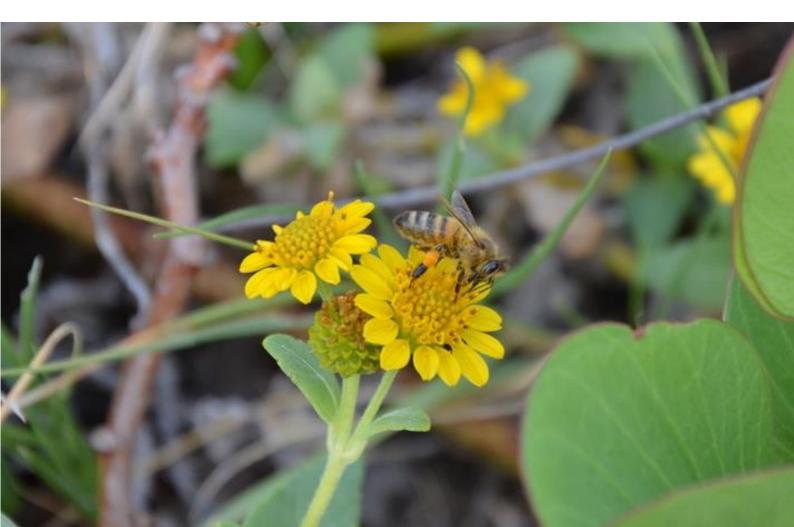




Norfolk Island bee pest survey 2021-2022

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Executive summary

Honey bees have long been an important part of the ecosystem and culture of Norfolk Island. They provide quality honey and hive products and essential pollination services that support the food security of this small remote island community. The health of Norfolk Island's honey bee population should be protected.

This report describes a recent pest and disease survey of Norfolk Island's honey bee population between December 2021 and April 2022. This work has given an updated bee health assessment from the previous Norfolk Island 2012-14 Quarantine Survey and filled critical gaps in the sentinel hive surveillance.

Key findings

- 67 bee colonies (~50% all managed colonies) were inspected and tested for 16 pests and diseases.
- No new honey bee pests or diseases were detected in Norfolk Island honey bees.
- All previously reported pests and diseases were detected, including
 - high prevalence and infection levels of the gut parasite, Nosema ceranae
 - high prevalence of Lake Sinai virus, a common bee virus group with no known disease
 - low detection of the lesser wax moth (*Achroia gresella*), a minor hive pest.
- Suspected European foulbrood was observed in one colony but found negative in all tests.
- Harmless *Acarapis* mites related to tracheal mite (*Acarapis woodi*) are prevalent on Norfolk Island honey bees and caused low frequency cross-reaction in DNA tests for tracheal mites.

Recommendations

The honey bee population on Norfolk Island is truly unique from a pest and disease perspective. No other honey bee population in the world has fewer pests and pathogens. Norfolk Island's isolation and limited import of bees and bee products, especially in the past 30 years, has been key to preserving this enviable health status. The following recommendations serve to further improve bee biosecurity for Norfolk Island, with detail provided in Section 4.

- 1. Permit only commercial importation of certified irradiated honey into Norfolk Island.
- 2. Resource ongoing surveillance in Norfolk Island as part of the National Bee Pest Surveillance Program.
- 3. Registration for all Norfolk Island beekeepers and encouraging beekeepers to perform regular hive inspections in line with Australia's *Honey Bee Industry Biosecurity Code of Practice.*

1 Introduction

Norfolk Island has a long history of beekeeping, with European honey bee (*Apis mellifera*) colonies introduced during European settlement on the island and in Australia. Since these early introductions and the establishment of the Norfolk Island Apiaries Act 1935, only limited imports of queen bees from Australia have occurred, under strong quarantine. There have been no queen imports since 1992 (Neil Tavener, pers. comm), although packaged honey continues to be imported commercially and by individual travellers to the island.

Beekeeping is a small industry on Norfolk Island with the majority of the island's estimated 120 colonies managed by two beekeeping operations and several recreational beekeepers managing between one and 10 colonies. Managed colonies typically stay at the same apiary site year-round. There is also a well-established feral honey bee population which is the main source of new managed colonies through swarm collection. There is no active queen production or breeding occurring on the island.

The last bee pest and disease survey was conducted as part of the Norfolk Island 2012-2014 Quarantine Survey and provided valuable baseline knowledge. That survey reported the lesser wax moth (*Achroia gresella*), the gut parasite *Nosema ceranae* and a strain of Lake Sinai virus (Malfroy et al. 2016). These pests and pathogens are common around the world and have not had observable impact in Norfolk Island. In the last few years, Australia's National Bee Pest Surveillance Program (NBPSP), has been extended to Norfolk Island to detect new incursions of exotic bee pests. There have been no new pest detections under this program in Norfolk Island, although only limited surveillance activities based on visual inspection of several sentinel hives has been possible.

The purpose and scope of this survey was to fill the gaps in current surveillance activities on Norfolk Island and update our knowledge from the Norfolk Island 2012-2014 Quarantine survey. The use of molecular testing also allowed more sensitive pest and disease detection and greater confidence in the health status of Norfolk Island's honey bees.

This project was conducted by Dr John Roberts of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and funded by the Department of Infrastructure, Transport, Regional Development, Communications, and the Arts with support from The Department of Agriculture, Fisheries and Forestry and Plant Health Australia.

