNA. Most of the material present will be honey bee genome, but there will also be DNA and RNA from viruses, bacteria, fungi and parasites.

Molecular testing is a great method for detecting pathogens (disease-causing organisms) because it is very specific, very sensitive, and it can be applied to large numbers of samples with ease. We wait until an entire sampling round is collected and then batch the samples together, which ensures consistency when comparing apiaries. We have also standardised the tests so that we can provide, with good accuracy, an estimate of the number of pathogens present per bee—better than just telling you if they are present or not.

Chronic bee paralysis virus (CBPV)

Chronic bee paralysis virus was present in one of every five apiaries (20%) in New Zealand during the autumn 2017 sampling, up from one in 14 apiaries (7%) six months earlier. It will be interesting to see if this is a seasonal trend, or if the increase continues as has happened over the last few years in the USA. CBPV causes trembling wings, adult bees crawl on the ground outside the hive, or may cause hairless bees that look black and shiny. This pathogen had no spatial distribution of note in the first two rounds of sampling.

Bees affected by chronic bee paralysis virus.

CBPV is said to be more pronounced when the colony is under stress. Overcrowding within the hive contributes to the spread of CBPV, as does undernutrition. It is important to fully clean dead-outs and to provide plenty of space within the hive, because the virus is passed via faeces and direct contact with infected bees.

For more information on CBPV, read this web page from UK's National Bee Unit: www.nationalbeeunit.com/downloadDocument.cfm?id=1158

Black queen cell virus (BQCV)

Black queen cell virus is found in all New Zealand apiaries in the Bee Pathogen Programme. Because our tests are so sensitive, virus can be present without any clinical signs of disease.

Clinical (observable) signs are the queen dying and turning yellow after capping, which can be confused with half-moon syndrome. With BQCV, the queen larvae eventually turn black, sometimes leaving a black spot on the outside of the cell.

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To reduce the damage this virus can do, keep bees well fed and clean grafting tools between uses with flame or ethanol. In further data analyses we will be looking at the association between the viral concentrations of BQCV and other variables we have collected.

Deformed wing virus (DWV)

Deformed wing virus and varroa.

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Nearly every apiary in New Zealand was found to have DWV (only three apiaries were free of DWV in each of the first two sampling rounds, and only one of those was negative in both data collection rounds).

DWV can be devastating to the colony, as its most severe outcome is the stumps that appear where the wings should be on adult bees. Without wings, such individuals represent a net loss to the colony. The deformed wings don't always appear in a colony that is carrying DWV. New international research has shown that there are different types of DWV, and that some types are more likely to be associated with wing deformities than others. In the Bee Pathogen Programme, our test doesn't differentiate between DWV types.

There is already strong evidence to support the association between DWV virulence and varroa mite numbers, but this can be misleading too, as DWV is able to survive in bees after varroa mites have been treated and eliminated from the colony.

DWV concentrations are very different between apiaries, so this research will allow us to understand why some apiaries succumb to symptomatic infection, yet others are able to sustain high concentrations of the virus without apparent harm.

Kashmir bee virus (KBV)

This virus was found in 35% of sampled apiaries in spring 2016 and increased slightly to 55% in autumn 2017. KBV can show the same clinical symptoms as CBPV, and both of these viruses can be present at high titres (concentrations) without showing any signs of disease.

KBV is most commonly spread by varroa mites but can also be brought back to the hive by foragers visiting the same flowers as infected bees. KBV has been associated with colony death since at least 2007. In our research, each bee from an infected colony will carry over one million viral particles.

Check out the results from the US National Apiculture Survey here:

https://bip2.beeinformed.org/state_reports/viruses/

For a good summary of bee viruses associated with the varroa mite, go to this web page: http://articles.extension.org/pages/71172/honey-bee-viruses-the-deadly-Varroa-mite-associates

Nosema ceranae

Nearly half of all apiaries had *Nosema ceranae* in spring 2016, with a slight drop to 40% in autumn 2017. There was only one instance of *N. ceranae* found in the South Island. In the apiaries that did have *N. ceranae*, most of them had a high concentration. The molecular data was consistent with the microscopy data mentioned above.

