Improving American Foul Brood disease detection and management

Project CleanHive Summary 2018 - 2021

SFF Project 405299

Marie Casey and John Scandrett

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Project CleanHive Key Findings

Otago and Southland beekeepers identified the opportunity available to them to pilot some innovative solutions for improved detection of American Foulbrood disease (AFB) using a mix of the tests that are currently available to them and some of the newer technology (qPCR tests for DNA). Currently the region has a low base level of AFB infection but there is a growing challenge from the increased movement of hives into the region from areas with higher levels of AFB.

This project was a pilot study to test whether the current testing methods can be calibrated with the proposed new tests (qPCR and detection dogs) to provide cost effective tools for detection and management of AFB at the pre-clinical stage.

The aim was to provide new opportunities for improved disease identification and control of AFB to help prevent the impact and spread of a notifiable disease. Cost effective testing will increase participation and effectiveness in both commercial and non-commercial beekeeping enterprises.

The CleanHive Project work plan evolved over the last three years as the discussion group's understanding of the potential uses for improved identification and management of AFB incursions grew. Below is a summary of the key findings, much of which is based on the use of swabbing the hive entrance to obtain samples for qPCR analysis (Foster method).

Use of qPCR for AFB management

Best time to swab

1. If clinical AFB present, (visual inspection) – as soon as possible to identify further at-risk hives, develop quarantine and management plans for the apiary
2. End of AFB incursion management – confirm quarantine processes have worked
3. Screening hives – 2 weeks prior to honey harvest to allow time for lab results to return and be acted upon
4. Purchase or lease of hives – any time
5. Screening for sale – by mutual arrangement
6. Test 'dead out' hives to confirm AFB free and safe to reuse – post winter or other times according to the situation.

Management Agency use of qPCR for AFB

7. Agency notified of local clinical case of AFB → target screening of the affected apiary and surrounding apiaries?
8. AP2 inspections – potential to screen widely, particularly high-risk areas and identify issues much earlier than waiting for clinical cases to develop. Could use composite testing of samples.

Composite qPCR testing of samples

To test apiaries

9. Identify background AFB spore loads
10. Confirm management practices are successful,
11. Screen larger numbers of hives (manuka apiaries, pre-lease or purchase of hives, confirm apiary hygiene).

Points to note – qPCR test

- Swabbing allows easy detection method for presence or absence of AFB DNA (but is not fully quantitative).
- **NOT** a stand-alone option for AFB detection – visual inspections are needed to confirm clinical cases and clinical cases may occasionally develop without being detected by swabbing due to a delay in AFB material reaching the hive bottom board.
- The financial cost of AFB elimination in an apiary is very significant - in loss of and replacement of hives, lost honey production, additional labour (note the time for Case Study Apiarist to visually inspect all the hives, p15). There is also additional stress and a mental health cost. It is therefore critical to identify any subclinical hives asap.
- Enables the beekeeper to develop a strategy of quarantine and management for AFB for the next 12 months that speeds up full elimination of AFB and reduces the chance of further infection. In addition, it can help identify the source of an infection as well as the management processes that enable spread within the apiary.
- Sampling nurse bees is generally the most reliable method for detecting AFB DNA but swabbing the hive bottom board is very time efficient. (Note sampling nurse bees is not 100% reliable in detecting AFB DNA, old hives can harbour AFB spores which take time to transfer to new bees to a hive.)

American Foul Brood in NZ

Introduction

One of the growing threats to the NZ honey industry is the management and control of American foulbrood (AFB) caused by the spore forming bacterium *Paenibacillus larvae*. The bacterium exists in a spore form and the vegetative (clinical) form.

American Foul Brood (AFB) lifecycle and Infection

AFB spores can be found at any time of the year in honeybee colonies or in beekeeping equipment. As a disease it is not considered highly contagious, but it is long lived (ranging from 35-70 years) and easily transferred i.e. *hard to catch, easy to spread!*

Adult bees are not infected. The infectious stage, in the hive, is through the transfer of spores **from nurse bees to the larvae that** are between 24 hours and 72 hours old. The infected brood die at the pupal or pre-pupal stage - but continue to be a source of spores.