2 Survey methods

2.1 Hive inspections and sample collection

Table 1. Summary of survey pest and disease targets and detection methods

Pests and Diseases	Detection methods		
Parasitic mites – Varroa and Tropilaelaps	Hive inspectionsSugar-shake of 300 worker bees		
Small hive beetle – <i>Aethina tumida</i>	 Hive inspections AJ Beetle-eater[™] traps with vegetable oil 		
Tracheal mite – <i>Acarapis woodi</i>	PCR testing of 60 worker beesTrachea dissection and microscope inspection		
Nosema – <i>N. apis</i> and <i>N. ceranae</i>	PCR testing of 60 worker beesMicroscope inspection for Nosema spores		
Brood diseases – American foulbrood, European foulbrood & Chalkbrood	 Hive inspections Field test-kits of suspected diseased brood PCR testing of suspected diseased brood PCR testing of honey 		
Bee viruses	PCR testing of 60 worker beesHigh-throughput sequencing		

There are estimated to be approximately 120 managed hives with the majority owned by two beekeeper operations, Ben Tomlinson (Norfolk Honey Company) and Merv Buffett and Clare McPherson. The remaining managed hives are kept in small numbers by up to a dozen other beekeepers on the island. Hives are typically kept in small numbers as sedentary apiary sites distributed across the island.

The survey was designed to capture two surveillance points, the first was conducted in December 2021, followed by surveillance in April 2022. Each time-point involved Dr Roberts visiting Norfolk Island to undertake field sampling and surveillance activities with assistance from local beekeepers (Figure 1).



Figure 1. Hive inspections of managed honey bee colonies on Norfolk Island.

Thirty-eight managed hives were inspected and sampled in December 2021 and 20 managed hives were inspected and sampled in April 2022. Six feral colonies were sampled in December 2021 and three managed hives were sampled but not inspected in April 2022.

Surveillance included hive inspections combined with molecular testing at CSIRO for several bee pests and diseases (Table 1). Hive inspections involved opening hives and making general observation for small hive beetle (SHB, *Aethina tumida*) under the hive lid and on frames. AJ Beetle-eater[™] traps containing vegetable oil were also placed into 23 inspected colonies during the December 2021 survey and inspected for SHB after 48 hours.

Brood frames were also inspected for any larvae or pupae showing signs of disease that could be caused by bacterial pathogens, American foulbrood (*Paenibacillus larvae*) and European foulbrood (*Melissococcus plutonius*), the fungal pathogen chalkbrood (*Ascosphaera apis*), and Sacbrood

virus. Any suspected diseased brood was collected with sterile forceps into collection tubes for diagnostic testing.

A sugar-shake was conducted on all inspected hives for surveillance of *Varroa* and *Tropilaelaps* mites (Dietemann et al. 2013). This involves collecting approximately 300 worker bees by shaking them from a brood frame into a tray and transferring a ½ cup of bees to a container with a mesh lid. A tablespoon of icing sugar is added to the container and rotated for 1 min to coat the bees and dislodge any attached mites. Loose icing sugar and any mites are shaken through the mesh lid into a tray for visual examination.

Samples of worker bees, honey and pollen were collected from all colonies when possible. Approximately 50-100 worker bees were collected from frames into a sterile container and preserved in RNAlater[®] or 70% ethanol and stored at -20°C until analysis. Honey was collected from a capped honey frame using a stainless-steel lab micro spoon to fill a 1.5 mL collection tube. Stored pollen was also collected from 10 randomly selected cells on a frame using a stainless-steel lab micro spatula into a 1.5 mL collection tube. Pollen was collected for analyses of plant diversity and plant pathogens as part of additional activities.

2.2 DNA and RNA extraction from adult bees and honey

Adult bee samples collected from the survey were rinsed with sterile water over a 250 μ m sieve to remove residual preservation buffer and as an additional screen for external parasitic mites. For each colony, 4 x 15 groups of bees were homogenised in 10 mL phosphate buffered saline in an extraction bag and Stomacher 80 biomaster[®] machine. This level of pooling was based on the PCR detection sensitivity for tracheal mites (Delmiglio et al. 2016). A volume of 200 μ L of the above extract was used for DNA extraction with a High Pure PCR Template Preparation Kit (Roche). A volume of 100 μ L was also combined from all four replicates per colony and used for RNA extraction using the Maxwell[®] RSC simplyRNA Tissue Kit (Promega).

Honey samples collected from the survey were first processed to concentrate environmental DNA (eDNA) before extraction. This involved warming the honey at 40°C for 30 minutes before diluting the honey by 50% with sterile water into two tubes. Diluted honey was then centrifuged at 4,000 g for 10 min before discarding the supernatants. Sterile water (500 µl) was added to the tubes and centrifuged again, and the supernatant discarded. CTAB (1 mL) was added to the pelleted eDNA and extracted using the Maxwell[®] RSC PureFood GMO and Authentication Kit (Promega).

2.3 Real-time PCR detection of bee pathogens

Extracted DNA and RNA was used for real-time PCR detection of microbial pathogens and the internal parasitic tracheal mite, *Acarapis woodi*. Primers and cycling conditions used for each target are given in Table 2. All assay were SYBR[®]-based followed by a melt curve analysis, except for the probe-based *A. woodi* assay developed by Delmiglio et al (2016).

