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Tiakitanga Pūtaiao Aotearoa

Supplementary Import Risk Analysis

Honey bee products

Draft approved for IHS development

Prepared for Ministry for Primary Industries
By Biosecurity Science and Risk Assessment
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Ministry for Primary Industries

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Manatū Ahu Matua

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Ministry for Primary Industries
PO Box 2526
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Email: brand@mpi.govt.nz
Telephone: 0800 00 83 33
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Ministry for Primary Industries
Pastoral House
25 The Terrace
PO Box 2526
Wellington 6011
New Zealand

Tel: 64 4 894 0100
Fax: 64 4 894 0731

Science and Risk Assessment
Ministry for Primary Industries

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DRAFT APPROVED FOR IHS DEVELOPMENT

July 2016

Acting Director, Biosecurity Science and Risk Assessment
Science and Risk Assessment
Ministry for Primary Industries

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Contributors to this risk analysis

Author

Lincoln Broad	Senior Adviser, Animals and Aquatic, Biosecurity Science and Risk Assessment	Ministry for Primary Industries, Wellington, New Zealand

Internal Review

Howard Pharo	Manager, Animal Imports and Exports	Ministry for Primary Industries, Wellington, New Zealand
Stephen Cobb	Manager, Animals and Aquatic	Ministry for Primary Industries, Wellington, New Zealand

External Review

Phil Lester	Insect ecologist	School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand
John McLean	Consultant Entomologist	Gisbourne, New Zealand
Joachim de Miranda	Honey Bee Research Group	Swedish University of Agricultural Sciences, Uppsala, Sweden
Eva Forsgren	Honey Bee Pathology Research Group	Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
Marrie-Pierre Chauzat	Honey Bee Disease Epidemiologist	French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Sophia-Antipolis, France

Contents

1. Executive summary

Imported honey bee products have the potential to harbour harmful exotic organisms. The risks associated with these were previously examined in MAF's 2004 honey bee products risk analysis.

However, surveillance in New Zealand conducted since 2007 has established the presence of *Deformed wing virus*, *Paenibacillus alvei* and *Nosema ceranae*. Further, a limited survey carried out over 2010 and 2011 provides evidence to support a claim of country freedom from *Israeli acute paralysis virus*.

This qualitative supplementary risk analysis updates MPI's 2004 assessment of the biosecurity risks associated with the importation of products derived from honey bees (honey, beeswax, pollen, propolis, bee venom, royal jelly).

With regard to hazard identification, an extensive list of organisms that could be associated with honey bees has been collated previously in MAF's 2004 honey bee products risk analysis. To update this list, literature review and consultation has been carried out to identify new organisms described in association with honey bees.

Endemic organisms have also been re-assessed concerning the possibility of newly emerged virulent strains that may now exist abroad. However, overseas strains of endemic organisms have not been identified as having greater pathogenicity.

Additionally, this biosecurity risk analysis assesses 15 new organisms that have been identified in association with honey bees since 2004. Of these, the newly described genotypes of *Paenibacillus larvae* (causative agent of American foulbrood) and new strains of *Melissococcus plutonius* (causative agent of European foulbrood) and Israeli acute paralysis virus are identified to be a hazard in certain honey bee products.

Nevertheless, the ability to discern the genetic structure of *P. larvae* into genotypes using molecular techniques does not alter the conclusions of the American foulbrood assessment carried out in MAF's honey bee products risk analysis of 2004. This is because the newly described genotypes of *P. larvae* that cause disease are already present in New Zealand.

Risk management options are presented for the causative agents of American foulbrood, European foulbrood and Israeli acute paralysis virus since these organisms are concluded to be a risk when importing specific honey bee commodities.

2. Introduction

This qualitative risk analysis examines the biosecurity risks associated with the importation of bee products derived from honey bees (*Apis mellifera*). These risks were previously examined in 2004 (MAF 2004). However, recognising technical advances, reports of newly identified pathogens of honey bees and changes to the OIE *Terrestrial Animal Health Code* over the intervening 11 year period, a supplementary biosecurity import risk analysis has been requested.

Moreover, surveillance in New Zealand conducted since 2007 has established the presence of *Deformed wing virus, Paenibacillus alvei* and *Nosema ceranae* (Bingham 2007; MPI 2010). Further, a limited survey carried out over 2010 and 2011 provides some evidence to support a claim of country freedom from *Israeli acute paralysis virus* (McFadden *et al.* 2014).

Accordingly, an extensive list of organisms of potential concern has been compiled from published risk analyses that had previously assessed pathogens of honey bees. Not all the organisms in these risk analyses are relevant to the commodity being examined in this supplementary biosecurity risk analysis.

Specific criteria were applied to organisms of potential concern in order to determine if they should be excluded from the preliminary hazard list. For example, organisms that are not present with the commodities being traded such as bee races. The criteria applied to derive the list of organisms of potential concern is given in Section 6.

Subsequently, Sections 7, 8 and 9 examine these organisms of potential concern (including endemic organisms for evidence of pathogenic exotic strains) to establish a preliminary hazard list (Section 10).

3. Purpose and Scope

The purpose of this supplementary biosecurity risk analysis is to update MAF's honey bee products risk analysis of 2004. The scope includes assessing newly identified organisms since 2004 that may be associated with imported honey bee products. Further, the scope also includes assessing whether there are more pathogenic strains of endemic organisms overseas.

4. Commodity definition

As defined in the *Terrestrial Animal Health Code* (hereafter referred to as the *Code*), the following products related to bees (obtained from apiculture or otherwise harvested) and international trade are considered:

- Honey
- Bee-collected pollen
    - Propolis
- Beeswax

- Royal jelly
- Honey bee venom

4.1. HONEY

Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature (Definition from the Codex Alimentarius, Revised Codex Standard for Honey, CODEX STAN 12-1981, Rev.1 [1987], Rev.2 [2001]). Three forms of honey can be found in the *Code* chapters: extracted honey, comb honey and strained honey.

Extracted honey: any honey removed from the comb.

Comb honey: honey kept inside the comb.

Strained honey: extracted honey that has at a minimum passed through a filter of pore size not greater than 0.42 mm diameter (35 mesh standard, see Townsend G.F. (1975) Processing and storing liquid honey. *In* Honey - a comprehensive survey, ed. E Crane, Heinemann, London, pp. 269-292).

When the term 'honey' is used, it refers to all the three forms.

Honey is traded mainly for human consumption. It may also be used externally (wound healing) and be further processed into a multitude of products containing various amounts of honey (ingredient in baked goods, sauces, or in cosmetics etc). Honey is not traded to feed honey bee colonies due to high purchase and shipping costs (Shimanuki and Knox 1997; Mutinelli 2011) and an increased risk of spreading diseases.

4.2. BEE-COLLECTED POLLEN

Pollen consists of the male reproductive cells of flowering plants. Bees use nectar or honey and salivary secretions to agglutinate and preserve pollen grains. For the purpose of the *Code*, bee-collected pollen is the pollen dislodged from the pollen basket of foraging honey bees and collected in a pollen trap or removed from the cells of honey bee or stingless bee colonies (bee bread, which is fermented pollen).

Pollen is traded mainly for human consumption, but may also be used for animal consumption (including bee consumption).

4.3. PROPOLIS

Propolis is a sticky material used by bees to seal gaps, encapsulate foreign objects and disinfect hive materials. It is derived from resins collected from plants and consists of a mixture of terpenes and other volatile substances. Two forms are found in the *Code* chapters: processed propolis and unprocessed propolis. Processed propolis is either alcohol extracted (tincture) or powdered.

4.4. BEESWAX

Beeswax is a complex mixture of lipids and hydrocarbons that is produced by the wax glands of honey bees. Two forms are found in the *Code* chapters: processed and unprocessed beeswax. Processed beeswax is beeswax produced by heating the raw wax to at least 60°C and then allowing it to solidify. Unprocessed beeswax is any wax coming from bees that has not followed the process described above. When the term 'beeswax' is used, it refers to both forms.

4.5. ROYAL JELLY

Royal jelly is a glandular secretion of honey bee workers that is placed in queen cells to feed queen-destined larvae. It is harvested and preserved by freezing or lyophilisation. Royal jelly is traded mainly for use in the cosmetic industry and in the human health food market.

4.6. HONEY BEE VENOM

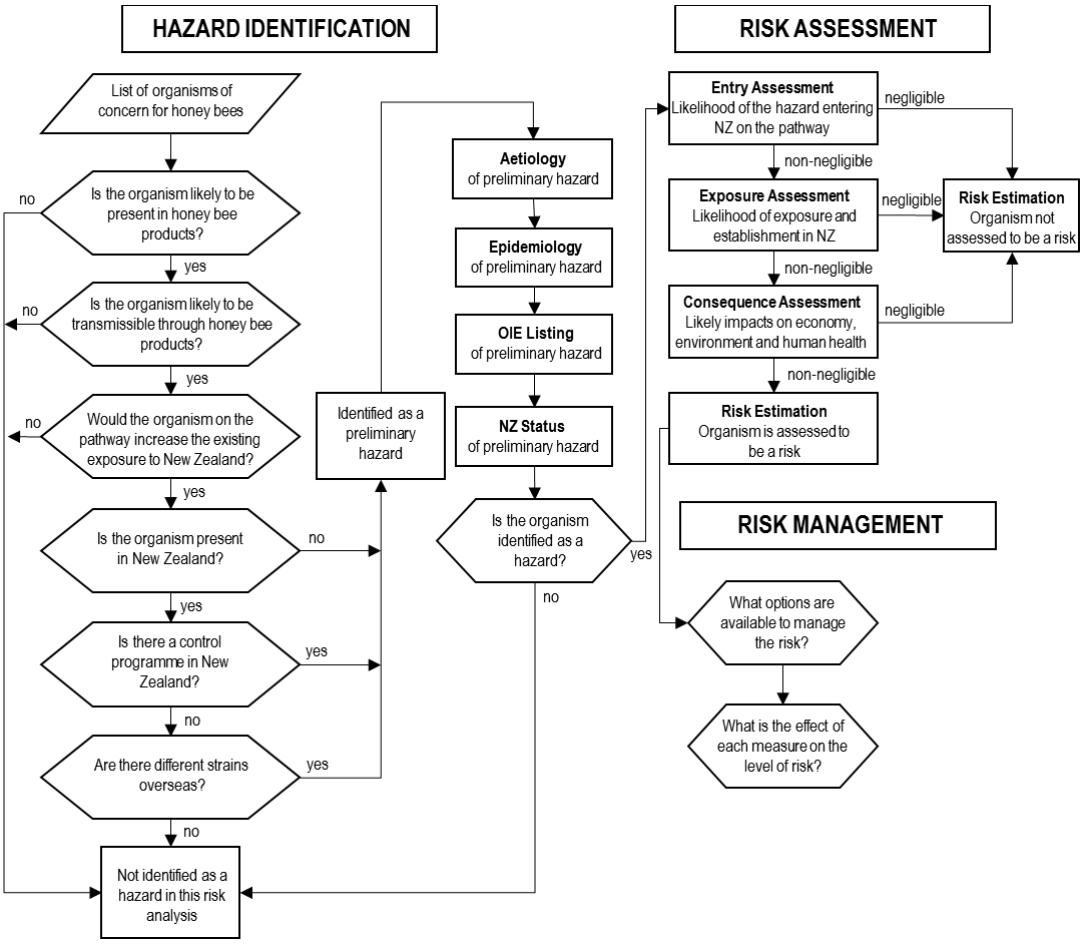
Bee venom is a complex mixture of proteins and low molecular weight components secreted by the venom glands of honey bees and used to defend the colony. It is collected by special collectors that are placed in or outside the hive, electrically stimulating the bees to sting through a membrane on a glass plate. Venom is used in the treatment of certain human medical conditions (apitherapy).