Infected larvae are also the only way the bacteria can multiply (increase the number of AFB *P. larvae* spores). A single diseased larva may contain more than 2.5 billion spores. AFB does not multiply in any other environment, but the spores can remain dormant in beeswax, honey, propolis, used hives and components.

AFB *P. larvae* spores are very resilient and can survive for a very long time outside the bee colony (more than 50 years). They can also withstand high temperatures (including boiling water), direct sunlight, desiccation, fermentation, and a range of disinfectants and veterinary drugs.

Spread of AFB P. larvae

How does AFB *P. larvae* spread, both in the hive and in the apiary?

Within the hive the worker bees actively clean cells and remove dead or diseased larvae. However, when cleaning infected cells, bees can then distribute spores throughout the colony.

It is important to note that AFB infections do not occur because a single bacterial spore finds its way into a colony; for infections to occur large numbers of spores are usually required. In controlled experiments it has been shown that several million spores need to be fed to a honey bee colony in either sugar syrup or honey to result in an infection of one or more larvae.

There is potential for the disease to spread rapidly throughout the hive as the bees, trying to remove the spore-laden dead larvae, contaminate brood food. Nectar stored in contaminated cells will contain spores and soon the brood chamber becomes filled with contaminated honey. As this honey is moved up into the honey supers, the entire hive becomes contaminated with spores.

Bees have an average foraging range of 3 to 5km and they occasionally rob honey. This honey may contain AFB spores from weak or dead managed and feral honeybee colonies, disposed unwashed honey containers, honey extracting facilities that are not bee-proof and infected hive material nearby.

There are also many interactions of the beekeeper/s working with their hives that create opportunities for spread of the disease. Management of beehives has a key role in both the spread and control of AFB.

American foulbrood field test

AFB and the honey industry

AFB is a notifiable bacterial disease of bees with a NZ National Management Plan in place. To be an effective plan this needs total industry participation and is reliant on vigilance and constant hive monitoring by beekeepers. Colonies with clinical AFB **must** be destroyed.

The industry has identified that beekeeper behaviour is the major factor in the spread and incidence of AFB. Consequently, beekeepers also have a major role in eliminating the disease but while they find more than 95% of the hives reported each year, the number of infected colonies is still growing year on year. The remainder are found during inspections by AP2 inspectors.

Potentially disease rates can be reduced quickly by both decreasing beekeeper spread of AFB and/or increasing detection rates, but a significant problem is that at present the only effective control is by removing clinically infected hives. Once clinical infection is detected or obvious there have been many opportunities to spread the disease i.e. these hives are not always found in time to prevent further spread of the disease via spores.

Management of AFB has become even more critical for beekeepers because there are some markets that will not accept honey that contains AFB spores. Hives with honey containing AFB spores because a clinical infection was not found early enough could be robbed out and spread to other hives or extracted and mixed with uncontaminated honey, thereby affecting the market opportunities and possibly value of that line of honey.

SFF Project CleanHive

Introduction

Project Clean Hive's objective was to identify and assess effective use of the currently available tools to improve risk assessment and proactive management of AFB in the Otago Southland region. The aim was to improve the outcomes currently achieved which rely on detecting the disease at the clinical stage in the hive before any action can be taken.

Included was the critical objective of calibration of a new laboratory testing methodology, Quantitative Polymerase Chain Reaction (qPCR), which had been demonstrated to be able to quantify the presence of AFB *P. Larvae* DNA in a bee sample, but no interpretation of the results was available.

Key Project objectives

- An overall aim to eliminate clinical AFB disease in the region
- Calibrate qPCR results against plate test results
- Increase detection of AFB in hives
- In a pilot trial compare the four tools currently available to identify presence or absence of AFB (a multi factor validation) – AFB detection dog, plate test, qPCR (quantitative polymerase chain reaction), and visual ID
- Refine the qPCR test as a cost-effective detection tool for the future.
- Develop some risk management strategies for the hives that are identified as potential AFB clinical risks to help beekeepers improve how they currently manage AFB
- Develop a composite testing protocol for early screening of hives for AFB DNA with qPCR
- Improve the biosecurity practices of both commercial and non-commercial beekeepers.

The project developed further into three distinct parts

1. Understanding AFB and the use of qPCR as a tool
2. In depth investigation of apiaries with an AFB infection
3. Development of Composite sampling methodology for qPCR and its potential for use in the wider industry (see page 13).