Target	Primers/probe	Cycling conditions	Reference
Apis mellifera	F: GGCAGAATAAGTGCATTG R: TTAATATGAATTAAGTGGGG	95°/2min, (95°/10s, 51°/15s, 72°/15s) x 35	(Utzeri et al. 2019)
AFB	F: GTGTTTCCTTCGGGAGACG R: CTCTAGGTCGGCTACGCATC	95°/2min, (95°/5s, 59°/15s, 72°/15s) x 35	(Han et al. 2008)
EFB	F: GTTAAAAGGCGCTTTCGGGT R: GAGGAAAACAGTTACTCTTTCCCCTA	95°/2min, (95°/5s, 59°/15s, 72°/15s) x 35	(Garrido-Bailón et al. 2013
Chalkbrood	F: GCACTCCCACCCTTGTCTA R: CAGGCTCGCGAGAACCC	95°/2min, (95°/15s, 56°/15s, 72°/15s) x 35	(Klinger et al. 2015)
Acarapis woodi	F: AATAAATCATAATGATATCCCAATTATCTGAGTAATG R: AATATCTGTCATGAAGAATAATGTC Probe: 6-FAM-ACC[+T]GT[+C]AA[+T]CC[+A]CCTAC-BHQ1	50°/2min, 95°/2min, (95°/10s, 59°/45s) x 35	(Delmiglio et al. 2016)
Acarapis spp.	F: TCAATTTCAGCCTTTTATTCAAGA R: AAAACATAATGAAAATGAGCTACAACA	95°/2min, (95°/10s, 52°/10s, 72°/30s) x 35	(Evans et al. 2007)
Nosema apis	F:CGTACTATGTACTGAAAGATGGACTGC R: AGGTCTCACTCTTACTGTACATATGTTAGC	95°/2min, (95°/5s, 59°/15s, 72°/15s) x 35	(Huang and Solter 2013)
Nosema ceranae	F:GAGAGAACGGTTTTTTGTTTGAGA R: ATCCTTTCCTTCCTACACTGATTG	95°/2min, (95°/5s, 59°/15s, 72°/15s) x 35	(Huang and Solter 2013)
BQCV	F: GATTCGTCTTGGGCGTCTGA R: GCCTGAAATGGTTGCGTCTG	45°/10min, 95°/2min, (95°/5s, 60°/10s, 72°/5s) x 35	This study
SBV	F: TCCAGCCTCACTGGATGAGA R: GAACAAACTCAACACGCGCT	45°/10min, 95°/2min, (95°/5s, 60°/10s, 72°/5s) x 35	This study
DWV-A	F: TACTAGTGCTGGTTTTCCTTT R: CTCATTAACTGTGTCGTTGAT	45°/10min, 95°/2min, (95°/5s, 60°/10s, 72°/5s) x 35	(Kevill et al. 2017)
DWV-B	F: TACTAGTGCTGGTTTTCCTTT R: CTCATTAACTGAGTTGTTGTC	45°/10min, 95°/2min, (95°/5s, 60°/10s, 72°/5s) x 35	(Kevill et al. 2017)
IAPV	F: CGTCGACCCATTGAAAAAGT R: GGTTGGCTGTGTGTCATCAT	45°/10min, 95°/2min, (95°/5s, 60°/10s, 72°/5s) x 35	(Palacios et al. 2008)
LSV	F: CKTGCGGNCCTCATTTCTTCATGTC R: CATGAATCCAAKGTCAAAGGTRTCGT	45°/10min, 95°/2min, (95°/5s, 60°/10s, 72°/5s) x 35	Iwanowicz (Iwanowicz et al. 2020)

Table 2. Primers and cycling conditions used for real-time PCR detection of bee parasites and pathogens.

2.4 High-throughput sequencing for bee viruses

RNA extracted from each sample was combined to create two pooled samples from the 44 colonies sampled in December 2021 and a pooled sample made from the 23 colonies sampled in April 2022. Another pooled sample was made from 5 bees x 20 colonies collected during the 2013 survey (Malfroy et al. 2016). Pooled RNA was sent to Azenta Life Sciences (Suzhou, China) for polyA library preparation and 150 bp paired-end sequencing on an Illumina[®] Novaseq.

Sequence data was quality trimmed and analysed using CLC Genomics workbench v20 (Aarhus Denmark). Trimmed reads were first mapped to the *A. mellifera* reference genome and unmapped reads collected. Unmapped reads were then *de novo* assembled into contigs (i.e. joining overlapping short sequences into a single longer sequence). Contigs were then compared to an online virus reference database using BLAST (NCBI) to identify virus sequences.

3 Survey results

3.1 Hive inspections

> No detection of parasitic bee mites, small hive beetle or brood disease

In December 2021, 38 hives were inspected for pests and diseases and a further 20 hives were inspected in April 2022 (Figure 2). There was no visible evidence for SHB or parasitic bee mites (*Varroa* and *Tropilaelaps* spp.) during inspections. This was further confirmed by conducting a sugar-shake of approximately 300 bees for each hive. SHB traps were also placed into 23 hives during the December 2021 surveillance and no beetles were found after at least 48 hours in the hive.



Figure 2. Map of Norfolk Island with bee colonies surveyed in 2021 (blue), 2022 (orange) or in both years (green).

Using a 1-stage freedom analysis (https://epitools.ausvet.com.au), this sampling level gave 95% confidence for freedom-from-disease at a 3 – 4% prevalence level, depending on estimates with numbers of hives inspected or samples lab-tested. Estimates from only SHB trapping gave 95% confidence of freedom-from-disease at a 12.5% prevalence level.

Inspection for brood disease found no evidence of American foulbrood or chalkbrood in any hive. However, there was suspected European foulbrood identified in several hives. In December 2021, one hive contained numerous larvae across several frames with possible European foulbrood symptoms such as discolouration and positioning on the cell wall (Figure 3). Multiple larvae were collected and tested for European foulbrood using an in-field lateral flow test (Vita Bee Health) and lab PCR testing at CSIRO. All tests were negative for European foulbrood.

In April 2022, single larvae in two colonies displayed European foulbrood symptoms. In-field testing and lab PCR testing at CSIRO found these both negative for European foulbrood.

The cause of this brood disorder remains unknown but these symptoms can have a genetic or environmental basis, rather than be the result of a pathogen. Environmental conditions were generally good and unlikely to be causing nutritional stress to hives. Similar brood disorders with unclear etiological agents have been observed elsewhere, including regions free of European foulbrood such as Western Australia and New Zealand.



Figure 3. Bee larvae with disease symptoms similar to European foulbrood but found negative in all tests.

3.2 Nosema detection

> High infections of *Nosema ceranae*, *Nosema apis* not detected

Worker bees were collected from each hive for lab detection of Nosema species. Pools of 4 x 15 bees per hive were DNA extracted and a single pooled DNA sample per hive was tested by PCR for *N. apis* and *N. ceranae*.