5. Risk analysis methodology

The methodology used in this risk analysis follows the guidelines as described in *Biosecurity New Zealand Risk Analysis Procedures – Version 1* (Biosecurity New Zealand 2006). This methodology takes into account, and is based on the recommendations made in Section 2 of the *Code* (OIE 2013a).

The process followed is shown in Figure 1 (overleaf).

Figure 1. The risk analysis process.



5.1. PRELIMINARY HAZARD LIST (ORGANISMS OF POTENTIAL CONCERN)

From consulting electronic journal databases and previous risk analyses, a list of organisms known to be associated with honey bees has been collated. From all the organisms of concern listed, preliminary hazards are identified by applying specific criteria to each organism to eliminate those where there is no available evidence that they constitute a risk. The remaining organisms are collated into a preliminary hazard list. The organisms in this list are subjected to hazard identification.

5.2. HAZARD IDENTIFICATION

Organisms in the preliminary hazard list were subjected to a more detailed hazard identification step. This step includes formal identification of the organism, whether it is the cause of an OIE listed disease, its New Zealand status, and a discussion on the relevant aspects of the epidemiology and characteristics of the organism. The hazard idenitification section is concluded by a determination of whether or not the organism is identified as a hazard. All hazards are subjected to risk assessment.

5.3. RISK ASSESSMENT

Risk assessment consists of:

a) *Entry assessment*: The likelihood of a hazard (pathogenic organism) being imported with the commodity.

b) *Exposure assessment*: Describes the biological pathway(s) necessary for exposure of susceptible animals or humans in New Zealand to the hazard. Further, a qualitative estimation of the probability of the exposure occurring is made.

c) *Consequence assessment*: Describes the likely consequences of entry, exposure and establishment or spread of an imported hazard.

d) *Risk estimation*: An estimation of the risk posed by the hazard associated with importing honey bee products. This is based on the entry, exposure and consequence assessments. If the risk estimate is assessed to be non-negligible, then the hazard is assessed to be a risk and risk management measures may be justified to effectively manage the risk.

Not all of the above steps may be necessary in all risk assessments. The OIE methodology makes it clear that if the likelihood of entry is negligible[A] for a certain hazard, then the risk estimate is automatically negligible and the remaining steps of the risk assessment need not be carried out. The same situation arises when the likelihood of entry is non-negligible but the exposure assessment concludes that the likelihood of susceptible species being exposed is negligible, or when both entry and exposure are non-negligible but the consequences of introduction are assessed to be negligible.

5.4. RISK MANAGEMENT

For each organism assessed to be a risk, options are identified for managing that risk. Where the *Code* lists recommendations for the management of a risk, these are described alongside options of similar, lesser or greater stringency, where available. In addition to the options presented, unrestricted entry or prohibition may also be considered. Recommendations for the appropriate sanitary measures to achieve the effective management of risks are not made in this document. These will be determined when the import health standard (IHS) and risk management proposal document are drafted.

As obliged under Article 3.1 of the World Trade Organization's Agreement on the application of Sanitary and Phytosanitary measures (the SPS agreement) the measures adopted in IHSs will be based on international standards, guidelines, and recommendations where they exist except as otherwise provided for under Article 3.3. That is, measure providing a higher level of protection than international standards can be applied if there is scientific justification, or if there is a level of protection that the member country considers is more appropriate following a risk assessment.

[A] Negligible and non-negligible are terms used as adjectives to qualify risk estimates. Negligible is defined as not worth considering; insignificant. Non-negligible is defined as worth considering; significant. Very low as a risk description means close to insignificant. Low means less than average, coming below the normal level. Medium means around the normal or average level, and high means extending above the normal or average level (Biosecurity New Zealand 2006a

5.5. RISK COMMUNICATION

After a biosecurity import risk analysis has been written, MPI analyses the options available and proposes draft measures for the effective management of identified risks. These are then presented in a draft IHS that is released together with a risk management proposal that summarizes the options analysis, the rationale for the proposed measures and a link to the risk analysis. The package of documents is released for a six-week period of stakeholder consultation. Stakeholder submissions in relation to these documents are reviewed before a final IHS is issued.

6. Organisms of potential concern and the preliminary hazard list

The first step in a biosecurity import risk analysis is the process of hazard identification. This identifies the pathogenic agents associated with the imported commodity that could potentially cause adverse consequences.

Organisms considered to be of potential concern included OIE-listed honey bee diseases of international concern and disease agents of honey bees identified in the previous MAF honey bee products risk analysis of 2004. In addition, electronic journal databases, external technical experts, and New Zealand stakeholders have been consulted to identify any new organisms that have been associated with honey bees since 2004.

The OIE lists 6 pests and diseases of honey bees, of which none are viruses. There is no evidence that any countries are free from these viruses and there is currently no data to demonstrate high colony mortality or morbidity caused by any specific virus in the absence of *Varroa* spp. mites.

The resulting updated list of organisms of potential concern is shown below (Table 1). All known viruses of honey bees have been included even though as discussed above, none are OIE-listed (OIE 2013b). The newly identified organisms from literature review that may be of potential concern have been added to the original risk analysis 2004 list and are highlighted in the column ‘newly identified since 2004’(overleaf).

Table 1. Organisms of potential concern

Common Name/ Disease	Scientific Name	Present in NZ?	OIE List?	Under official Control or Unwanted?	Newly identified since 2004
VIRUSES					
Acute paralysis	Acute paralysis virus	Yes	No	No	
Aphid lethal paralysis virus	Aphid lethal paralysis virus	No (1)	No	No	Yes
Apis Iridescent disease	*Apis* iridescent virus	No (1)	No	No	
Arkansas bee virus	Arkansas bee virus	No (1)	No	No	
Bee virus X	Bee virus X	Yes	No	No	
Bee virus Y Berkeley bee virus	Bee virus Y Berkeley bee virus	Yes No (1)	No No	No No	
Big Sioux River virus	Big Sioux River virus	No (1)	No	No	Yes
Black queen cell	Black queen cell virus	Yes	No	No	
Chronic bee paralysis virus and associate	Chronic paralysis virus and associate	Yes	No	No	
Cloudy wing	Cloudy wing virus	Yes	No	No	
Deformed wing	Deformed wing virus	Yes (2)	No	No	
Egypt bee virus	Egypt bee virus	No (1)	No	No	
Filamentous virus	*Apis mellifera* Filamentous virus	Yes	No	No	
Israeli acute paralysis virus	Israeli acute paralysis virus	No (3)	No	No	Yes
Kashmir bee virus	Kashmir bee virus	Yes	No	No	
Kakugo	Kakugo virus	No (1)	No	No	Yes
Lake Sinai virus 1	Lake Sinai virus 1	Yes	No	No	Yes
Lake Sinai virus 2	Lake Sinai virus 2	Yes	No	No	Yes
Sacbrood	Sacbrood virus	Yes	No	No	
Slow paralysis	Slow bee paralysis virus	No (1)	No	No	
Tobacco ringspot virus	Tobacco ringspot virus	Yes	No	No	Yes
Thai sacbrood	Thai sacbrood virus	No (1)	No	No	
Turnip ringspot virus	Turnip ringspot virus	No (1)	No	No	Yes
Turnip yellow mosaic virus	Turnip yellow mosaic virus	No (1)	No	No	Yes
Bee macula-like virus	Bee macula-like virus	No (1)	No	No	Yes
Varroa destructor virus-1	*Varroa destructor* virus-1	No (1)	No	No	Yes
BACTERIA					
American foulbrood	*Paenibacillus larvae* (molecular genotypes have been recently described)	Yes	Yes	Official control	Yes
European foulbrood	*Melissococcus plutonius* (new strains recently described)	No (4)	Yes	Unwanted	Yes
Paenibacillus alvei	*Paenibacillus alvei*	Yes (5)	No	No	
Septicaemia	*Pseudomonas aeruginosa*	Yes	No	No	
Spiroplasmas	*Spiroplasma melliferum, S. apis*	No (1)	No	No	
FUNGI					
Chalkbrood	*Ascosphaera apis*	Yes	No	No	
Stonebrood	*Aspergillus* spp.	Yes	No	No	

ARTHROPOD PARASITES					
Bee louse	*Braula coeca*	No (1)	No	Unwanted	
External acarine mites	*Acarapis dorsalis, A. externus*	Yes	No	No	
Phorid fly	*Apocephalus borealis*	No (1)	No	No	Yes
Small hive beetle	*Aethina tumida*	No (1,4)	Yes	Unwanted	
Tracheal mite	*Acarapis woodi*	No (4)	Yes	Unwanted	
Tropilaelaps spp.	*Tropilaelaps clareae, T. koenigerum*	No (4)	Yes	Unwanted	
Varroa destructor	*Varroa destructor*	Yes	Yes	Unwanted	
Other *Varroa* species	*Varroa jacobsoni, V. underwoodi, V. rindereri, Euvarroa sinhai, E. wongsirii*	No (2)	No	Unwanted	
Wax moths	*Galleria mellonella; Achroia grisella*	Yes	No	No	
PROTOZOA					
Amoeba disease	*Malpighamoeba mellificae*	Yes	No	No	
Neogregarine	*Apicystis bombi*	No (1)	No	No	Yes
Crithidia mellificae	*Crithidia mellificae*	No (1)	No	No	Yes
Gregarine disease	*Gregarinidae*	No (1)	No	No	
Nosema disease	*Nosema apis*	Yes	No	No	
	Nosema ceranae	Yes (6)	No	No	
OTHER HONEY BEES & RACES					
Africanised bee	*Apis mellifera scutella*	No	No	Unwanted	
Cape honey bee	*Apis mellifera capensis*	No	No	Unwanted	
Other honey bee races	*Apis mellifera carnica and Apis mellifera caucasia*	Yes	No	No	
Honey bees other than *A. mellifera*	*Apis* spp. other than *A. mellifera*	No	No	Unwanted	

Note 1: Not reported in New Zealand.
Note 2: Bingham P (2007). Quarterly report of investigations of suspected exotic diseases. *Surveillance* 34 (3), 27-31.
Note 3: McFadden AMJ, Tham K, Stevenson M, Goodwin M, Pharo H, Taylor B, Munro G, Owen K, Peacock L, Stanslawek WL, Stone M (2014). Israeli acute paralysis virus not detected in *Apis mellifera* in New Zealand in a national survey. *Journal of Apicultural Research*, 53 (5), 520-527.
Note 4: Not found in specific surveys or routine exotic disease surveillance in New Zealand.
Note 5: Spence R, Demchick P, Hornitzky M, Pharo H, Peacock L, McFadden A, Stone M (2013). Surveillance of New Zealand apiaries for *Paenibacillus alvei*. *New Zealand Entomologist*, 36 (2) 82-86.
Note 6: Bingham P (2010). Quarterley report of investigations of suspected exotic diseases. *Surveillance*, 37 (4), 30-40.