What is qPCR

Quantitative Polymerase Chain Reaction *(qPCR) is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR). It monitors the amplification of a targeted DNA molecule during the PCR (i.e., in real time). Real-time PCR can be used quantitatively (quantitative real-time PCR) and semi-quantitatively (i.e., above/below a certain amount of DNA molecules) (semi-quantitative real-time PCR) (ref. Wikipedia).*

The discussion group had been made aware of the new technology and its potential in discussions with Mark Goodwin, Plant and Food Research.

To proceed the Project team had to find a laboratory to perform the qPCR test and assist with calibration and analysis. John Mackay at dnature (a skilled molecular biologist) agreed to support the project. The qPCR test designed and used by dnature, the laboratory, looks for two AFB *P. larvae* markers. The test for marker one is significantly more sensitive than marker two. Consequently, we have concentrated on calibrating marker one. In addition, dnature volunteered the use of their new

swab method (now named the Foster[1] AFB method) as part of the project, as an alternative to sampling bees – but still using the qPCR test.

The test results (Cq) are presented on a numeric scale from 1 to 37.9. The smaller the number the higher the AFB DNA concentration present.

In this project the observed qPCR sample test results range from 17 to 37.5, with ND for not detected (i.e. no AFB DNA detected in the sample).

Sampling techniques

Bee samples

Bee sampling is the standard testing methodology for both plate and qPCR tests.

The laboratory provides jars for nurse bee collection and instructions for collecting samples. Thirty nurse bees per hive are collected in a sample jar, euthanized by freezing and should be sent to the laboratory as soon as practical.

Attached is a link to the YouTube video for sampling bees
https://www.youtube.com/watch?v=JGvuVFMp8rM

Sample Collection for qPCR

Individual sampling tubes and swabs were provided to beekeepers to sample hives individually to prevent any cross contamination. Forms were provided to record sample and hive details.

Sampling involved swabbing the hive entrance from side to side and rotating the swab at the same time.

While sampling bees is the gold standard, swab sampling from the hive entrance will give repeatable results if it is done carefully to ensure a representative sample is collected.

When collecting samples, take care to avoid cross-contamination between the samples. PCR is a very sensitive testing technique, and even small amounts of cross contamination can result in a 'false positive' result if there are bacteria or spores present in some samples.

Data collection

When collecting samples each numbered tube is assigned to one hive. In addition, apiarists should record for each sample tube - hive number/ID, yard location/ID, sampling date, and hive details e.g. frames of brood, brood comments, and hive health comments. Knowing the age of the hive can also be useful when interpreting results.

Methodology

The project has been underway since October 2018 and over 834 hives samples have been submitted for testing. Generally, where possible, the samples included nurse bee samples and swab samples from hive woodware for each hive. There have been some situations where only swab samples or only bee samples were collected. For example, only swab samples can be collected from a dead-out hive (one where the entire bee colony has died).

[1] Utilisation of a novel dual-target qPCR assay for American Foulbrood to detect clinically relevant hive infections by rapid and non-invasive eDNA sampling means: the Foster method. JF Mackay, R Hewitt, T Waters, J Scandrett (In press)

One objective of the project was to evaluate the accuracy of the detector dog in detecting the presence of AFB in hives in an apiary. The detector dog operates similarly to a drug or customs dog, trained to detect against a known sample.

Swab samples and bee samples were taken in parallel from hives in apiaries located near clinical AFB apiary sites. This happened for 2 years during the project.

Clinical hives and hives next to clinical hives were also tested within apiaries and this was followed in June 2020 by testing all the hives within a business that had suffered a severe AFB incursion. This is documented as a case study within this report.

Note: for clinical hives, samples were collected immediately upon visual identification of AFB and before they were destroyed, thereby following the protocols required by the AFB Management Agency.

Results and Outcomes

Comparing the 'detector dog' vs plate testing and qPCR

Samples were collected from 77 hives that the "AFB detector dog" had indicated on at the start of the project. The dog was run past as many as 2,000 hives at this time. As stated, subsequent sampling included swabs and bees. The swabs and bee samples were tested using qPCR (dnature laboratory) and a subsample of bees was sent to the National Animal Health laboratory, MPI, Wallaceville for plate testing (using standard culture test methodology).