Previous testing had only detected *N. ceranae*. PCR testing for both *N. apis* and *N. ceranae* again only detected *N. ceranae* in Norfolk Island honey bees. Detection levels based on real-time PCR, found that all hives had a relatively high infection level, with higher colony infections found for the December 2021 surveillance period (Figure 4, Mann-Whitney U = 236.5, p = 0.0005). While there was no clear visible effect from Nosema on colonies, high infections can reduce the lifespan of adult bees and cause young bees to prematurely become foragers, which can lead to population decline in the colony (Fries et al. 2013; Holt and Grozinger 2016).

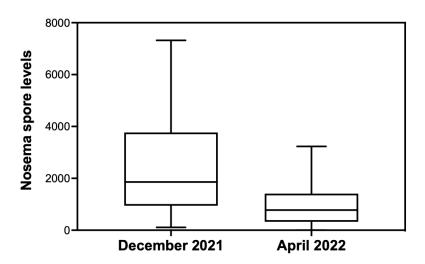


Figure 4. Comparison of mean (SEM) *Nosema ceranae* spore levels in December 2021 colonies and April 2022 colonies estimated by quantitative real-time PCR. Nosema levels were significantly higher in December 2021 colonies than April 2022 colonies, Mann-Whitney U = 236.5, *p* = 0.0005.

3.3 Honey testing for brood diseases

> No DNA traces of brood diseases in Norfolk Island honey

Honey was collected from 55 inspected hives and 4 feral colonies across the surveillance periods for PCR detection of brood diseases. In addition, 15 past honey samples collected from harvests between 2014 and April 2021 were also received for brood disease testing.

PCR testing for American foulbrood, European foulbrood and chalkbrood found no evidence of these brood diseases in any Norfolk Island honey. However, a sample taken from honey seized by biosecurity staff brought into Norfolk Island from Australia was also tested and had a positive detection for American foulbrood (C_T = 31.6) which was confirmed by Sanger sequencing.

While the risk of disease spread was prevented in this case, it does highlight the risk of brood diseases being introduced to Norfolk Island through untreated imported honey.

3.4 Tracheal mite detection

> No detection of tracheal mite in Norfolk Island honey bees

Tracheal mite (*A. woodi*) is an internal parasite infesting the bee's breathing tubes (trachea). It is a common pest around the world but is not found in Australia, New Zealand or Scandinavia (Delmiglio et al. 2016). It has not been detected previously in Norfolk Island using bee dissection methods. Molecular methods have been developed for tracheal mite detection and were used as the primary technique in this survey.

The real-time PCR probe assay is sensitive for *A. woodi* detection but has known imperfect specificity. PCR testing of Norfolk Island samples with this assay identified three positive colonies from December 2021. Detection levels were low and from only one or two replicates, suggesting a

low incidence of mites giving a positive reaction. The PCR products (113 bp) of these positive samples were Sanger sequenced and found to match 100% to *A. woodi*.

This result was followed by dissections of remaining bees to find visual evidence of tracheal mite infestation. Twenty bees were dissected from each colony with no bee showing signs of damaged or infested trachea (Figure 5).

These three colonies (located at two apiary sites) were re-sampled in April 2022 and PCR testing found one colony was positive again for *A. woodi*. As before, dissections of 20 additional bees found no visual evidence for tracheal mite infestation.

The lack of visual confirmation of tracheal mite in any sample, suggests a cross-reaction to an external *Acarapis* species may be responsible for these false positives. During bee surveillance in New Zealand, Delmiglio et al. (2016) also found that approximately 10% of colonies cross-reacted to this test from a rare genotype of a related external *Acarapis* species.

We further tested the Norfolk Island samples with a generic PCR assay that detects all three *Acarapis* species (Evans et al. 2007), with *A. externus* and *A. dorsalis* being commensal external parasites. Most samples were positive and Sanger sequencing confirmed both *A. externus* and *A. dorsalis* are present in Norfolk Island.

For further investigation, we also collected 11 individual external mites from the dorsal groove of bees from the positive hive sampled in April 2022 (Figure 4). Each mite was DNA extracted using Chelex[®] resin and tested with the tracheal mite-specific assay. One external mite sample gave a positive signal (C_T = 26.55), demonstrating that cross-reaction from an external *Acarapis* mite is responsible for the false positives observed in these Norfolk Island colonies.

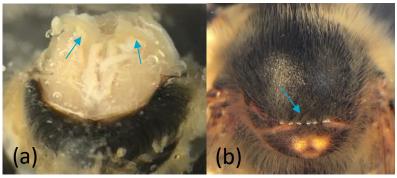


Figure 5. Dissection showing healthy bee trachea (a). External *Acarapis* mites tested from bee thorax (b).

3.5 Bee virus detection

> Lake Sinai virus is the only bee virus found in Norfolk honey bees

Worker bees were collected from each hive for lab detection of viruses. There are several common RNA viruses that infect honey bees, although only Lake Sinai virus (LSV) was previously detected in Norfolk Island. Five bee viruses are prevalent in Australia; Black queen cell virus (BQCV), Sacbrood virus (SBV), Israeli acute paralysis virus (IAPV) and Lake Sinai virus (LSV) (Roberts et al. 2017). Deformed wing virus (DWV-A and DWV-B) is not in Australia but is the most serious viral pathogen in association with *V. destructor* around the world.

RNA extracted from each sample was combined to create two pooled samples from the 44 colonies sampled in December 2021 and a pooled sample made from the 23 colonies sampled in April 2022. Another pooled sample was made from 5 bees x 20 colonies collected during the 2013 survey (Malfroy et al. 2016). These pooled samples were first screened for LSV, SBV, BQCV, IAPV and DWV by real-time PCR. LSV was the only virus detected. Individual samples were subsequently tested for LSV and 43/44 (97%) colonies in 2021 and 19/23 (83%) colonies in 2022 were virus infected.