Regarding the column "Present in NZ?", there has been no systematic surveillance for many of the organisms listed and the new organisms have only been recently described in bees overseas. Therefore, a designation of Note (1) "Not reported in New Zealand" takes into account that the presence or absence of the organism in New Zealand is uncertain.

From the above list, only organisms that are likely to be associated with the commodities defined above (Section 4) will be subjected to further consideration. Several organisms discussed in the 2004 import risk analysis for bee products are not associated with any traded commodity (e.g. honey bees and races), and are not hazards in honey bee products.

The new organisms of potential concern identified have in almost all cases been detected only in honey bees or various other insects. While there are no reports of these organisms in honey bee products, there do not appear to be specific studies to examine this possibility.

7. Pathogenic strains of endemic organisms

At this stage, endemic organisms including those that have been assessed in 2004 as posing no biosecurity risk, have been retained as organisms of potential concern. This is because there has been no recent review of whether or not more pathogenic exotic strains have emerged.

Consequently, this Section examines these endemic organisms and assesses any new evidence that may show the existence of more pathogenic exotic strains. In this regard, for many of the organisms endemic to New Zealand there is little or no new research since 2004. Accordingly, a short summary of any new information is sufficient for most endemic organisms.

Nevertheless, in regards the newly identified Lake Sinai viruses, these have only recently been detected in New Zealand. For completeness a chapter has been dedicated to these viruses.

7.1 VIRUSES

Acute bee paralysis virus and Kashmir bee virus

The bee products risk analysis (MAF 2004) concludes there is no evidence to suggest that more pathogenic strains exist abroad for these viruses.

There are publications since 2004 on *Acute bee paralysis virus* (ABPV) and *Kashmir bee virus* (KBV) in relation to their genetic similarity to the newly discovered Israeli acute paralysis virus. These references are included in chapter 13 that examines primarily Israeli acute paralysis virus but also discusses these three closely related viruses and their pathogenicity. This chapter includes discussion and references to the new information on *Acute bee paralysis virus* and *Kashmir bee virus* (refer to chapter 13 for more information).

Although there are geographic variants of these viruses throughout the world, there is no evidence that any of these are more pathogenic than the strains already present in New Zealand. Therefore, *Acute bee paralysis virus* and *Kashmir bee virus* are not identified as hazards.

Bee virus X and *Bee virus Y*

The bee products risk analysis (MAF 2004) concludes there is no evidence to suggest that more pathogenic strains of *Bee virus X* or *Bee virus Y* exist abroad. There is no new information about these viruses (Forsgren 2014). Accordingly, *Bee virus X* and *Bee virus Y* are not identified as hazards.

Black queen cell virus

The bee products risk analysis (MAF 2004) concludes there is no evidence to suggest that more pathogenic strains of *Black queen cell virus* (BQCV) exist abroad.

Since 2004, overseas researchers have carried out genetic analysis and phylogenetic comparisons of BQCV genotypes. Different strains have been described corresponding to different countries, most likely arising through natural recombination events (Noh *et al.* 2013; Tapaszti *et al.* 2009).

While there are geographic variants of this virus throughout the world, there is no evidence that any of these are more pathogenic than the strains already present in New Zealand.

Since no more pathogenic exotic strains of BQCV are recognized, they are not identified as hazards.

Chronic bee paralysis virus and Chronic bee paralysis virus associate

The bee products risk analysis (MAF 2004) concludes there is no evidence to suggest that more pathogenic strains exist abroad for both *Chronic bee paralysis virus* (CBPV) and *Chronic bee paralysis virus associate* (which is always associated with CBPV).

Since 2004, new PCR technology that takes into account the internal genomic variability of CBPV has been able to detect up to 40% more CBPV isolates in French honey bees than earlier assays (Blanchard *et al.* 2008).

Phylogenetic analysis of two particular genes of CBPV detected in honey bees from 9 European countries, led to the description of four genotypes of CBPV (Blanchard *et al.* 2009).

Morimoto *et al.* (2012) demonstrate a geographic separation of Japanese isolates of CBPV from European, Uruguayan and mainland US isolates. The authors suggest that the lack of major trade between Europe and the mainland US with Japan over the past 26 years may explain the results. Indeed, Japan has been importing most of its honey bee queens from Hawaii, New Zealand and Australia for the past 26 years (Morimoto *et al.* 2012). Theoretically, the isolates of CBPV in Japan could have originated from, and therefore be similar to New Zealand strains.

While there are geographic variants of this virus throughout the world, there is no evidence that any of these are more pathogenic than the strains already present in New Zealand.

Therefore, chronic bee paralysis virus and its associate, are not identified as hazards.

Cloudy wing virus and *Filamentous virus*

The bee products risk analysis (MAF 2004) concludes there is no evidence to suggest that more pathogenic strains exist abroad. Since there is no new information, *Cloudy wing virus* and *Filamentous virus* are not identified as hazards.

Deformed wing virus

Phylogenetic analysis of nucleotide sequences has revealed geographic differences in *Deformed wing virus* (DWV) between countries (Reddy *et al.* 2013; de Miranda and Genersch 2010). Ryabov *et al.* (2014) demonstrated that *Varroa destructor* is capable of carrying a diverse range of DWV strains, but that particular strains replicate preferentially when directly inoculated experimentally or by *Varroa* into honey bees. Gisder *et al.* (2009) note that the

appearance of individual disease symptoms is not only dependent on DWV transmission by *Varroa*, but also on viral replication in *Varroa* and on the DWV titre in the parasitising mite.

Although there is evidence that *V. destructor* may select for specific strains from a heterogenous population of *Deformed wing virus* strains, this does not necessarily imply that overseas strains are more pathogenic than the ones already found in New Zealand. Indeed, it is highly likely that the introduction of *V. destructor* into New Zealand will also have brought the corresponding varroa-adapted DWV strains. Moreover, the same process of selecting for or against particular strains of DWV due to the presence of varroa is also occurring here (Martin *et al.* 2012; Mondet *et al.* 2014).

DWV has previously been assessed as having a negligible likelihood of being present in imported commodities (MAF 2004). Singh *et al.* (2010) reported the detection of DWV in pollen pellets collected from bees using PCR. This molecular technique is very sensitive but does not distinguish between viable and non-viable forms of the virus.

A field experiment to determine if DWV contaminated pollen or honey might be infectious to honey bees gave inconclusive results. The results were undermined since a negative control honey bee colony in the experiment that was not exposed to contaminated pollen or honey returned positive PCR results to DWV (Singh *et al.* 2010). Recently, Mazzei *et al.* (2014) showed the presence of DWV on pollen sampled directly from visited flowers and that, following experimental injection into individual honey bees, it is able to establish an active infection, as indicated by the presence of replicating virus in the head of the injected bees.

Further, Genersch *et al*. (2006) Singh *et al.* (2010) Evison *et al.* (2012) and Fürst *et al.* (2014) have reported detecting DWV by PCR in various other pollinators such as bumble bees and wasps. Apart from honey bees, DWV has also been detected in wasps and Argentine ants here in New Zealand. Replication of the virus was shown to occur in these ants, indicating a true infection (Lester *et al*. 2015; Sébastien *et al*. 2015). However, for most of the surveys carried out in other insects it is not known if the PCR detection of DWV represents true infection or passive acquisition of the virus or its nucleic acid by the honey bee, or possibly contamination as samples of foraging bees and wasps were collected together at flowers or in traps.

DWV detection in bumble bees has been associated with typical DWV symptoms (Genersch *et al*. 2006) and reduced longevity in artificially infected bumble bees (Fürst *et al.* 2014), establishing these bumble bee species as true hosts for DWV. There is no evidence that the DWV infecting bumble bees is fundamentally distinct from that infecting honey bees (Fürst *et al.* 2014).

In conclusion, there is sufficient evidence to conclude that DWV may well be present in multiple species of insect in New Zealand, of which some are true hosts. While there are geographic variants of this virus throughout the world, there is no evidence that any of these are more pathogenic than the strains already present in New Zealand.

Accordingly, *Deformed wing virus* is not identified as a hazard.

Sacbrood virus

Several PCR methodologies have recently been developed to detect *Sacbrood virus* (Grabensteiner *et al.* 2007; Kukielka and Sánchez-Vizcaíno 2009; Yoo *et al.* 2012; Mingxiao *et al.* 2013).

Recently, complete genomic sequencing and phylogenetic analysis of the *Sacbrood virus* has been carried out (Nguyen and Le 2013; Xia *et al.* 2015). Phylogenetic analysis of the *Sacbrood virus* circulating among honeybees in the European region of the Russian Federation revealed two geneotypes (Lomakina and Batuev 2012). In Korea, Choe *et al.* (2012a) described two strains of sacbrood virus in circulation there. Genetic and phylogenetic analysis of the Korean strains revealed a low level of sequence variation among isolates regardless of the genome regions studied or the geographic origins of the strains. Multiple sequence comparisons indicated that Korean sacbrood virus strains are most closely related to Chinese and SBV strains, and more distantly to Thai, UK and European strains (Choe *et al.* 2012b).

The complete genome of sacbrood virus isolated in China was analysed and phylogenetic analysis showed that the virus segregated into three distinct groups (Xia *et al.* 2015). A novel strain of *Sacbrood virus* discovered in New Guinea is concluded to be most likely derived from a European, rather than Asian strain of SBV (Roberts and Anderson 2014).

In summary, in recent times researchers have described the natural geographic variation between *Sacbrood virus* isolates.

Such variation is entirely natural and expected. While there are geographic variants of this virus throughout the world, there is no evidence that any of these are more pathogenic than the strains already present in New Zealand.

Accordingly, exotic strains of *Sacbrood virus* are not identified as hazards.

7.2 BACTERIA

American foulbrood and powdery scale disease

Although American foulbrood is present in New Zealand, it is a preliminary hazard since it is under official control.

The causative organism of powdery scale disease (*Paenibacillus larvae pulvifaciens*) has been reclassified. As a result, the distinction between causative organisms of American foulbrood and powdery scale disease is no longer valid. The reclassification into one species (*Paenibacillus larvae*), eliminates the subspecies designations (*Paenibacillus larvae pulvifaciens* and *Paenibacillus larvae larvae*) (de Graaf *et al.* 2006).

Nevertheless, the newly discovered molecular genotypes of *Paenibacillus larvae* that have been described (causative agent of American foulbrood) are identified as preliminary hazards (included in preliminary hazard list, Section 10).

Paenibacillus alvei

There is no evidence that more pathogenic strains of *P. alvei* exist abroad. Accordingly, this organism is not identified as a hazard.

Septicaemia (*Pseudomonas aeruginosa*)

The ubiquitous bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen. Certain isolates infect a broad range of host organisms from plants to humans (Jianxin *et al.* 2004). There is no evidence that more pathogenic strains of *P. aeruginosa* exist in honeybees abroad.

Accordingly, this organism is not identified as a hazard.