After analysis of the results from the 77 hives, it was determined that the results from the qPCR bee samples showed this technique was much more sensitive than the plate test. AFB DNA was found by qPCR in 7 samples. No AFB was cultured by plate testing. Given the sensitivity of qPCR compared to the AFB plate test and it was decided to just use qPCR sampling and compare bee and hive swab samples for the rest of the project.

In addition, it was observed that the detector dog indicated on a significant number of hives, but the hives were not necessarily clinical or potentially clinical AFB cases. Often the hives were observed to be in "poor health" for a range of reasons, but for nearly all hives this was not because of AFB.

The issues that arose using a detector dog included:

- access to a pure sample of AFB *P. larvae* spore to train dogs. Access to pure infected material is controlled as a notifiable disease. Sample material taken from infected hive is likely to have other material including virus and bacterial that confuses the training of the dog.
- limited number of dogs likely to be trained
- dogs do not like bees
- use of the dog is often better at night when apiaries are quiet (bees asleep)
- the number of hives /apiaries a dog can screen can be limited by these limitations

No further detector dog testing was done after the first round of testing as ongoing monitoring of the 77 hives showed there were a high number of false positives and a few false negatives. This simplified the trial to a comparison of qPCR sampling and visual detection of clinical AFB and gaining experience with interpreting the qPCR results.

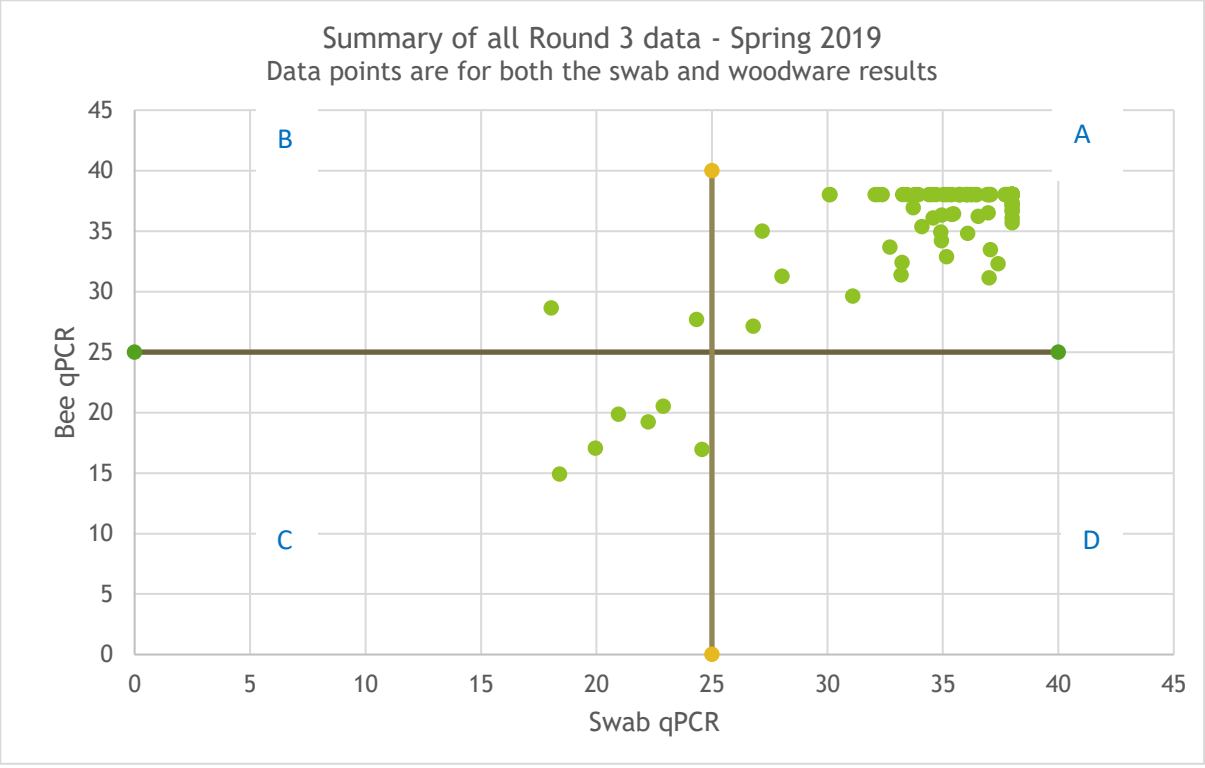
Key observations

- **An indication of the loading of AFB spores** in a hive can now be estimated through qPCR analysis and the experience to date is that the data generally appears reliable, both for bee samples and swab samples from hive woodware. The majority of hives tested do not have spores or if there are

spores, they are at a very low level, such that a clinical infection is extremely unlikely to develop. Experience to date using dnature's test method is that a qPCR test result of less than 27 has a high to very high risk of developing clinical AFB. A test result over 30 has a low to nil chance of developing clinical AFB.