Pooled RNA samples were also analysed by HTS for untargeted virus discovery. This data was consistent with the real-time PCR results with LSV being the only known bee viruses detected. The LSV strain found in Norfolk Island is most similar to LSV-3 strains found in Australia and overseas but is clearly a distinct strain with only around 85% shared genetic identity.

Many diverse strains of LSV have been identified in honey bee populations around the world through HTS and this virus group has likely existed in honey bees for a long time. There is no clear evidence of any significant impact on bee colonies, although there has been some association with weaker colonies overseas (Daughenbaugh et al. 2015).

3.6 Data records

Surveillance data from this survey has been provided to Plant Health Australia for addition to AUSPestCheck, which is a national plant pest surveillance virtual coordination centre - https://www.planthealthaustralia.com.au/resources/auspestcheck/

4 Recommendations

The honey bee population on Norfolk Island is truly unique from a pest and disease perspective. No other honey bee population in the world has fewer pests and pathogens. Norfolk Island's isolation and limited import of bees and bee products, especially in the past 30 years, has been key to preserving this enviable health status. The following recommendations serve to further improve bee biosecurity for Norfolk Island.

1. Permit only commercial importation of certified irradiated honey into Norfolk Island.

Imported honey is a high-risk source for many bee diseases that could decimate Norfolk Island's bee population and the only way to fully mitigate this risk is to ensure that only irradiated honey is imported. Pasteurisation of honey is only effective for European foulbrood but not for American foulbrood. It is recommended that import conditions be updated and modelled on Western Australia's policy (Appendix 1), which requires all honey imports be accompanied by a health certificate declaring it is sourced from disease-free areas or effectively treated. These changes are also consistent with the original Norfolk Island Apiaries Act 1935 (Appendix 2), which requires disease-freedom certification of imported honey.

Arguably, Norfolk Island has avoided introduction of these brood diseases under the current policies and the risk of hive exposure to imported personal use honey may be low. However, the impact from an incursion would be high for local honey production and free pollination. These changes also simplify decisions for DAFF biosecurity officers, who currently must assess personal use honey imports that typically have no information on how the honey is processed and treated.

2. Resource ongoing surveillance in Norfolk Island as part of the National Bee Pest Surveillance Program.

Current hive surveillance outside of this survey relies on sentinel hives located at four key locations (Kingston, Cascade, Ball Bay and the Airport) under the NBPSP. Monthly inspections of these hives are a valuable contribution to Norfolk Island's bee biosecurity. This work is volunteered by local beekeepers (Merv Buffet and Clare McPherson) but there is a need to foster bee biosecurity capability in DITRDCA and/or DAFF in preparation of an incursion. This could initially involve staff accompany sentinel hive inspections but ultimately requires a transition to independent ownership and surveillance of sentinel hives, consistent with other NBPSP locations.

Ongoing surveillance for Norfolk Island would benefit from molecular testing of samples sent to Australia under the NBPSP. Quarterly samples of bees and honey, pooled from all sentinel hives, is recommended to test for for tracheal mite, viruses and brood diseases. On-island biosecurity staff should also make available rapid antigen test kits for AFB and EFB for beekeepers to test any unusual symptomatic brood.

Surveillance for exotic bee swarms (*A. mellifera* and *A. cerana*) remains challenging with the low sensitivity of current methods, e.g. catch-boxes, sweep netting, and high level of local bee swarms.

Without more sensitive surveillance techniques, local awareness campaigns to report unusual bee swarms are likely the best approach.

3. Registration for all Norfolk Island beekeepers and encouraging beekeepers to perform regular hive inspections in line with Australia's *Honey Bee Industry Biosecurity Code of Practice.*

Registration for all beekeepers is key tool for communication around bee biosecurity. In the event of a pest incursion, it allows biosecurity officers to quickly inform beekeepers and ideally contain and eradicate any introduced pest and disease. It also helps share important biosecurity, such as how to check your hives for pests and diseases and how to report any suspicious observations. Having free registration encourages compliance and could require all registered beekeepers to conduct and report at least two pest and disease inspections per year, for example, spring and autumn. This involves full brood inspections and a method for Varroa mite surveillance, for example, sugar-shake or drone uncapping. Reporting this information will provide additional ongoing freedom-from-disease evidence. Providing new beekeepers with basic beekeeping training (online or in-person) would assist with inspection compliance and strengthen bee biosecurity.

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6 Appendices

Appendix 1. Western Australia honey importation conditions

QUARANTINE WA Import Requirements Search

Important Notes

• All organisms must be listed as 'Permitted (s11)' to be allowed entry into Western Australia. If the organism is not listed as 'Permitted (s11)' an application for an Import Permit must be submitted. To check the status of the organism or to apply for an Import Permit check the WA Organism List. Some species may also require an import permit under the Wildlife Conservation Act, please check with the Department of Biodiversity, Conservation and Attractions on +61 (0)8 9219 9000.

• Where certification is stated to be an import requirement, each consignment to be accompanied by an Interstate Plant Health Certificate issued by the quarantine authority in the exporting state or territory or under a quality assurance scheme approved by the Director of Plant Biosecurity for the Department of Primary Industries and Regional Development, Western Australia. Approved quality assurance schemes include an accepted Interstate Certification Assurance arrangement, Certification Assurance arrangement or Biosecure HACCP. Certificates must be originals and be presented to a Quarantine WA Inspector prior to inspection, unless import conditions or ICA procedures state that copies are permitted.

• All consignments to Western Australia must be labelled with 'Product name (full botanical name where applicable), Producer (Packer or Agent) and the district of production.'

• The chemical treatments listed as satisfying import requirements is not meant to imply that these products are registered or approved by the Australian Pesticides and Veterinary Medicines Authority (APVMA). This will need to be confirmed with the APVMA for the particular jurisdiction in which the treatment is planned. Uses approved by the APVMA are changing all the time and confirmation of approved uses can be made via their Pubcris database at www.apvma.gov.au. Some treatments required on imported commodities and plants, and in particular fumigation with methyl bromide, may be phytotoxic and damage, kill or render the goods unsaleable.