7.3 FUNGI

Chalkbrood

The bee products risk analysis (MAF 2004) noted "While there is limited evidence of strain variation in virulence of *Ascosphaera apis* under experimental conditions, there is no evidence of this being linked to severity of chalkbrood under natural conditions". Vojvodic *et al.* (2012) examined the effect of multiple infections of several *Ascosphaera* species on *in vitro* honey bee larvae. Artificially, bee larvae were exposed to a spore suspension containing millions of either single or mixed species of *Ascophaera*. This generally did not cause any disease, with only very rare disease occurrence.

Therefore, the new information does not change the conclusion reached in the 2004 risk analysis. Consequently, *Ascophaera* spp. are not identified as hazards.

Stonebrood

Aspergillus spp. were not classified as a potential hazard in MAF's 2004 bee products risk analysis. At that time, there was no evidence to suggest that strains that are more virulent exist abroad. Foley *et al.* (2014) confirm the ubiquitous nature of *Aspergillus* spp. in the apiary environment. Experimentally, *Aspergillus flavus*, *A. nomius* and *A. phoenicis* could be pathogenic to honey bee larvae. All these species are present in New Zealand (New Zealand Organisms Register 2015).

Since no more pathogenic strains in honey bees have been identified overseas, *Aspergillus* spp. are not identified as hazards.

7.4 PROTOZOA

Amoeba disease

In the bee products risk analysis (MAF 2004) this protozoan is concluded not to be a hazard with no more pathogenic strains existing abroad. Since there is no new information that alters this conclusion, amoeba disease is not identified as a hazard.

Nosema disease

Two microsporidian parasites are described from honeybees, *Nosema apis* and *Nosema ceranae*. Both of which are present in New Zealand (MPI 2010).

N. apis has historically been associated with the European honey bee *Apis mellifera* and *N. ceranae* with the Asian honey bee *Apis cerana*. Experimental infections show that both parasites are cross-infective to both the European and Asian honey bee. Samples from across the world now demonstrate that the infection of *N. ceranae* in *A. mellifera* is worldwide (Klee *et al.* 2007; MPI 2010).

Experimentally, Forsgren and Fries (2010) investigated whether *N. ceranae* is more virulent than *N. apis* in *A. mellifera*. Their results did not indicate a higher virulence of *N. ceranae* compared to *N. apis* since bee mortality was not significantly different. Furthermore, *N. ceranae* had no competitive advantage within *Apis mellifera*, which had been subject to mixed infections. In contrast, in nature *N. ceranae* now appears to be widespread in the United States of America, having replaced *N. apis*. It is not known why *N. ceranae* has become the dominant organism in the US (Huang *et al.* 2015). Indeed, Ritter (2014) concludes that *N. ceranae* has now replaced *N. apis* virtually worldwide.

Based on the results of cage experiments, Higes *et al*. (2007) reported rapid mortality of individually infected honey bees and suggested that this demonstrated high virulence of *N. ceranae*. However, several later cage experiments have not been able to replicate this finding (Fries 2010). While Paxton *et al.* (2007) did demonstrate lower survival of bees infected with *N. ceranae* compared to those infected with *N. apis*, they advised caution in making conclusions about relative virulence of these organisms as the results were based on a single cage per treatment observation. Further, *in vivo* experiments have led to the opposite conclusion. For instance, Forsgren and Fries (2010) showed that *N. ceranae* was less virulent than *N. apis*.

Higes *et al.* (2008; 2009) report a regional phenomenon of high mortality events in Spanish bee colonies attributed to infection with *N. ceranae*. However, *N. ceranae* is commonly detected in healthy hives with no mortality caused by its presence (Cox-Foster *et al*. 2007; Invernizzi *et al*. 2009). Fernández *et al.* (2012) found 100% presence of *Nosema* spp. in some Spanish locations, indicating that these parasites were widespread throughout Spain. Two years of monitoring indicated that 87% of the hives with *Nosema* spp. remained viable, with normal honey production and biological development during that period of time. Fernández *et al.* (2012) concluded that the results indicated that both *N. ceranae* and *N. apis* could be present in beehives without causing any disease.

To explain the inconsistent results from the Spanish studies, it was hypothesised that differences in honey bee mortality may be because of differences in virulence of *N. ceranae* strains from different geographic regions.

To test this hypothesis, Dussaubat *et al.* (2013) performed a genetic variability study between Spanish and French strains of *N. ceranae* in resident honey bees (*A. mellifera iberiensis*). Their results revealed no specific genetic or virulence differences. Dussaubat *et al.* (2013) concluded that maybe the genetic origin of the honey bee was important in determining virulence since this could determine host susceptibility and how the honey bee responds to infection with *N. ceranae*.

In summary, recent experimental studies have specifically examined whether there is a difference in virulence between *N. apis* and *N. ceranae* in *Apis mellifera*. It appears there is no significant difference in virulence. Furthermore, there are no particular strains of *Nosema apis* or *N. ceranae* in any region of the world recognised as being more virulent than any other.

Accordingly, since no more pathogenic strains of *Nosema* spp. have been identified overseas, they are not identified as hazards.

8. Exotic organisms assessed as a negligible risk in 2004

8.1 VIRUSES

Apis iridescent virus, *Arkansas bee virus*, *Berkeley bee virus*, *Egypt bee virus*, *Slow bee paralysis virus* and *Thai sacbrood virus* have been assessed to have a negligible likelihood of being present in imported commodities (MAF 2004).

Slow bee paralysis virus is probably primarily a virus of (certain) bumble bee species, particularly *B. lapidaries* and *B. hortorum*, with honey bees probably secondary, occasional hosts since it is rarely, if ever, detected in honey bee surveys (McMahon *et al*. 2015; de Miranda *et al*. 2010).

Nevertheless, there is no new information on *Slow bee paralysis virus* or any of these viruses that alters the previous conclusion reached in 2004. Therefore, *Apis iridescent virus*, *Arkansas bee virus*, *Berkeley bee virus*, *Egypt bee virus*, *Slow bee paralysis virus* and *Thai sacbrood virus* are not identified as hazards.

8.2 SPIROPLASMAS

Spiroplasmas were assessed to have a negligible likelihood of being present in the imported commodities (MAF 2004). Since there is no new information, spiroplasmas are not identified as hazards.

8.3 PROTOZOA

Although the causative agent of gregarine disease could be associated with honey bee products, the consequences of introduction were assessed as negligible (MAF 2004).

There is no new information that alters the negligible consequence assessment. Therefore, the protozoa that cause gregarine disease require no further assessment.

9. Hazards identified in MAF's 2004 bee products risk analysis

The hazards assessed to be risks in the 2004 risk analysis were:

- American foulbrood
- European foulbrood
- Bee louse
- Small hive beetle
- Parasitic mites of the family *Varroidae*

American foulbrood

The recently described genotypes of American foulbrood are of potential concern and a risk assessment has been carried out. Further, in view of the new information on American

foulbrood, updated risk management measures have been proposed which differ from the 2004 risk analysis (see Chapter 19 of this risk analysis for full details).

European foulbrood

As far as variation in virulence of *Melissococcus plutonius* is concerned (causative agent of European foulbrood), there appears to be some recent evidence that suggests different strains exist across the globe, possibly with variations in pathogenicity.

Additionally, there is new information on quantitative PCR methods being developed which, unlike traditional PCR methods, may allow quantification of pathogen load.

Since there is additional information on *Melissococcus plutonius*, Chapter 20 of this risk analysis has been dedicated to this organism.

Arthropod parasites

For the arthropod parasites, *Braula coeca* (bee louse) *Aethina tumida* (small hive beetle) and parasitic mites of the family *Varroidae*, the following summarizes the new information since 2004.
Recently, infestation with *Aethina tumida* (small hive beetle) has been listed by the OIE within bee diseases, infections and infestations. Moreover, the *Code* makes recommendations for the safe trade in bees and bee products to prevent small hive beetle being introduced into an importing country. The internationally agreed recommendations set out in the *Code* reflect the risk recommendations made in MAF's 2004 honey bee products risk analysis.

For *Braula coeca* (bee louse), there is little new information. The recent literature reports that *Braula* is found nearly worldwide, but that they are not harmful to honey bees and of no economic importance (Ebejer 2012; Ellis 2008). Nevertheless, the conclusion of MAFs 2004 non-negligible consequence assessment remains valid. This is because comb honey products could aesthetically be damaged by tunneling larvae potentially causing economic loss.

Therefore, the new information does not alter the previous conclusions reached in the MAF risk analysis of 2004.

For parasitic mites of the family *Varroidae*, *Varroa destructor* is of economic importance due to the impact on colony health. It is considered the major pest of honey bees and no other pathogen has so far been a greater threat to apiculture (Mondet and Le Conte 2014).

MAF's 2004 honey bee products risk analysis examined all the parasitic mites of the family *Varroidae*. There is no new information that alters the conclusions or risk management recommendations made.

In conclusion, there is no new evidence that would indicate that the risk management recommendations in MAF's 2004 bee products risk analysis for the arthropod parasites are not appropriate. As noted above, the recently described genotypes of *Paenibacillus larvae* (causative agent of American foulbrood) and European foulbrood will be subject to further assessment (see chapters 19 and 20).

10. Preliminary hazard list

Taking into account the elimination of endemic and exotic organisms through Sections 7, 8 and 9, the following organisms are identified as preliminary hazards:

Viruses

Aphid lethal paralysis virus
Big Sioux River virus
Israeli acute paralysis virus
Lake Sinai virus 1 and 2
Kakugo virus
Varroa destructor virus-1
Turnip ringspot virus and *Turnip yellow mosaic virus*
Bee macula-like virus

Bacteria

Paenibacillus larvae (recently described molecular genotypes)
Melissococcus plutonius (new strains recently described)

Arthropod parasites

Apocephalus borealis

Protozoa

Apicystis bombi
Crithidia mellificae

All organisms that comprise the preliminary hazard list will be subject to hazard identification.

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11. Aphid lethal paralysis virus

11.1. HAZARD IDENTIFICATION

11.1.1. Aetiological agent

Runckel *et al.* (2011) detected a nucleotide sequence that closely aligned with *Aphid lethal paralysis virus* (ALPV) (named ALPV strain Brookings) from honey bees.

ALPV belongs to the family *Dicistroviridae*, genus *Cripavirus* (International Committee on Taxonomy of Viruses 2014).

The ALPV sequences discovered by metagenomics techniques are not dissimilar enough from the reference strain to meet the International Committee on Taxonomy of Viruses criteria as being new dicistroviruses. They therefore represent different isolates of ALPV and not new virus species (Liu *et al.* 2014).

11.1.2. OIE list

ALPV infection of honey bees is not listed.

11.1.3. New Zealand status

ALPV is not listed as an unwanted organism and there is no record of it being present in this country.

However, ALPV is a common intestinal virus of a number of aphid species, including *Rhopalosiphum padi*, *Metopolophium dirhodum*, and *Myzus persicae* which are present in New Zealand (Teulon *et al.* 1999).

Accordingly, since aphid vectors are present, ALPV is likely to be present.

11.1.4. Epidemiology

From honey bees in the United States of America, Runckel *et al.* (2011) detected a nucleotide sequence that closely aligned with ALPV (named ALPV strain Brookings). ALPV is common in aphids but had not previously been reported in association with honey bees.

