TECHNICAL SUMMARY

Testing for AFB

Geoff Corbett

Analytica Laboratories presents an overview of American foulbrood and explains its testing regime for honey.

Match hanging off AFB ropy material. Photo: AFB Pest Management Agency.

WHAT IS AFB?

American foulbrood (AFB) is a bacterial disease affecting beehives, with serious consequences. In New Zealand, it is a notifiable disease and governed by a national pest management plan. AFB is a disease caused by the spore-forming bacterium *Paenibacillus larvae*. The disease primarily affects and kills the developing pre-pupae and pupae, or brood, subsequently weakening the bee colony. Spore-forming bacteria are extremely robust and resilient. The spores themselves are the key to the bacterium's long survival under extreme conditions, having the ability to remain viable for decades. Billions of spores are produced by the dying larvae, and their remains are difficult to remove by worker bees and are a continuous source of infection for the new brood. The disease can be easily spread between hives and apiaries. One major problem for the control of AFB is that clinical symptoms appear late in the epidemic when the colony's hygienic behaviour to remove infected larvae can no longer keep up. It is therefore important to keep on top of any infection and use an array of tools for early detection and prevention.

RECENT DEVELOPMENTS

The new Overseas Market Access Requirement (OMAR) document that relates to honey exported from New Zealand to China outlines that imported honey may be tested for AFB. The Ministry for Primary Industries' (MPI) memorandum note F53/20, released in November 2020, states that AFB is on the list of Animal Quarantine Epidemics of the People's Republic of China. The General Administration of China Customs (GACC) monitors imported foods for these quarantine organisms and can order product destruction if they are detected. While AFB is not a food quality issue, it is certainly a biosecurity issue. MPI advises, "to avoid detections at the border, and the threat of escalation, exporters are advised to only export honey to China that is in line with China's management of their quarantine requirements". Developments since the release of this OMAR document means that you cannot export to China without a non-detect result.

TESTING HONEY FOR AFB

How is honey tested for AFB?

Analytica offers an IANZ (International Accreditation New Zealand)- and RLP (Recognised Laboratory Programme)-approved test for AFB using a modern DNA testing technique called Real-Time PCR. While not the same as conventional microbiological plating techniques, it is faster and more sensitive, allowing for the sub-clinical detection of AFB in honey. The test is specific for AFB bacteria spores in a sample and presence can be detected without the risk of confusion with other types of microorganisms. Real-Time PCR works by copying the target region of DNA specific to AFB. This allows a small amount of DNA to be detected and provides a mechanism to calculate the original concentrations of AFB present.

Real-Time PCR results are often displayed as a Cq value. The Cq value is the number of PCR cycles it takes for the original amount of target DNA in the sample to reach the defined threshold set for that test. In the case of AFB it is 36 cycles. A high Cq value means that there are fewer DNA copies in the sample, and it takes more PCR cycles to amplify the DNA to a point where it is detectable; e.g., detection at 35 cycles would show that the starting material had few copies.

A low Cq value means that there were greater numbers of DNA copies in the sample and the threshold is therefore reached in a fewer number of PCR cycles eg. detection at 20 cycles would indicate >1,000-fold more starting copies than at 35 cycles. The Cq value is generally equated to how many original copies of the target DNA were present in the sample, which is then approximated to the number of bacteria or spores per gram of honey.

WHAT ANALYTICA HAS OBSERVED OVER THE LAST FEW MONTHS SINCE AFB WAS ADDED TO THE OMAR

Since the OMAR regarding China was introduced late last year, Analytica has performed over a thousand AFB tests on honey samples from New Zealand. Approximately 86% of the New Zealand samples we have tested resulted in a non-detect. Of the 14% of the New Zealand honeys that tested positive for AFB, approximately 80% displayed a relatively low spore count of <360 per gram of honey, 5% had an intermediate count >360<2,000, and 14% had a high spore count of >2,000.

While the dataset used to create these statistics is not huge, it does indicate that there is still a reasonable incidence of AFB and the industry should maintain their vigilance in management of the disease.