• The identification of products as suitable treatments does not constitute an endorsement.

• Import requirements displayed apply to goods sourced from the selected state, where those goods have not been subject to possible infection or contamination whilst in transit through other states. Where goods have been stored, unpacked, re-packed or otherwise disturbed whilst in transit, additional requirements specific to the transit state(s) may apply.

Import Requirements Summary

Please note: This requirement may not be valid after Monday, October 31, 2022

Condition No.H02

Honey, honeycomb, propolis, royal jelly, pollen, beeswax, bees (dead), bee larvae (dead), used beehives, beekeeping appliances/equipment, queen candy and other honey products REQUIREMENTS FOR THE IMPORT OF PRESCRIBED POTENTIAL CARRIERS OF DECLARED PESTS OF HONEY BEES (Apis mellifera)

Declared Pests of honey bees

- European foulbrood (Melissococcus plutonius EFB)
- American foulbrood (Paenibacillus larvae AFB)
- Nosema ceranae
- Small hive beetle (Aethina tumida SHB)

Prescribed Potential Carriers of European foulbrood and American foulbrood

- Honey;
- Honeycomb;
- Beeswax;
- Propolis in a quantity per individual unit that exceeds 200ml or 200g;
- Royal jelly in a quantity per individual unit that exceeds 150ml or 35g;
- Bee collected pollen for use in beekeeping;

• Bee collected pollen for uses other than beekeeping in a quantity per individual unit that exceeds 150ml or

100g. Used beehives and beekeeping appliances/equipment;

• Honey bees (including queens, packaged bees, drones, working colonies, brood, bee comb).

Note I Bee venom is not a potential carrier of European foulbrood or American foulbrood and is permitted entry Note - Propolis, royal jelly and bee collected pollen, in capsules or tablets in ready retail packaging and intended for human consumption, is not a prescribed potential carrier.

Note - Consignments of propolis may comprise of more than one individual unit, subject to the total weight of each unit amounting to not more than the lesser of 200ml or 200g. Consignments of royal jelly may comprise of more than one individual unit, subject to the total weight of each unit amounting to not more than the lesser of 150ml or 35g. Note I Exporters of bee collected pollen for uses other than beekeeping may be required to provide evidence of its intended use.

Prescribed Potential Carriers of Nosema ceranae

Used beehives and beekeeping appliances/equipment

• Honey bees (see Condition 01a - Permit required under r72)

• Bee semen (see Condition 01a - Permit required under r72)

Note 🛛 Bee venom is not a potential carrier of Nosema ceranae and is permitted entry.

Prescribed Potential Carriers of Small hive beetle

• Used beehives and beekeeping appliances/equipment

• Honey bees (see Condition 01a - Permit required under r72) Specific Requirements

Prescribed potential carriers of European foulbrood, American foulbrood and Nosema ceranae to be imported into Western Australia must satisfy the following requirements:

1. Honey, honeycomb, propolis or royal jelly where it is the single greatest ingredient by volume

Each consignment must be accompanied by an Interstate Health Certificate issued by the quarantine authority in the exporting state or territory, or under a quality assurance arrangement approved by the Chief Plant Biosecurity Officer, certifying that the following requirements have been met:

1.1 the product

1.1.1 has been treated with irradiation at a rate of at least 15 kilogray (AFB, EFB);

OR

1.1.2 (a) has originated from, and packaged in a country, state or territory that is free from European foulbrood; and (b) has been declared by the exporter as derived from apiaries that have been inspected by the beekeeper and found free of American foulbrood;

OR

1.1.3 (a) has been treated with irradiation at a rate of at least 10 kilogray (AFB); and (b) has originated from, and packaged in a country, state or territory that is free from European foulbrood;

OR

1.1.4 (a) has been declared by the exporter as derived from apiaries that have been inspected by the beekeeper and found free of American foulbrood; and

(b) has undergone heat treatment at a minimum temperature for the corresponding minimum time as specified in this table (EFB):

Temperature (IC) Minimum time

50 54 hours

60 - 65 8 hours

70 1 hour and 48 minutes

80 22 minutes

82 20 minutes

90 or more 5 minutes

OR

1.1.5 (a) has been treated with irradiation at a rate of at least 10 kilogray (AFB); and (b) has undergone heat treatment at a minimum temperature for the corresponding minimum time as specified in this table (EFB):

Temperature (PC) Minimum time

50 54 hours

60 - 65 8 hours

70 1 hour and 48 minutes

80 22 minutes

82 20 minutes

90 or more 5 minutes

AND

1.2 The machinery and equipment used to pack the product has only processed prescribed potential carriers meeting the relevant import requirements for Western Australia or has been cleaned and washed free from contaminating risk material prior to the packing.

Note - Irradiation of food products is regulated by the Australia New Zealand Food Standards Code.

2. Honey, honeycomb, propolis, royal jelly, pollen, beeswax samples, dead bees and dead bee larvae imported for the purpose of diagnostics or analysis. Each consignment must be consigned to a quarantine facility approved for that purpose and be:

2.1 packaged in a secure manner in accordance with International Air Transport Association Dangerous Goods Regulations Packing Instructions, with packaging clearly marked on the outside with the name and address of the exporter and the recipient approved quarantine facility; and 2.2 accompanied by the relevant form available from DPIRD.