ALPV was infrequently detected and at very low levels in adult honey bees throughout the year, with an increase during late summer when bees often feed on honeydew (secreted by aphids) during low nectar flows (Runckel *et al.* 2011; de Miranda *et al.* 2013).

The discovery of ALPV strain Brookings resulted from next generation sequencing technologies that have revolutionised methods for the discovery and identification of new viruses from insects (Liu *et al.* 2011). Runckel *et al.* (2011) screened RNA from honey bees against a comprehensive database (GenBank) that contains nucleotide sequences for all known insect viruses.

New molecular techniques (such as metagenomic surveys, arthropod pathogen microarrays and ultra-deep sequencing), are powerful means to detect genetic material from numerous viruses within honey bees and for describing new viruses (Benson *et al.* 2013; Liu *et al.* 2011).

Nevertheless, although next generation sequencing technologies have profoundly changed the methodology for virus discovery in insects, interpreting results obtained from such metagenomic surveys requires caution, as there are limitations to this technology. For instance, there are no standard methods for creating the sequences from extracted RNA and interpreting the results obtained from querying the generated sequences against GenBank or other genetic databases. Different researchers have used different approaches for data mining viral sequences that hit sequences in the genetic databases. Therefore, since there are no clearly established guidelines for the use of this technology, investigators applying different techniques may cause detection of different viruses (Liu *et al.* 2011).

Further, next generation sequencing cannot identify novel viruses that lack homology to known viruses (Liu *et al.* 2011). This inadequacy is important since there are a number of previously identified viruses of honey bees that have been described based on serology and electron microscopy (Bee virus X, Bee virus Y, Arkansas bee virus and Berkeley bee virus) for which no nucleic acid sequence information is available. Without these nucleic acid sequences, it is not possible to investigate whether one of these previously described viruses may in fact be ALPV (Runckel *et al.* 2011).

Accordingly, since there is no nucleic acid or serological reagent to explore this possibility, it is inconclusive as to whether ALPV is indeed a new discovery in bees.

Since ALPV is common in aphids and because bees may seasonally acquire the virus when feeding on contaminated honeydew, it cannot be determined whether ALPV is incidental or truly infectious to bees (de Miranda *et al.* 2013). Detection of viral sequences is not of itself sufficient evidence to show infection or replication of the virus in the host (Liu *et al.* 2011).

In any case, the detection of ALPV in honey bees seems of no significance since infection in bees is subclinical. Runckel *et al.* (2011) consider that further investigation is required to determine whether ALPV is a honey bee pathogen. However, no such work has yet been published.

Recently, Ravoet *et al.* (2013) and Granberg *et al.* (2013) also detected ALPV nucleic acid in bees in Belgium and Spain respectively using the new molecular techniques that Runckel *et al.* (2011) applied in the United States of America. Similarly, no clinical signs were observed in the infected honey bees in Belgium or Spain.

11.1.5. Hazard identification conclusion

Whether ALPV is exotic to New Zealand is not conclusively known. However, since aphid vectors are present, ALPV is likely to be present.

Honey bee products have not been implicated as a medium for the transmission of ALPV.

It is not known whether ALPV is incidental or truly infectious to honey bees. The available evidence indicates that it is an aphid virus and not a true pathogen of honey bees. Indeed, there have been no reports of ALPV causing disease in honey bees.

For the above reasons, ALPV is not identified as a hazard in honey bee products.

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12. Big Sioux River virus

12.1. HAZARD IDENTIFICATION

12.1.1. Aetiological agent

Big Sioux River virus (BSRV) has been reported as being genetically similar to *Rhopalosiphum padi virus* (RhPV) (Runckel *et al.* 2011).

RhPV is a common intestinal *Dicistrovirus* of aphids (D'Arcy *et al.* 1981) in the genus *Cripavirus*. BSRV has also been detected in the aphid *Rhopalosiphum padi* (Pal 2014).

BSRV is claimed to be a novel virus and named after its place of discovery (Runckel *et al.* 2011). However, BSRV is not officially recognised as a species of *Cripavirus* (International Committee on Taxonomy of Viruses 2013).

Since BSRV is not officially recognised as a species, it appears to be most closely related to, and synonymous with RhPV.

12.1.2. OIE list

BSRV infection of honey bees is not listed.

12.1.3. New Zealand status

Runckel *et al.* (2011) describe Big Sioux River virus (BSRV) as being genetically most similar to *Rhopalosiphum padi* virus (RPV), a common intestinal dicistrovirus of several aphid species including *R. padi, R. maidis* and *R. rufiabdominalis,* which are present in New Zealand (D'Arcy *et al.* 1981).

BSRV has also been detected in the aphid *Rhopalosiphum padi* (Pal 2014).

Neither BSRV nor RPV have been reported in New Zealand. These viruses are not listed on the unwanted organisms register.

Nevertheless, since aphid vectors are present and carry these viruses without clinical signs, BSRV and RPV could also be present.

12.1.4. Epidemiology

In the United States of America, Runckel *et al.* (2011) utilised a new molecular technique that detected BSRV in honey bees. This involved screening bee samples against a comprehensive database (GenBank) containing nucleotide sequences for all known insect viruses (Runckel *et al.* 2011). These new molecular techniques (described as metagenomic surveys, arthropod pathogen microarrays and ultra-deep sequencing) are powerful means to detect genetic material from numerous viruses within bees and for describing new viruses [somewhat related to an already known genetic sequence] (Benson *et al.* 2013; Liu *et al.* 2011).

Runckel *et al.* (2011) describe BSRV as being genetically most similar to *Rhopalosiphum padi* virus (RPV). RPV is a common intestinal virus of several aphid species whereby the plant vascular system is utilised to transmit infection horizontally between aphids (de Miranda *et al.* 2013). BSRV RNA has been detected in the aphid species *Rhopalosiphum padi* (Pal *et al.* 2014) and based on its genetic similarity to RPV, presumably, BRSV is a virus of aphids and its epidemiology is not dissimilar to RPV.

In adult honey bees, BSRV was detected infrequently at very low levels throughout the year, with a sharp increase during late summer when bees often feed on honeydew (aphid excreta) during low nectar flows. This indicates that since BSRV is probably common in aphids and because bees seasonally acquire the virus when feeding on contaminated honeydew, it cannot be determined whether BSRV is incidental or truly infectious to bees (de Miranda *et al.* 2013).

Moreover, interpreting results obtained from next generation molecular surveys requires caution since there are limitations to this technology. The previous chapter on aphid lethal paralysis virus has a more indepth discussion on these limitations.

In particular, a significant inadequacy of this technology is that next generation sequencing cannot identify novel viruses that lack homology to known viruses (Liu *et al.* 2011). In honey bees, there are a number of previously identified viruses described that are based on serology and electron microscopy (e.g. Bee virus X, Bee virus Y, Arkansas bee virus and Berkeley bee virus) for which no nucleic acid sequence information is available.

Therefore, it is inconclusive as to whether BSRV is indeed a new discovery in honey bees.

It is thought that BSRV may be related to Berkeley bee virus (de Miranda *et al.* 2013). However, without nucleic acid sequences, it is not possible to investigate whether Berkeley bee virus or another previously described bee virus is in fact BSRV or RPV.

Nevertheless, the detection of BSRV in honey bees seems to be of no significance since infection in honey bees is not associated with any noticeable clinical signs of disease (Runckel *et al.* 2011).

12.1.5. Hazard identification conclusion

BSRV has not been reported in New Zealand. However, since aphid vectors are present, BSRV and RPV could also be present.

BSRV is reported as being genetically most similar to *Rhopalosiphum padi* virus (RPV) of aphids. However, due to limitations in the molecular technique applied, it is inconclusive as to whether BSRV is indeed a new discovery in honey bees.

Further, it is not known whether BSRV is incidental or truly infectious to honey bees. Based on the genetic similarity to RPV, and the temporal association with bees feeding on honey dew (aphid excreta), the evidence indicates honey bees are incidentally infected.

Honey bee products have not been implicated as a medium for the transmission of BSRV and there are no reports of BSRV causing disease in bees.

Therefore, BSRV is not identified as a hazard in honey bee products.

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13. Israeli acute paralysis virus

13.1. HAZARD IDENTIFICATION

13.1.1. Aetiological agent

Israeli acute paralysis virus (IAPV) is a single-stranded RNA virus recently added to the genus Aparavirus, which is a member of the family Dicistroviridae (International Committee on Taxonomy of Viruses 2013).

IAPV was incidentally identified in 2004 as a consequence of virus propogation in white-eye honey bee pupae, as were the closely related acute bee paralysis virus (ABPV) and Kashmir bee virus (KBV) (Maori et al. 2007; de Miranda *et al*. 2010).

IAPV, ABPV and KBV naturally have high mutation rates and are recent descendents or variants that can be best described as a complex of related species (de Miranda *et al*. 2010).

13.1.2. OIE list

IAPV, ABPV and KBV infections of honey bees are not listed.

13.1.3. New Zealand status

A survey for IAPV carried out during 2010 and 2011 did not detect the presence of IAPV (McFadden *et al*. 2014). Honey bee samples were tested from 1050 hives from 499 apiaries. The power of the study was such that there was a 92% probability of detecting the virus if it was present at a 65% colony prevalence in 1% of apiaries.

Such specific surveys to assess the probability of the absence of bee diseases are rarely carried out. Nevertheless, a recent United Kingdom survey detected IAPV, but at an extremely low prevalence of only one colony out of 16,500 colonies screened. The affected colony did not show any clinical signs of infection (Budge 2012).

As with the United Kingdom, the varroa mite is present in New Zealand. Since the varroa mite vectors ABPV, KBV and IAPV (de Miranda *et al*. 2010), it is possible that IAPV could be present here but at a lower prevalence than was detectable given the power of the New Zealand survey carried out.

13.1.4. Epidemiology

IAPV was initially described in Israel in 2002 from a single dead honey bee (de Miranda *et al*. 2010; Maori *et al*. 2007). IAPV is very similar to two closely related honey bee viruses already present in New Zealand (ABPV and KBV). It appears that IAPV is more closely related to KBV than to ABPV (Cox-Foster *et al*. 2007).

However, IAPV has been shown retrospectively to have been circulating in United States of America since at least 2002 (Chen and Evans 2007).

IAPV is reported to have a worldwide distribution, having been reported in North America, Israel, Russia, France, Spain, United Kingdom, China, and Australia (de Miranda *et al*. 2010;

Runckel *et al*. 2011; Budge 2012; Granberg *et al*. 2013). Recent genetic analysis of bee virus isolates that have been considered to be KBV (from Australia, France and Russia) suggests that many viruses that have been classified as KBV in the past may in fact be IAPV (Palacios *et al*. 2008). Caution is required when diagnosing the presence of these viruses by PCR since the original primers designed in 1995 are based on KBV, but continue to be used widely. Therefore, there is the potential for cross-amplification of related viruses when using these primers (Baker and Schroeder 2008; de Miranda *et al*. 2010).

To investigate whether other pollinators might be hosts for IAPV, Singh *et al*. (2010) housed colonies of honey bees and bumble bees together in experimental greenhouse rooms. The honey bee colonies were experimentally infected by feeding sugar solutions containing IAPV. About a week after co-habitation and co-foraging the infected honey bees with bumble bees, the bumble bees began returning IAPV PCR positive results. There was no mention of clinical disease being expressed in honey bees or bumble bees during the time it took to carry out the experiment.