- **When interpreting swab sample test results** it is important to know the hive history, particularly how long the bees have been in that hive. It is quite possible to have "clean" bees in a spore contaminated hive and vice versa.
- **Spore contaminated woodware** has been seen to cause a clinical AFB infection when new, "clean" bees are transferred into a hive.
- **Woodware can be tested** from dead-out hives to determine if it is safe to use, or to determine if AFB was present and the likely cause for the dead-out.
- **qPCR sampling** enables beekeepers to gain an indication of what is happening within hives at a pre-clinical stage. Hives with high AFB DNA levels and no clinical symptoms can be quarantined and monitored and appropriate management applied depending on whether they develop clinical symptoms or not.

Figure 1: Combined data from 3 rounds of hive sampling across Otago/Southland; data points are for both bee and swab results



Legend

Quadrant A – both the bee and hive swab results are above 25 – **No AFB**

Quadrant B -the bee result is above 25 but the hive swab result is below 25 (environmental,) **Potential for AFB to develop**

Quadrant C- both bee and swab results are below 25 – **Problem identified** – 6 hives identified as clinical

Quadrant D – the bee result is less than 25 but the hive swabs above 25 – **Potential for AFB disease to develop**

- **Brood must be present** for clinical AFB to develop. It appears unless a high spore loading occurs in the autumn and carries through the winter, clinical infections after winter generally do not seem to occur until November.

  For hives that pick up a large spore loading in the autumn, clinical infections can occur any time thereafter when there is brood present. The ability of bees to manage the disease and the relationship of this to nutrition, stressors and general hive management is an ongoing discussion.

- **Timing of sampling:** The results from the combined data set from 3 rounds of hive sampling to spring 2019, Fig 1 above, show that in businesses where there is no recent clinical AFB history the spring sampling test results will show no or low AFB DNA levels.

  Although AFB is aseasonal i.e. disease can occur at any time, the earliest indication of AFB at clinical levels in a hive in our sampling, either in the bees or swab sample is late Nov/early Dec.

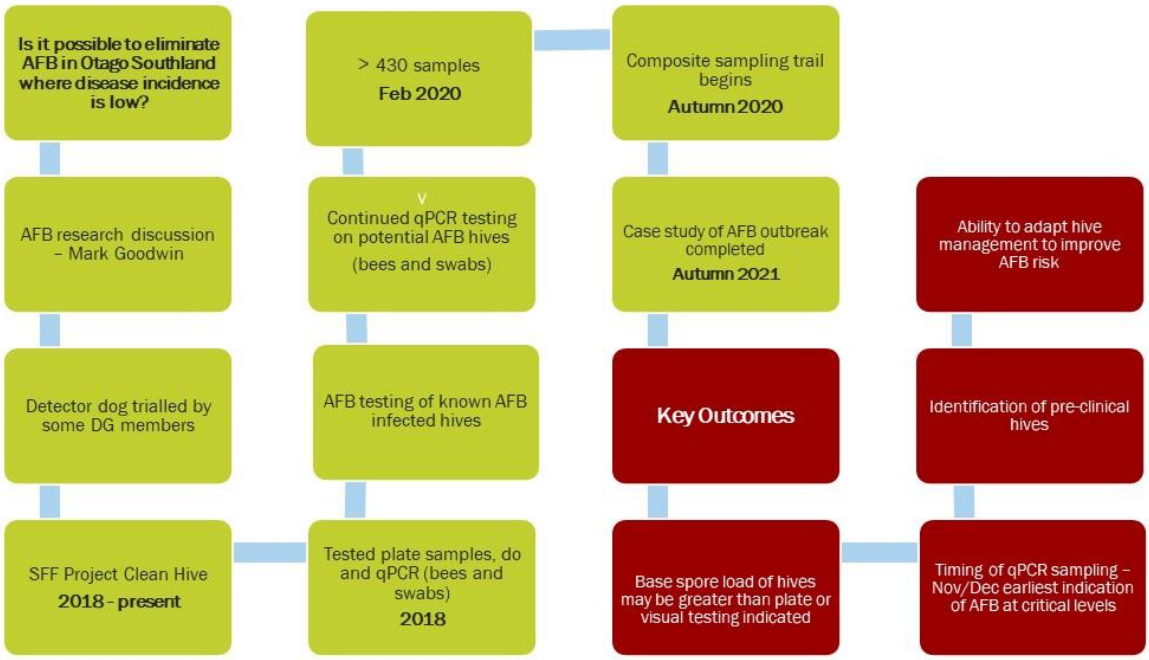
  Note that although a qPCR result of 25 was used in the graph below to define four result groups, subsequent testing suggests 27 should be the cut off. A hive with a qPCR result less than 27 for bees or swabs is almost certain to develop clinical AFB.