3. Beeswax and products in which beeswax is the single greatest ingredient by volume.

For use other than beekeeping

3.1 The product to be clarified and refined by heat treatment to melting point and free from extraneous matter; or

3.2 Each consignment to be accompanied by an Interstate Health Certificate issued by the quarantine authority in the exporting state or territory, or under a quality assurance arrangement approved by the Chief Plant Biosecurity Officer, certifying that the following requirements have been met:

3.2.1 The product has been treated with irradiation at the rate of at least 15 kilogray; (AFB, EFB) or

3.2.2 (a) declared by the exporter as derived from apiaries that have been inspected by the beekeeper and found free of American foulbrood; and

(b) The product has originated from a country, state or territory that is free from

European foulbrood.

For use with beekeeping

Not permitted except in accordance with the terms and conditions of, an import permit issued under the Biosecurity and Agriculture Management Act 2007.

4. Honey used in queen candy and food for bees that contains bee products (including but not limited to honey, honeycomb, propolis or royal jelly)

Each consignment to be accompanied by an Interstate Health Certificate issued by the quarantine authority in the exporting state or territory, or under a quality assurance arrangement approved by the Chief Plant Biosecurity Officer, certifying that the following requirements have been met:

4.1 The product has been treated with irradiation at the rate of at least 15 kilogray (AFB, EFB); and

4.2 The machinery and equipment used to pack the product has only processed prescribed potential carriers meeting the relevant import requirements for Western Australia or has been cleaned and washed free from contaminating risk material prior to the packing.

5. Bee collected pollen

(A) for use in beekeeping, or

(B) for uses other than beekeeping in units of greater than 150ml or 100g.

Each consignment to be accompanied by an Interstate Health Certificate issued by the quarantine authority in the exporting state or territory, or under a quality assurance arrangement approved by the Chief Plant Biosecurity Officer, certifying that the following requirements have been met:

5.1 The product has been treated with irradiation at the rate of at least 15 kilogray (AFB, EFB); and

5.2 The machinery and equipment used to pack the product has only processed prescribed potential carriers meeting the relevant import requirements for Western Australia or has been cleaned and washed free from contaminating risk material prior to the packing.

6. Used beehives; beekeeping appliances/equipment

Each consignment to be accompanied by an Interstate Health Certificate issued by the quarantine authority in the exporting state or territory, or under a quality assurance arrangement approved by the Chief Plant Biosecurity Officer certifying that the product has been treated with irradiation at the rate of at least 15 kilogray (AFB, EFB, Nosema ceranae, SHB).

Note - Beehives; beekeeping appliances/equipment claimed to be new will be inspected on arrival to verify freedom from biosecurity risk organisms and material.

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without prior written permission of the Western Australian Agriculture Authority.

Appendix 2. Norfolk Island Apiaries Act 1935

- Apiaries Act 1935
- No. 4, 1935
- **Compilation No. 2**
- Compilation date: 13 August 2019
- Includes amendments up to: Norfolk Island Continued Laws Ordinance 2015
- (No. 2, 2015) as amended up to Norfolk Island
- Legislation Amendment (Fees and Other Matters)
- Ordinance 2019 (F2019L01048)
- NORFOLK ISLAND

APIARIES ACT 1935

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- 11A. Provisions inapplicable to certain bees
- 12. Registration of apiaries
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- 16. Recovery of expense

NORFOLK ISLAND

Apiaries Act 1935

An Act to regulate the bee industry and to prevent the spread of disease in bees.

Short title

1. This Act may be cited as the Apiaries Act 1935. Definitions 2. In this Act, unless the contrary intention appears — "apiary" means any place where bees are kept; "bee-keeper" means any person who keeps bees, or any person in charge of bees; "disease" means foul brood, bee-moths, or any other disease or pest declared by the Chief Executive Officer, by notice published in the Gazette, to be a disease within the meaning of this Act; "frame-hive" means a hive containing movable frames in which the combs are built and which may be readily removed from the hive for examination; "inspector" means an inspector appointed in pursuance of this Act. Inspectors 3. The Chief Executive Officer may, by written instrument, appoint such inspectors as are necessary to carry out the provisions of this Act. Importation of bees 3A. (1) A person shall not bring into, or cause to be brought into, Norfolk Island — (a) bees of a species other than the species Apis mellifera (L.); or (b) bees of the species Apis mellifera (L.) from a country other than Australia, Canada, New Zealand or the United States of America, unless, in a particular case -(c) the Chief Executive Officer has certified, by instrument in writing, that he is satisfied — (i) that the bees are to be brought into Norfolk Island for scientific purposes or in special circumstances; and (ii) that the arrangements proposed for keeping, dealing with and treating the bees are such that their presence in Norfolk Island is not likely to lead to the spread of disease in Norfolk Island; and (d) the instrument is produced to the Collector of Customs or to an inspector. (2) A person shall not bring into, or cause to be brought into, Norfolk Island bees of the species Apis mellifera (L.) from Australia, Canada, New Zealand or the United States of America unless, in a particular case -NORFOLK ISLAND 2 Apiaries 1935 (a) an inspector has certified, by instrument in writing, that, in the circumstances of the case, he is satisfied that their presence in Norfolk Island will not introduce disease into Norfolk Island; and (b) the instrument is produced to the Collector of Customs or to an inspector.

Infected bees, etc, not to be kept or sold or brought into Norfolk Island

4. (1) A bee-keeper shall not -

(a) keep or allow to be kept upon any land occupied by him any bees, bee-combs, hives, or appliances known by him to be infected with or liable to spread disease without immediately taking the steps approved by the Chief Executive Officer to cure or eradicate the disease; or

(b) sell, barter, give away, or, otherwise than in the manner approved by the Chief Executive Officer, dispose of any bees or appliances from an apiary known by him to be infected with or liable to spread disease.

(2) A person shall not bring into, or cause to be brought into, Norfolk Island, any bee-combs, hives, honey or appliances unless and until he has made an application to the Chief Executive Officer for the purpose and the Chief Executive Officer has consented thereto.