Further, two bumble bee species and some paper wasps collected from flowering plants near IAPV PCR positive honey bee apiaries were also found to be IAPV PCR positive (Singh *et al*. 2010).

Nevertheless, the reports of detection of IAPV nucleic acid sequences from bumble bees under the two scenarios described by Singh *et al*. (2010) shows foraging may act as a common source of exposure to IAPV, but does not demonstrate cross-species transmission of IAPV from honey bees to bumble bees (or vice versa).

Levitt *et al*. (2013) present molecular evidence that IAPV replicates in Bombus impatiens but what this means for virus transmissibility is not known. IAPV has recently been shown to be a truly infectious agent of bumble bees, capable of causing disease (Meeus *et al*. 2014; Piot *et al*. 2015). Further, ABPV and KBV (close relatives of IAPV) have also been naturally found in a variety of bumble bee species (McMahon *et al*. 2015; Meeus *et al*. 2014).

Based on molecular evidence, Yanez *et al*. (2012) suggests IAPV could be replicating in *Vespa velutina* (hornet wasps which are predators of honey bees) in China.

Die-offs of honey bees in the United States of America were reported to begin in late 2006. In September 2007, Cox-Foster *et al*. (2007) reported that IAPV was strongly correlated with the die off phenomenon that came to be known as colony collapse disorder (CCD) (United States Department of Agriculture 2013).

There is no clear definition of CCD and its main manifestation is simply no or a low number of adult honey bees present but with a live queen and no dead honey bees in the hive. Often there is still honey in the hive, and immature bees (brood) are present (United States Department of Agriculture 2013; VanEngelsdorp *et al*. 2009). American beekeepers routinely lose about 30-35% of bee colonies each winter, and about 30% of these losses are thought to be due to CCD (United States Department of Agriculture 2013). In recent years, American beekeepers are still facing concerning losses, but without the typical CCD manifestations described above (Kaplan 2015).

Although IAPV was initially identified as a predictive marker for colony losses, expanded studies could not confirm this result (United States Department of Agriculture 2013). For

instance, vanEngelsdorp *et al*. (2009) reported that IAPV was scarce in both normal and collapsed colonies whereby no single pathogen could be identified as being consistently associated with collapse. Likewise, Cornman *et al*. (2012) also reported that IAPV was not associated with colony collapse disorder.

A retrospective study revealed that IAPV was already present in the United States of America, long before the first reports of colony collapse disorder (Chen and Evans 2007; Ravoet *et al.* 2013). Runckel *et al*. (2011) infrequently detected IAPV nucleic acid in bees in the United States of America, but no clinical signs of disease were associated with infection. Granberg *et al*. (2013) also detected IAPV nucleic acid in bees in Spain and similarly, no clinical signs were observed in the infected honey bees.

Although a number of factors continue to be associated with CCD, including parasites and pathogens, poor nutrition, pesticides, bee management practices, habitat fragmentation, and agricultural practices, no single factor or pattern of factors has been proven to be "the cause" of CCD (United States Department of Agriculture 2013). Nevertheless, a consensus is emerging that CCD is a combination of poor nutrition, varroa infestation and a number of viruses transmitted by varroa (Mutinelli 2011).

The study of RNA viruses of honey bees is difficult since some are extremely fragile and do not survive long outside live bees. As cell culture systems for these viruses are not available they cannot be grown in research laboratories. The same PCR methodology applied in Israel was employed in the testing of CCD-affected colonies in the United States of America, leading to the identification of IAPV there.

However, as further work was undertaken following the initial diagnosis of IAPV in the United States of America, it emerged that there are several strains of IAPV, and several clades of this virus appear to be cycling in North America (Palacios *et al*. 2008). In the United States of America there appears to be one strain of IAPV in western States and two strains in eastern States, one of which is very similar to that found in Australian bees (Cox-Foster *et al*. 2008).

In Israel in 2002, where IAPV was first identified, it was reported to be associated with honey bees exhibiting behaviour such as twitching wings outside the hive and a loss of worker bee populations. These signs are very similar to those associated with a number of other bee viruses, especially in countries where the varroa mite is present.

Experimentally, IAPV is reported to be highly virulent with about 80% of adult bees infected by the oral route or by injection dying within 7 days (de Miranda *et al*. 2010). Apart from the original report of clinical disease associated with IAPV from Israel in 2004, there has also been a large scale field experiment with colony-level infection of IAPV, as part of a clinical trial of a novel anti-IAPV treatment (Hunter *et al*. 2010) which clearly showed the negative impact of IAPV infection also at the colony level. These and similar studies with IAPV's sister species ABPV and KBV show that this complex of related viruses can cause elevated bee mortality also outside the laboratory, when present at high enough incidence and titre, conditions that are readily met through varroa-mediated transmission (Di Prisco *et al*. 2011).

The transmission routes and epidemiology of IAPV are considered to be the same as for KBV and ABPV (de Miranda *et al*. 2010). All these viruses persist predominantly as subclinical infections unless in association with varroa mite that may act as vector and amplifier of these

viruses whilst providing a means of transmission by the injection route (Shen et al. 2005; Genersch and Aubert 2010; de Miranda *et al*. 2010; Mutinelli 2011). KBV is recognised as the most virulent of the honey bee viruses (Anderson and Gibbs 1988; Todd *et al*. 2007).

There is no consistent information on the persistence and viability of honey bee RNA viruses outside the bee host. When isolated in the laboratory, purified viruses gradually lose their viability and infectivity, with some viruses (ABPV, KBV, IAPV among them) more resistant to such loss of viability than others (such as DWV) (Bailey *et al*. 1979; Anderson 1986).

However, considering the physical and biochemical processes that honey is subjected to during and after extraction, it is very unlikely that extracted honey would harbour infective virus. Similarly, considering the standard processing of bee venom, propolis, pollen and beeswax, it is also unlikely that viable IAPV would be present in these imported honey bee products at high enough concentrations to be considered capable of initiating infection in exposed honey bees.

The detection by RT-PCR of IAPV RNA in fresh refrigerated royal jelly from China (Cox-Foster *et al*. 2007) raises the possibility of a more realistic infection risk. Although the detection method used (RT-PCR) is unsuitable for establishing virus viability or infection risk, the commodity itself (royal jelly) is very much a possible, even a likely carrier of viable bee viruses. This is because many honey bee viruses, including the Iflarviruses and the Dicistroviruses of which IAPV is a member, replicate in the honey bee brain, from where they are actively secreted into the hypopharyngeal and salivary secretions that become royal jelly (Chen *et al*. 2014), with which the young larvae and particularly the queen larvae are fed. Thus, through the royal jelly secretions these viruses are transmitted to the young honey bee larvae as part of the oral transmission pathways by which these viruses persist within the honey bee colony. Royal jelly is a big part of virus epidemiology within the hive (de Miranda 2016).

13.1.5. Hazard identification conclusion

IAPV has not met the criteria for listing within the OIE list of honey bee diseases.

However, IAPV has the potential to be pathogenic and infectious to honey bees and bumble bees. In association with varroa-mediated transmission, it may be lethal at the colony level, just like its sister species ABPV and KBV (de Miranda 2016).

Accordingly, IAPV is identified as a hazard in honey bee products.

13.2. RISK ASSESSMENT

13.2.1. Entry assessment

There is no consistent information on the persistence and viability of honey bee RNA viruses outside the bee host.

However, considering the physical and biochemical processes that honey is subjected to during and after extraction, it is very unlikely that extracted honey would harbour infective virus. Similarly, considering the standard processing of bee venom, propolis, pollen and

beeswax, it is also unlikely that viable IAPV would be present in these commodities at high enough concentrations to be considered capable of initiating infection in exposed honey bees.

IAPV RNA has been detected by RT-PCR in fresh refrigerated royal jelly from China (Cox-Foster et al. 2007). Royal jelly is recognised as a possible, even a likely carrier of viable bee viruses (de Miranda 2015).

IAPV is orally infectious to young honey bees (larvae and adults) this requires extremely high titres of virus unlikely to be ever found in any honey bee products with the exception of royal jelly.

Accordingly, the likelihood of IAPV entering with royal jelly is assessed to be moderate to high.

In view of the physical and biochemical processes honey bee products have undergone, the likelihood of entry is assessed to be very low for honey, propolis, pollen, bee venom and beeswax.

13.2.2. Exposure assessment

Honey is imported for human consumption and not as a feed for honey bees. The high purchase and shipping costs preclude the use of honey as feed for honey bees (Shimanuki and Knox 1997; Mutinelli 2011). The practice of exposing hives to imported honey would be an unlikely event since beekeepers do not generally feed honey of an unknown source to their hives.

Further, honey, propolis, pollen, bee venom and beeswax have not been implicated as a source of IAPV transmission when trading these products. Further, taking into account the conclusion of the likelihood of entry assessment above, the likelihood of these imported honey bee products being deliberately exposed to New Zealand hives and having viable IAPV at a titre that could initiate infection is assessed to be negligible.

Accordingly, the exposure assessment is negligible for honey, propolis, pollen, bee venom and beeswax.

In regards royal jelly, it is mostly imported for use as a food supplement for humans (Mutinelli 2011) or for use in cosmetics. However, it is also a common and key component of the honey bee queen rearing industry, where it is used to seed queen cups for the subsequent grafting of honey bee larvae destined to become new queens. It is through this route that, if any viable viruses are present, exposure of New Zealand's honey bee population may occur.

Royal jelly may contain titres of IAPV capable of transmitting infection and has a high likelihood of exposure since it is purposefully fed to honey bee larvae.

Accordingly, the exposure assessment for royal jelly is assessed to be high.

13.2.3. Consequence assessment

In view of the entry and exposure assessments above, only royal jelly is assessed to have the potential to cause harmful consequences. Negative consequences to honey bee health could result due to exposure of imported royal jelly to hive larvae for the purposes of queen rearing.

In a hive infected with IAPV, clinical disease (and ABPV, KBV) is simply highly elevated mortality which is difficult to observe, since the colony simply replaces the lost honey bees (de Miranda 2016). Nevertheless, IAPV has the potential to be pathogenic and infectious to

honey bees and bumble bees. In association with varroa-mediated transmission, it may be lethal at the colony level, just like its sister strains ABPV and KBV (de Miranda 2016).

Since there is a direct exposure pathway into hives when importing royal jelly, it is likely there would be ensuing harmful consequences if the commodity is harbouring IAPV at an infectious dose.

This notwithstanding, no evidence could be found that honey bees or bumble bees naturally infected with IAPV experience more pathogenic effects than the closely related ABPV or KBV (present in New Zealand). Indeed, the available evidence indicates that the consequences of introducing IAPV would not be very different to those of ABPV and KBV.

In regards the phenomena of CCD being caused by IAPV, this was a speculative association, made without convincing evidence to support the claim. The initial suggestions that IAPV may be a possible cause of CCD in the United States of America (Cox-Foster *et al*. 2007) are now recognised to be incorrect. From literature review and consultation with honey bee experts during the development of this risk analysis, it is concluded that there is no causal link to CCD.