Nosema ceranae can be a significant cause of colony losses, especially during overwintering or in the early spring. MPI recently investigated a case where hive losses over winter were greater than 70%. In this case, the concentrations of *N. ceranae* in live bees registered at the highest level of those found in the 60 apiaries from all rounds of the Bee Pathogen Programme. Furthermore, dead bees from this apiary had *N. ceranae* concentrations that were several orders of magnitude higher than other apiaries we sampled.

Since this fungal parasite originated from tropical Asia, one reliable method for destruction of *N. ceranae* is freezing. Other ways to reduce the impact of *N. ceranae* include: making sure bees are well stocked with pollen and honey going into winter, overwintering hives in sunny areas to encourage cleansing flights, and sterilising beekeeping equipment between hives.

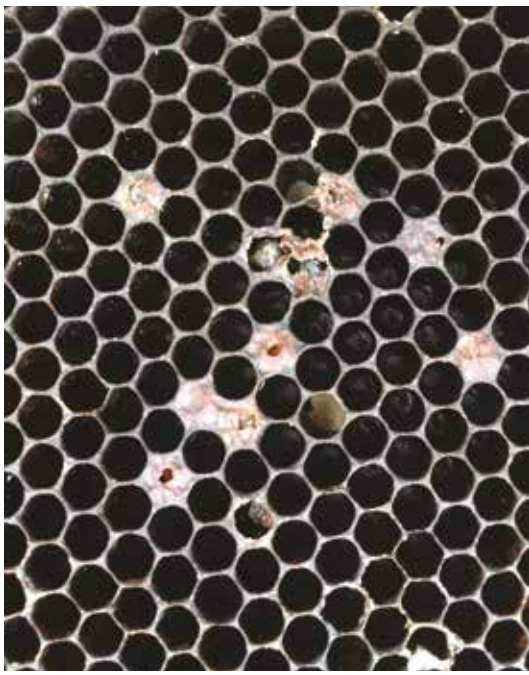
To read more about *Nosema ceranae,* see this article: http://entomology.ucdavis.edu/files/147621.pdf

Nosema apis

Nosema apis was nearly ubiquitous in spring 2016, with only two apiaries (3%) free of it. By the autumn sample round in 2017, *N. apis* was only present in 42% of apiaries (just as was observed for the total nosema spore counts in our microscopy data).

Like its cousin *Nosema ceranae, Nosema apis* seems to be present either in high concentrations of over one million per bee by quantitative PCR, or not at all. It appears to be less likely to cause harm to a colony when compared to *Nosema ceranae.*

American foulbrood (AFB)



American foulbrood.

At the beginning of this research we didn't have a molecular test for the DNA of *Paenibacillus larvae*, also known as American foulbrood (AFB). It was a state of science achievement to participate in developing the AFB Duo Assay with dnature in Gisborne. Since clinical observation is still the gold standard in identifying AFB, we have matched our data generated by the AP2s to the results of the AFB duo test.

In the first two sample rounds, six of our 60 apiaries produced a positive laboratory result matched by clinical signs of AFB according to the AP2 inspection. (One of these didn't appear in the results sent to beekeepers.) We will be further comparing the AFB duo test

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with another commercially available assay and letting you know the results.

The epidemiology of AFB is further complicated by the discovery of different types of AFB, described recently as Enterobacterial Repetitive Intergenic Consensus (ERIC) types.

We know from overseas research that New Zealand honey has both ERIC-I and ERIC-II types. The difference between these is that AFB ERIC-I has a longer incubation period, meaning brood cells are capped before the larvae are dead, whereas AFB ERIC-II has a faster incubation period and larvae die before capping. It is easier for nurse bees to detect AFB ERIC-II and remove infected larvae, which also makes it more difficult for beekeepers to detect.

Having a molecular test to distinguish between ERIC types will improve the detection and traceability of AFB infections.

For more information on AFB types, see this web page by New Zealand's own Dave Black: https://www.nzbees.net/blogs/entry/10-another-look-at-american-foul-brood/

In the meantime, we have determined that AFB is present in one out of every 10 apiaries over a 12-month period (where clinical observation and laboratory molecular tests agree). This is an annual incidence rate of 10% of apiaries sampled. Total apiaries sampled were 60. The managers of these 60 participating apiaries represent 13% of all registered hives throughout New Zealand, which is approximately 99,777 colonies.