(3) Any application for the consent of the Chief Executive Officer in pursuance of subsection 4(2) shall be accompanied by a certificate in writing from an apiculturist of the Department of Agriculture in the State or country of origin, or from such person as the Chief Executive Officer considers to be appropriate in the circumstances, certifying that the bee-combs, hives, honey, or appliances come from a district in which foul brood (Bacillus larvae, Bacillus pluton, or Bacillus alvei) and Isle of Wight disease (Acarine disease) do not exist.

(4) The lessee, holder or occupier of any land on to which any bee-combs, hives, honey or appliances are to be brought from outside Norfolk Island shall forthwith notify that fact to the Chief Executive Officer.

(5) Subsections 4(1), 4(2), 4(3) and 4(4) do not apply to honey —

(a) that is brought into Norfolk Island for commercial purposes;

(b) that is packed in a container that is either effectively sealed or closed in such a way as to prevent the honey from escaping; and

(c) as to which an inspector is satisfied that the honey will not introduce disease into Norfolk Island.

Bee-keeper to give notice of disease

5. Every bee-keeper in whose apiary any disease appears shall immediately after first becoming aware of its presence, send written notice thereof to the Chief Executive Officer or to an inspector.

Powers of entry and inspection

6. Any inspector may, after giving reasonable notice to, or with the permission of the bee-keeper concerned, enter and inspect any premises where bees are kept, and may inspect any bees, bee-hives, fitting, apparatus, appliances, or any articles used in connection therewith.

Power to destroy bees

7. (1) If an inspector certifies, in writing to the Chief Executive Officer, that any bees are diseased and, in his opinion, are a source of danger to other bees, and that

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they ought to be destroyed, the Chief Executive Officer may make an order directing the bee-keeper to destroy the bees.

(2) If, at the expiration of 7 days after the service of the order upon the bee-keeper, the bees are not destroyed, any inspector may cause them to be destroyed at the bee-keeper's expense.

Bee-hives, etc, liable to spread disease to be disinfected

8. (1) If an inspector finds that any bee-hive, fittings, apparatus, appliances, or any other articles are, in his opinion, liable to spread disease, he may order —

(a) that all or any of them be cleansed, disinfected, or readjusted in such manner and within such time as he directs, at the bee-keeper's expense; and

(b) that the articles, or such of them as he specifies, shall not be sold or otherwise alienated or removed for a further specified period of not more than one month, except with his written consent.

(2) If the inspector certifies in writing to the Chief Executive Officer that any of the articles mentioned in this section cannot be effectively cleansed, disinfected, or readjusted, and that they ought to be destroyed, the inspector may cause the articles to be destroyed at the cost of the bee-keeper.

(3) Where the value of the articles ordered to be destroyed exceeds \$50, the articles shall not be destroyed except with the approval in writing of the Chief Executive Officer.

After date to be fixed only frame-hives to be used

9. Any person keeping bees, other than native or indigenous bees, in any hive other than a frame-hive, shall be guilty of an offence.

Penalty: 2 penalty units.

Transfer of bees to frame-hives

10. (1) If an inspector finds any bees hived otherwise than in frame-hives, he may by notice in writing require the beekeeper to transfer the bees to frame-hives, within a time specified in the notice.

(2) If, at the expiration of that time, the bees are not so transferred, he may cause the bees to be so transferred at the bee-keeper's expense, and the bee-keeper shall in addition be guilty of an offence.

Penalty: 2 penalty units.

Alteration of hive, frame, etc

11. If an inspector finds that the bee-combs in any hive cannot, without cutting, be separately and readily removed from the hive for examination, he may order the bee-keeper to readjust the hive, comb, or frame, in such manner and within such time as he specifies.

Provisions inapplicable to certain bees

11A. Sections 9, 10 and 11 do not apply with respect to bees brought into Norfolk Island as mentioned in subsection 3A(1).

Registration of apiaries

12. (1) A bee-keeper shall apply to have his apiary registered.

(2) The application for the registration of an apiary established at the commencement of this Act shall be made within one month thereafter.

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(3) The application for the registration of an apiary established after the commencement of this Act shall be made within one month after the establishment thereof.

(4) A bee-keeper who removes his apiary shall within 14 days of the removal give notice thereof.

(5) Applications and notices under this section shall be given to the Chief Executive Officer in accordance with a form approved by the Chief Executive Officer.

(6) The Chief Executive Officer may register or refuse to register any apiary.

(7) A bee-keeper who fails to comply with any of the provisions of this section shall be guilty of an offence.

Penalty: 2 penalty units.

Inspectors not liable except for wilful damage

13. (1) An inspector acting in the execution of this Act shall not be deemed to be a trespasser by reason of any entry or removal or destruction authorised by this Act nor be liable for any damage occasioned in carrying out the provisions of this Act, unless the damage was occasioned by the inspector wilfully and without necessity.

(2) A person shall not be entitled to receive any compensation in consequence of any measures taken for the eradication of any disease or the destruction of any bees or any articles ordered to be destroyed under this Act, or in respect of any damage that may result to him therefrom, either directly or indirectly, unless the damage was occasioned wilfully and without necessity.

Service of orders, etc

14. Every direction or order by the Chief Executive Officer, or an inspector, shall be in writing, and, in the case of a direction or order by an inspector, signed under his hand, and shall be either delivered to the bee-keeper personally or sent to him at his last known place of abode.

Offences and penalties

15. Every person who -

(a) obstructs an inspector in the exercise of his duties under this Act; or

(b) fails to comply with any order or direction given under the provisions of this Act; or

(c) commits any other breach of this Act,

shall be guilty of an offence, and shall, where no other penalty is provided, be liable to a penalty not exceeding 2 penalty units and in the case of a failure to comply with any such order or direction the inspector may himself carry out the necessary work at the expense of the person failing so to comply.

Recovery of expense

16. Where by this Act it is provided that anything may be done at the bee-keeper's expense, the cost of such action shall be deemed to be a debt due to the Administration and may be recovered by an inspector suing in his own name, in any Court of competent jurisdiction.