There are no public health consequences since humans are not susceptible to infection with IAPV.

In view of the above, the consequences for importing IAPV contaminated royal jelly are assessed to be low.

13.2.4. Risk estimate

For royal jelly, the likelihood of entry, exposure and consequences are assessed to be non-negligible.

As a result, the risk estimate is non-negligible and IAPV is assessed to be a risk in royal jelly. As the risk is non-negligble for this commodity, the following risk management measures have been identified for risk managers to consider.

For bee venom, propolis, pollen, beeswax and honey, the likelihood of IAPV entering with these commodities is very low and the exposure assessment is negligible. Thus, the risk estimate is negligible for these commodities. Therefore, no risk management measures are justifiable for honey, beeswax, propolis, pollen or bee venom.

13.3. RISK MANAGEMENT

The following points were considered when drafting risk management options for IAPV in royal jelly:

- IAPV is not an OIE-listed disease. There are no international standards recommended by the OIE for the safe trade in honey bee products. Further, the virus has a worldwide distribution, having been reported in North America, Israel, Russia, France, Spain, United Kingdom, China, and Australia (de Miranda *et al*. 2010; Runckel *et al*. 2011; Budge 2012; Granberg *et al*. 2013). There are no countries or zones internationally recognised as free from IAPV.

- The standard measure used in Australia to prevent European foulbrood (EFB) transmission between Eastern Australia (infected with EFB) and Western Australia (EFB free State) when trading honey is to apply heat treatment at 65°C for 8 hours. MPI commissioned a research study (carried out in the UK) specifically designed to examine thermal inactivation of EFB and IAPV in honey. The results of this study demonstrate that this time temperature combination inactivates IAPV. If this temperature and time duration were applied to royal jelly, it could reasonably be assumed that IAPV would be inactivated.

- Since royal jelly could be specifically imported with the intention of being fed to honey bee larvae, other risk management options could be implemented to prevent this practice from occurring. That is, allowing the importation of royal jelly only for use in industries where there would be no exposure to honey bees or bumble bees. For example, the cosmetics and human health industries.

- Each batch of the commodity to be imported could be tested to show that it is free from IAPV. However, there are no internationally recognised diagnostic tests for IAPV in royal jelly. Further, caution is required when diagnosing the presence of IAPV by PCR since the original primers designed in 1995 are based on KBV, but continue to be used widely. Therefore, there is the potential for cross-amplification of related viruses when using these primers (Baker and Schroeder 2008; de Miranda *et al*. 2010). Finally, a positive PCR result does not indicate whether the virus is still viable or not and whether or not it is present at a titre that could pose a transmission risk. Suitable tests for use in royal jelly for showing freedom from IAPV for trading purposes, may be under development and be available in the future. Such tests could be used after bilateral agreement.

13.3.1. Risk management options

For royal jelly

One or a combination of the following measures could be considered to effectively manage the risk:

Each consignment could be either:

(1) Directed into a Transitional Facility where it is packed into a form that could not expose honey or bumble bees. Including for instance, but not restricted to, capsules for human consumption or for use as an ingredient in cosmetics.

or

(2) Been subjected to a heat treatment at a temperature of 65°C for 8 hours (or an equivalent treatment) to inactivate IAPV.

or

(3) Subjected to an agreed diagnostic test for IAPV with negative results shown.

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14. Lake Sinai viruses

14.1. HAZARD IDENTIFICATION

14.1.1. Aetiological agent

A mass metagenomic sequencing survey of honey bees detected two closely related nucleotide sequences that did not match any sequences in GenBank. The two novel sequences were named *Lake Sinai virus-1* (LSV-1) and *Lake Sinai virus-2* (LSV-2) (Runckel *et al.* 2011).

However, the Lake Sinai viruses are not officially classified (International Committee on Taxonomy of Viruses 2014).

Even so, de Miranda *et al.* (2013) deduce that the Lake Sinai viruses' genome organisation and sequences place them with chronic bee paralysis virus, in a unique family somewhere between the Nodaviridae and Tombusviridae.

14.1.2. OIE list

Infection of honey bees with Lake Sinai viruses is not listed.

14.1.3. New Zealand status

Lake Sinai viruses (LSVs) are not listed as unwanted organisms. Using molecular techniques, multiple LSV strains have recently been confirmed as present in New Zealand, although the detection has not yet been published (Mackay 2015).

14.1.4. Epidemiology

The LSVs are closely related to one another, being first identified through a mass metagenomic sequencing survey of honey bee colonies in the United States of America (Runckel *et al.* 2011).

Cornman *et al.* (2012) similarly detected strains of LSV in bees in the United States of America using metagenomics but could not determine the significance of their presence on colony health. Most recently, Granberg *et al.* (2013) and Ravoet *et al.* (2013) also using metagenomic approaches, described the first detections of nucleotide sequences similar to LSV in honey bees in Spain and Belgium.

LSVs appear distantly related to chronic bee paralysis virus, at family level (de Miranda 2016). They could also be part of those honey bee viruses that have been historically characterised through serology, microscopy and physico-chemical parameters, but for which no genetic comparison can be made (Bee virus X and Y, Arkansas bee virus and Berkeley bee virus) (Runckel *et al.* 2011; de Miranda *et al.* 2013; Granberg *et al.* 2013).

LSV-1 is reported to be more common than LSV-2. Each appears to reach peak incidence at differing times of the year, with LSV-1 common and present throughout the year with high

titres and incidence in early summer. Similarly, LSV-2 is also present throughout the year, but it has a peak incidence in late winter (de Miranda *et al.* 2013).

Despite being newly identified through metagenomics screening (Runckel *et al.* 2011), LSV-1 and LSV-2 have also been detected, with similar incidences and titres, in historical European honey bee samples (de Miranda *et al.* 2013). There are a number of similarities between the predicted physico-chemical properties of LSV-1 and LSV-2 and those of the previously described Bee virus X and Y. Additionally, there are similarities in seasonal incidences that suggest that LSV-1 and LSV-2 could be the same as the previously described Bee virus X and Y. Runckel *et al*. (2011) have acknowledged this possibility.

Bee virus Y and X are well known historical viruses of honey bees that are present in New Zealand. They are not known to cause significant disease in honey bees.

Despite several investigators detecting LSV in the United States of America and Europe, there have been no recognisable impacts on honey bee health with no clinical signs of infection reported in infected honey bees and no noticeable effects on colony winter losses (Runckel *et al.* 2011; Ravoet *et al.* 2013; Granberg *et al.* 2013).

14.1.5. Hazard identification conclusion

The evidence to support LSVs being truly novel viruses of honey bees is incomplete. There have been no tests conducted to establish whether these viruses had not already been described but in a different pretext. Considering the available evidence, there is reasonable circumstantial evidence to conclude that LSV-1 and LSV-2 may in fact prove to be Bee virus X and Y (de Miranda *et al.* 2013). Both viruses have already been shown to be present in New Zealand.

Additionally, multiple strains of Lake Sinai virus have recently been detected in New Zealand using molecular techniques (Mackay 2015).

Further, LSVs belong to an unclassified group of viruses. There is no reliable information as to their possible impacts on individual honey bee or colony health. However, they are not recognised pathogens of honey bees and are not OIE-listed.

Finally, no information could be found whether honey bee products facilitate the transmission and spread of LSVs.

Accordingly, LSVs are not identified as a hazard in honey bee products.

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15. Kakugo virus

15.1. HAZARD IDENTIFICATION

15.1.1. Aetiological agent

Fujiyuki *et al.* (2004) identified a novel RNA nucleotide sequence (named Kakugo RNA) in honey bees that encodes for a protein with structural features and sequences similar to insect picorna-like viruses.

Kakugo virus is not officially recognised by the International Committee on Taxonomy of Viruses (International Committee on Taxonomy of Viruses 2014). It did not satisfy the criteria for unique species status and is best considered to be a strain of deformed wing virus (de Miranda 2016).

15.1.2. OIE list

Kakugo virus infection of honey bees is not listed.

15.1.3. New Zealand status

Kakugo virus has not been reported and it is not listed as an unwanted organism.

15.1.4. Epidemiology

Kakugo virus RNA was first described by Fujiyuki *et al.* (2004) whilst screening for genes associated with aggression in healthy Japanese honey bees (Fujiyuki *et al.* 2004; de Miranda and Genersch 2010).

Kakugo virus was detected at elevated levels in the brains of guard honey bees attacking a dead decoy of the giant hornet (a natural enemy of bees). The detection of Kakugo virus RNA in the brain but not the thorax or abdomen was hypothesised to indicate a relationship between viral infection in the brain and aggressive worker bee behaviours (Fujiyuki et al. 2004).

Subsequent examination of brain tissues taken from naturally and experimentally infected bees with Kakugo virus (KV) determined that the virus was present in natural infections but only upon experimental infection was the virus detected in various brain regions, and also in the hypopharangeal glands and fat bodies suggesting systemic infection (Fujiyuki *et al.* 2009). The investigators again implied KV infection may affect brain functions or physiological states in honey bees (Fujiyuki *et al.* 2009). Despite this, the study could not discriminate KV-infected worker bees from uninfected bees in naturally maintained hives based on behavioural differences suggesting that, at the infectivity doses employed, KV does not greatly affect their behaviour.

Further, there was no effect on survival rate of honey bees experimentally inoculated with KV. Fujiyuki *et al.* (2009) concluded that at the dose used, KV infection does not cause elevated mortality in honey bees.

Terio *et al.* (2008) detected RNA from Italian bees about 95% comparable to the newly described Kakugo virus (KV) but more than 99% similar to deformed wing virus (DWV). The honey bee samples were taken from 5 hives that were presumed to be displaying increased aggressiveness. The assumption of increased aggressiveness was subjectively based on repeated attacks on humans, in the absence of any other apparent dangers. It would seem this means no additional threats were posed to the honey bees apart from the humans themselves. In any case, the sequence found had intermediate features between DWV and KV. Terio *et al.* (2008) termed their discovery a KV/DWV-like virus.

There are no reports of KV causing clinical disease. Lanzi *et al.* (2006) particularly note the absence of any clinical or behavioural signs in honey bees with KV RNA. Despite the close similarity with DWV, wing deformities have so far not been associated with KV (Fujiyuki *et al.* 2004; de Miranda and Genersch 2010). Wing deformities nearly always arise from infections during the pupal stage, in association with varroa infestation, which was not investigated in these studies. The original DWV isolate, extracted from deformed adult honey bees from Japan (Bailey and Ball 1991), is genetically practically identical to the Kakugo virus sequence (de Miranda and Genersch 2010), thereby establishing a link between confirmed adult symptoms and the KV sequence.

Additionally, Lanzi *et al.* (2006) also notes that the concentration of honey bee viruses such as KV in the hypopharangeal glands in honey bee brains is not an unexpected phenomenon. This is a recognised means in which honey bee viruses are transmitted to the larvae as part of the glandular secretions (royal jelly) to feed young larvae.

Phylogenetic analysis concluded that KV is not particularly unique (Lanzi *et al.* 2006). KV found in Italian honey bees is considered a geographic variant of DWV since the RNA was 97% similar to the original Japanese DWV reference sequence.