Discussion

There are two potential sources of bias in the current national sample:

1. Beekeepers may have wanted to participate if they had a 'hospital apiary' and wanted to know what pathogens they might be able to identify or treat for. If the majority of managers participated for this reason, it is possible that our research would overestimate the prevalence and incidence of pathogens in our results.
2. Experienced beekeepers who are conscientious about biosecurity may have been more willing to participate than others. This would be described as a 'healthy volunteer' effect, where 'good' beekeepers might apply regular varroa treatments, make sure their bees have plenty of feed and don't aggressively split colonies, etc. If this were the case for the majority of participants, then we would expect our results to underestimate the true prevalence and incidence rates of disease.

While we cannot say for sure that the 60 sampled apiaries are representative of all beekeepers in New Zealand, we expect that the biases described above are inconsistent among the 60 apiary managers in this research. A realistic assumption would be that both sources of bias are operating, and even changing, over the two-and-a-half-year period of research.

Because of the density-matched sampling protocol we used and the cohort (five sample rounds) data we are collecting, we will be able to identify risk-based criteria in New Zealand apiaries that are unique in apiculture research worldwide.

There is a trade-off between getting more apiaries or more detail on fewer apiaries. MPI prioritised a sample size that would allow us to conduct a detailed apiary analysis and still find a rare disease, or an exotic pathogen.

Where to from here?

The Bee Pathogen Programme has two more rounds of data collection, ending in 2018. We will be reporting results through *The New Zealand BeeKeeper* journal and in person where possible. Enrolled apiaries can share their individual data if they choose to.

The 2018 sampling rounds will introduce a new trapping method for detecting small hive beetle (SHB). We fully expect our traps to come back to the lab without SHB since this pathogen has not been detected in New Zealand. Putting the traps out now will tell us how well they are tolerated by the bees and the beekeepers, and whether our 60 beekeepers will send them back to us for analysis. If successful, the traps could be used in future surveillance for exotics.

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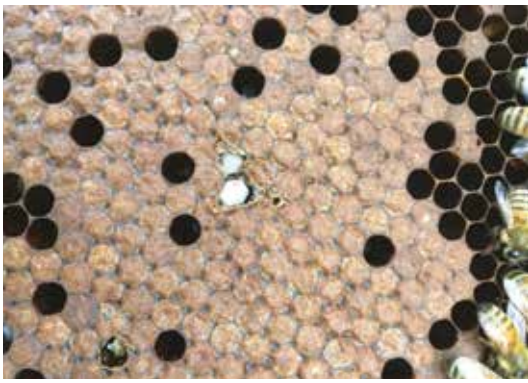

Chalkbrood. Photos courtesy of the Ministry for Primary Industries.

Further molecular testing is being introduced on freezer-stored samples to look for exotic pathogens that are not currently known to exist in New Zealand, such as European foulbrood *(Melissococcus plutonius)* and Israeli acute paralysis virus (IAPV).

We look forward to doing a more detailed analysis of the pathogen results with the data we have collected from apiary managers and the AP2s conducting the inspections.

Acknowledgements

The authors would like to thank the 60 apiary owners who participate in the programme and the AP2s who conduct field investigations and collect data from apiary managers: Dan Lambert, Kim Kneijber, Stephen Black, Allan Richards, John Maynard, Trevor Bryant, Norbert Klose, Phil Sutton and Grant Hayes.

We would also like to thank the Technical Advisors who have provided valuable insights into the questionnaire design and sampling process: Oksana Borowik, Marco Gonzalez, Peter Ferris, Frank Lindsay, John McLean, Barry Foster, Paul Badger and Phil Lester.

The MPI team in Auckland would like to thank Stacey Lamont and Santha France for processing the samples in rounds one and two, Jane Martin for preparing the equipment and Sherly George for allocating the facilities.

The MPI team at Wallaceville would like to thank Grant Munro (in memoriam), Wlodek Stanislawek and David Pulford for their scientific advice, Abbas Munshi, Emma Bramley, Amy McBride and the Animal Health Laboratory staff for their support of the project.