Project Progress against objectives

Actions	Project objectives	Outcomes
Calibration	qPCR against the plate test	qPCR more sensitive and plate test was slow and expensive*
Accuracy check	AFB detector dog	Tested hives indicated on by dog (bees and swabs), *dog is unreliable*
Swab sampling	Compared against bee samples	Not in original project methodology until discussed with lab*, trialled with good results*
Sampling at risk hives	1-2km from known AFB infections to check AFB spread	Generally, no AFB DNA found
Sample all hives	Apiary where clinical cases AFB identified	Other hives within the apiary often had AFB DNA present
Sample clinical cases	Calibrate qPCR	This gave vital calibration data for qPCR prior to hive destruction
Sample all hives	2nd apiary in project that had clinical AFB (30% invasion)	Case study outlined in report (p15)
Composite sampling	Tested composite sampling of swabs (8 and 12 samples)	Method to reduce cost of sampling large numbers of hives, found to be reliable
Trialled swab technique	Tested cursory vs intensive swabbing (on a small number of hives)	The swabbing technique had little effect on the result

* the AFB plate test is free for apiarists but is a slow diagnostic tool

AFB TIMELINE 2018-2021



Composite Testing protocol (qPCR)

One of the aims within this project was to trial a methodology for composite testing of a larger number of hives especially where an apiarist may have concerns about the health of hives and there are no clinical AFB signs. Sample collection follows laboratory protocols as outlined above.

The objectives of a composite test using qPCR screening are:

- To reduce the cost of individually testing large numbers of hives to identify potential AFB infection
- Allow a stepwise screening process, if a composite sample tests positive all samples forming the composite are tested.
- To increase the ability to identify and manage potential issues early
- To increase the timeliness of getting test results back

If either clinical or subclinical AFB is detected, then further investigation of the individual hive(s) can be carried out, and movement control of hives and beekeeping equipment can be implemented to control the spread of disease.

Methodology

In discussion with John Mackay at dnature the following methodology was trialled to see if it would be effective. Note: both bees and swabs were taken to get the best possible data about each hive before the composite results were interpreted.

1. **At the apiary site**: - the hives were sampled individually as usual with a sample of bees collected as well as a swab sample from each hive.
2. **At the laboratory:** - eight swab samples were combined for composite testing. Each swab had any AFB DNA extracted and then a subsample was taken to form the composite sample to be tested. The bees were not composite tested but were tested per individual hive. They did provide an extra check on each individual hive's AFB DNA level.
3. The eight individual samples were retained so that they can be tested as individual hives if the qPCR test gave a positive AFB result for the composite sample.
4. Eight samples had been calculated at this stage as the maximum number that can be combined and still yield a sensitive enough result.
5. For the composite results that were positive for AFB DNA, the individual swab samples were tested, and their results compared to the bee sample result. The hive history was also studied, where possible. It is possible to have an apparent disparity between the swab and bee results, depending on recent hive management. In the few cases where this has happened the difference in results have generally been able to be explained by the recent management of the hive. A more reliable interpretation of the swab result can be made if the hive has had a stable history for at least the last 3 months, i.e. no splitting or changing of frames.

Results

200 hives were swabbed and tested using the 8-sample composite method.

8 sample composites gave reliable results, all composite test results were positive where one or more individual hives tested positive.

In this case it was found all dead out hives contained AFB DNA. As a result, dead out hives should be left out of composite testing and instead be tested individually to confirm whether the hive was suitable for reuse.

The bee and swab sampling identified 3 groups of hives:

1. Those hives that have no or extremely low levels of AFB DNA and would not be expected to develop clinical symptoms.
2. Those hives that have marginal AFB DNA levels where it is possible clinical AFB might develop. These hives were quarantined, and both inspected and sampled regularly after the initial test. More than 30% of these hives ended up being destroyed.
3. Those hives that have high AFB DNA levels where a clinical infection is expected will be quarantined and destroyed or monitored and then destroyed, as required.

Based on the success of the 8 sample composite 180 hives were tested using a 12 sample composite. This was also successful in that all composite tests were positive when one or more individual hives tested positive.

Conclusion

Early intervention when clinical cases are discovered within a business along with qPCR screening should potentially allow for eradication of clinical AFB in one season.

Screening hives by swabbing and using 12 sample composites seems feasible and sensitive enough to be used commercially to identify the likely AFB status of a hive. Hives can then be segregated into groups and managed on the potential for clinical AFB development.

If a commercial test using qPCR with a swab test of hive woodware was adopted by more than one lab it would be important to ensure standard methodologies are used between labs and for reporting results.

It is critical to know the hive history when interpreting AFB DNA test results. More work is required monitoring AFB DNA levels over time in hives that have low to moderate AFB DNA in them (Cq number larger than 30?) to see what trends occur. Anecdotal evidence is AFB DNA levels decline over time, although we have seen clinical infections develop in hives within this range.

AFB Composite testing Case study

History of the AFB infection Case Study Apiary

Date 2018	From March 2016 to March 2018, 180 hives were purchased from 3 different sources.
	Visual inspection for AFB was carried out and this was followed by an inspection by an independent DECA holder as an additional check. No 'clinical' AFB was detected.
Aug 2019	Purchased more hives including apiary sites, from a retiring beekeeper – 55 hives and 12 'dead' hives with brood honey, sealed off from the previous autumn ("dead" hives purchased for the woodware and honey stores). At the time of purchase he was warned not to keep hives in the Wyndham area due to AFB disease issues so he moved hives to a site near Clinton where good spring sites were available. 12 Wyndham hives were added to 11 hives at an existing yard to total 23 hives.
Dec 2019	Had a target of 300 hives by utilising strong spring honey flow to grow hive numbers. Used the purchased dead-out woodware after stripping and cleaning; honey in these boxes was used as feed.
	With splitting the result was 292 hives achieved by December 2019
	No general quarantine practice used (except for hives from Wyndham).
	Note: Bee keeping storage shed is open and not bee proof, recycled woodware stored here as well.
Jan 2020	Putting on super boxes – noted weak hive and failed queens. Did not check for AFB at this time as thorough checks had been made in spring and the symptoms appeared to be due to varroa.
Feb/Mar 2020	No brood check when took the honey off. The issue of being a single operator (no staff to assist). Taking honey off took 5 days. The aim was to do the inspections next.
March 2020	Neighbour 400m away from the beekeeping shed who is a hobby beekeeper asked the case study beekeeper to check their hives as they were concerned they had AFB. Visual inspection confirmed this.
March 2020	Found first AFB on own property.
1st check	59 clinical cases were found and they were spread over 60% of the apiary sites.
	The process took 14 days to check the hives visually (Varroa strips in at the same time.
2nd check 11-21 April	19 clinical cases
	In total 78 hives (26.5% of the hives) were burnt; 9 hives heavily infected or dead and the remainder only just infected. In the Clinton Apiaries – across 5 yards 21 hives were burnt, and the worst yard had 12 clinical AFB hives.
	An AP2 inspector checking the Clinton yards picked up (3) clinical AFB hives during 2 extra inspection visits to these sites between the beekeeper's inspection dates.
3rd check 2nd June	Use of qPCR testing to identify further potential AFB issues. 200 hives were sampled.
	Two samples per hive were taken for qPCR analysis – bee samples and swabs of the hive entrance
August	Interim qPCR results provided (covid caused delays)
2 Sept 2020	Full qPCR results available

Apiary setup following AFB infection

Hives were categorised into four groups. All apiary sites went into lockdown (Covid-19) with only hives that had to be destroyed and hives that were being shifted to yard D being moved. No other hives were shifted.

A yards No AFB detected in hives (ND qPCR result for bees and woodware swabs)

B yards everything else – hives with indication of AFB spore loads (qPCR +ve for AFB but at low levels i.e. Cq number higher than 31) Also included were yards where 1 or two clinical hives had been removed.

C yards The highly suspect hives as a result of qPCR swabs (Cq number 27 - 32). These are isolation yards with no honey production for next summer.

D yard Very high chance they are likely to be clinical (Cq less than 27)

Using qPCR sampling in an AFB outbreak

The AFB outbreak in this Apiary has provided the opportunity to do 2 things:

- screen a large number of hives for AFB DNA after a clinical AFB incursion and
- trial a composite testing methodology for qPCR.

Sampling Case Study apiaries

As a result of the severe incursion of AFB in this business, 200 hives were sampled in June 2020, both swabs and bees where possible, and sent to the lab for analysis. Five hives had died out and were swab sampled only. The swab results indicated whether the hives woodware is safe to use in the future or whether they will need to be destroyed.

The hives were tested in composite groups of 8 swabs. In the lab this means each swab sample is prepared separately for qPCR and then subsampled to provide the composite sample. The bee samples were then tested for each individual hive (see Composite Testing protocol). If any composite sample returned positive the individual swabs could be checked.

Results

The bee samples were tested and of the 194 results provided 45 had AFB DNA detected. Of the 45 only 5 had test levels below 30 and these were separated for monitoring. Two results suggested clinical AFB was imminent, one in fact developed AFB soon after and both were destroyed. A third hive is dead leaving 2 hives to monitor. See Apiary setup above, - D yard.

The honey supers were also categorised based on hive qPCR results, either “A Category” which are from hives with no AFB DNA detected and “B category” which covers the remainder.

Conclusion

The use of qPCR to test all the hives in this honey business has been of benefit to both the owner and the CleanHive project.

Visual inspection of all hives was carried out as per the AFB Management guidelines when purchased. However, once the infection was identified the ability to sample all the hives, using a rapid and non-invasive swab method, gave an early indication of which hives were at risk with high *P. larvae* spore loads. The qPCR results then enabled the beekeeper to proactively manage the remaining hives using a quarantine system.

Additionally, taking the swabs is a simple task and a significant labour saving process as well which is important for both small businesses (1-2 staff) and large businesses relying on transitory or inexperienced staff.

The testing has enabled the owner to be aggressive in culling risky hives and return his business to AFB free faster than the traditional approach using visual inspection and hive destruction.

The CleanHive project was able to use a large outbreak in this apiary, in an area with a traditionally low incidence of clinical AFB, to advance the sampling methodology for qPCR. This involved trialling, with dnature, a composite sampling test using qPCR to screen larger numbers of hives for the presence of AFB *P. Larvae* spore DNA.

The lessons learned from this case study highlight many of the known danger points in AFB spread and incursion.

Appendix 1

Otago Southland Beekeepers Discussion Group

This group covers the wider region from Invercargill to The Southern Lakes (including apiaries on the West Coast) and Otago through to mid Canterbury. The group is made up primarily of beekeepers that cover many facets of the honey industry and this has been important for the reach and extension of the project into the wider beekeeping community. Some of the beekeepers also work in supporting beekeeping industries.

Nineteen are commercial beekeepers – ranging in scale from 50 hives (nearing retirement) to 3-7000 hives.

Two are AP2 inspectors

Several rear queens including some for sale

Four extract/pack honey for other beekeepers

Two are organic producers

Several are involved in mentoring and training beekeepers, including hobbyists and provide support for hobbyists, including honey extraction

Collectively they have or have had involvement in all levels of the industry and APINZ over the years, for example two members are past presidents of the National Beekeepers Association and one is chairman of The Management Agency National American Foulbrood Pest Management Plan

Additional input and support to the group has been provided from FutureBees, BettaBees, Gribble's laboratory (Mosgiel) and John Mackay, dnature, Richard Hall and Hayley Pragert (MPI)

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