Lanzi *et al.* (2006) state it is clear that DWV, KV, and *Varroa destructor virus-1* (VDV-1) are variants of a single major virus, with little geographic differentiation between the European, American, and Japanese versions of KV since they are practically identical. Lanzi *et al.* (2006) contend that KV and possibly VDV-1 are biological and geographic variants of a virus that historically has been known as DWV.

From molecular characterisation and phylogenetic analysis, a South Korean isolate of DWV was reported to be very closely related to KV (96% similar) (Reddy *et al.* 2013).

Subsequent phylogenetic analysis suggests that the Kakugo sequence is very closely related to, but distinct from DWV and VDV-1 (Fujiyuki *et al.* 2006; Rortais *et al.* 2006; Berènyi *et al.* 2007).

15.1.5. Hazard identification conclusion

Different investigators have carried out several phylogenetic analyses on KV sequences. Results have been contradictory on the genetic phylogeny and classification of KV. However, there is a general consensus that KV is closely related to, and most likely a strain of, DWV.

Officially, KV is not recognised by the International Committee on Taxonomy of Viruses as a separate named entity (International Committee on Taxonomy of Viruses 2014).

Although DWV is associated with characteristic wing deformities, KV does not cause clinical signs of disease (Fujiyuki *et al.* 2004; Lanzi *et al.* 2006; de Miranda and Genersch 2010).

Behaviourally, Fujiyuki *et al.* (2004) originally inferred that there might be a relationship between viral infection in the brain and aggression in guard bees. However, further experimental investigation has not been able to confirm this hypothesis. In fact, inoculating bees with KV did not cause any adverse effects on bees (Fujiyuki *et al.* 2009).

Likewise, Lanzi *et al.* (2006) reported the absence of any behavioural or clinical signs in honey bees infected with KV.

Further, from expert consultation sought through the development of this risk analysis (see *Contributors to this risk analysis*, page 45), no compelling evidence has been able to confirm that KV causes aggression in honey bees.

Therefore, aggression in honey bees caused by KV seems a speculative association with no convincing evidence to support the claim.

Finally, KV is not an OIE listed disease and no evidence could be found that honey bee products facilitate the transmission and spread of this virus.

Accordingly, KV is not identified as a hazard in honey bee products.

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16. Varroa destructor virus-1

16.1. HAZARD IDENTIFICATION

16.1.1. Aetiological agent

Varroa destructor virus-1 is a species within the genus *Iflavirus*, family Iflaviridae (International Committee on Taxonomy of Viruses 2013).

16.1.2. OIE list

Varroa destructor virus-1 infection of honey bees is not listed.

16.1.3. New Zealand status

Varroa destructor virus-1 has not been reported and it is not listed as an unwanted organism.

16.1.4. Epidemiology

Ongus *et al.* (2004) extracted an RNA sequence from *Varroa destructor* mites in the Netherlands. By phylogenetic analysis, the sequence was determined to be most closely related to deformed wing virus (DWV) and Kakugo virus. Ongus *et al.* (2004) proposed the virus be named *Varroa destructor virus-1*.

Varroa destructor virus-1 (VDV-1) appears to be more host-specific to *V. destructor* than to bees (Ongus *et al.* 2006). However, DWV and VDV-1 replicate in varroa mites as well as in honey bees (Ongus *et al.* 2004). It is thought that these two viruses co-exist in bees and mites as part of the same species-complex (de Miranda and Genersch 2010; Gauthier *et al.* 2011). Both DWV and VDV-1 have been linked to wing and body deformities typically associated with DWV (Zioni *et al*. 2011) and they have been shown to naturally form viable recombinants (Moore *et al*. 2011; Wang *et al*. 2013; Zioni *et al*. 2011) with differences in virulence (Ryabov *et al*. 2014).

There is clear phylogenetic separation between VDV-1 and DWV (Lanzi *et al*. 2006; Berènyi *et al*. 2007; de Miranda and Genersch 2010).

In 2008, the International Committe on Taxonomy of viruses (ICTV) recognised VDV-1 as a species within the genus *Iflavirus*. This designation is based on the capsule protein sequence, which was about 82% similar to DWV. In accordance with the species demarcation criteria of the ICTV, (strains of a species have above 90% similarity in their capsule protein sequence) the capsule protein sequence of VDV-1 is sufficiently different from deformed wing virus for the creation of the new species (International Committee on Taxonomy of Viruses 2008).

Recently, a South Korean isolate of DWV was reported to be very closely related to VDV-1 (about 82% similar) (Reddy *et al.* 2013) which supports the ICTV determination.

Gauthier *et al.* (2011) reported viral particles in association with degenerating ovarian follicles in affected queen bees. Viral sequences that matched DWV and VDV-1 were recovered from the affected ovaries. Gauthier *et al.* (2011) concluded that these viruses are common in bees and that despite the association of these viruses with ovarian pathology they

were of low virulence. This was due to there being little effect on queen function or fitness, even at very high viral titres.

Moreover, since both functioning and non-functioning queens had very high viral titres, no association between the presence of VDV-1 or DWV and ovarian pathology or egg-laying deficiency was found. Gauthier *et al.* (2011) suggests that other factors are most likely involved in causing the ovarian pathology.

16.1.5. Hazard identification conclusion

Different investigators have carried out phylogenetic analyses on VDV-1 sequences. All of these clearly identify VDV-1 as genetically distinct from DWV, although there are different opinions as to whether these differences are sufficient to classify these viruses as distinct species or strains of a single species. The discovery of viable natural recombinants between the viruses in mixed infections supports the single-species hypothesis (Moore *et al*. 2011; Zioni *et al*. 2011; Ryabov *et al*. 2014).

Nevertheless, the International Committee on Taxonomy of Viruses currently recognises VDV-1 as distinct from DWV.

Further, from expert technical consultation sought through development of this risk analysis (see *Contributors to this risk analysis*, page 48), there is no compelling evidence to show that VDV-1 causes a unique disease in honey bees, substantially different from DWV. Both have been associated with typical wing deformities, varroa transmission and associated colony mortality.

Finally, VDV-1 is not an OIE listed disease of honey bees and no evidence could be found that honey bee products facilitate the transmission and spread of this virus.

Accordingly, VDV-1 is not identified as a hazard in honey bee products.

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17. Turnip ringspot virus and Turnip yellow mosaic virus

17.1. HAZARD IDENTIFICATION

17.1.1. Aetiological agent

Turnip ringspot virus (TuRSV) is the provisional name for what has been proposed as a novel *Comovirus* detected in a turnip. The genetic sequence detected in the turnip is most similar to radish mosaic virus (Rajakaruna *et al.* 2007).

However, TuRSV is not recognised by the International Committee on Taxonomy of Viruses. *Turnip yellow mosaic virus* (TuYMV) belongs to the genus *Tymovirus*, family Tymoviridae (International Committee on Taxonomy of Viruses 2014).

17.1.2. OIE list

TuRSV and TuYMV infection of honey bees is not listed.

17.1.3. New Zealand status

TuRSV and TuYMV have not been reported.

Further, these viruses of turnips are not listed as unwanted organisms.

17.1.4. Epidemiology

TuRSV and TuYMV genetic sequences were detected in Spanish honey bee samples through metagenomic techniques. No clinical signs of viral infection were noticed in the honey bees (Granberg *et al.* 2013).

These plant viruses are sap-transmitted or vectored through particular plant biting insects (for instance species of flea beetle in the genus *Phyllotreta* (Markham and Smith 1949).

The samples for genetic analysis were prepared from whole honey bees that had been homogenised in a blender (Granberg *et al.* 2013).

Honey bees are exposed naturally whilst foraging to plant viruses and to plant pollen and nectar collected or ingested by honey bees. Any viral residues from such exposure can be detected by metagenomic analysis.

Therefore, the detection of plant viruses associated with the honey bee homogenates is very likely to be incidental as a consequence of honey bees being in direct contact with plants.

Detection of nucleic acid sequences in honey bee homogenates does not provide evidence to conclude that insects can be infected with plant viruses.

17.1.5. Hazard identification conclusion

There is only the one report of TuRSV and TuYMV in association with healthy honey bees detected through metagenomic analysis of whole honey bee homogenates.

TuRSV and TuYMV are plant viruses and are not OIE listed diseases of honey bees. No evidence could be found that honey bee products facilitate the transmission and spread of these viruses.

Accordingly, TuRSV and TuYMV are not identified as hazards in honey bee products.

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18. Bee macula-like virus

18.1. HAZARD IDENTIFICATION

18.1.1. Aetiological agent

Bee macula-like virus (BeeMLV, formerly referred to as Varroa destructor Macula-like virus; VdMLV) is not officially recognised (International Committee on Taxonomy of Viruses 2014).

de Miranda *et al.* (2015) describes BeeMLV as belonging to the family Tymoviridae, and provides evidence that this virus is infectious in honey bees and possibly also in *Varroa destructor*.

18.1.2. OIE list

BeeMLV infection of honey bees is not listed.

18.1.3. New Zealand status

Neither *Varroa destructor* macula-like virus nor BeeMLV have been reported in New Zealand or are listed as unwanted organisms.

18.1.4. Epidemiology

BeeMLV is a newly reported virus in honey bees and varroa mites (de Miranda *et al.* 2015) with an extensive presence in French honey bees and mites (samples from 2002), as well as in *Varroa destructor* RNA from the USA. Ravoet *et al.* (2013) showed that Bee MLV was present in Belgian honey bees. In the Belgian bees, molecular evidence showed Bee MLV had a high prevalence and that it may perhaps be replicating in honey bees. Most recently, Erban *et al*. (2015) detected BeeMLV proteins in mites from the Czech Republic.

However, BeeMLV was not associated with clinical disease and there was no effect of its presence on colony winter losses (Ravoet *et al.* 2013).

Although a high prevalence was reported in Belgian honey bees, Gauthier *et al.* (2011) studied 30 queen bees from various French locations but did not detect VdMLV. Likewise, Strauss *et al.* (2013) studied 13 honey bee apiaries in the Gauteng region of South Africa over 14 months and did not detect VdMLV in either honey bees or varroa mites. The origin and epidemiology of BeeMLV are still unclear (de Miranda *et al.* 2015).

18.1.5. Hazard identification conclusion

Honey bees infected with VdMLV do not show clinical signs of disease.

Further, VdMLV is not an OIE listed disease of honey bees and no evidence could be found that honey bee products facilitate the transmission and spread of this virus.

Accordingly, VdMLV is not identified as a hazard in honey bee products.

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19. *Paenibacillus larvae*

19.1. HAZARD IDENTIFICATION

19.1.1. Aetiological agent

At the time of the 2004 MAF risk analysis on honey bee products, the causative agents of American foulbrood (AFB) and powdery scale disease were considered to be *Paenibacillus larvae* subsp. *larvae* and *P. larvae* subsp. *pulvifaciens* respectively.

Using PCR methods, Genersch *et al.* (2006) genotyped reference strains of *P. larvae* subsp. *pulvifaciens* and reference strains and field isolates of *P. larvae* subsp. *larvae*. The PCR genotyping technique called ERIC (enterobacterial repetitive intergenic consensus) utilises primers corresponding to highly conserved regions of the bacterial genetic sequences (Alippi *et al.* 2004; Genersch *et al.* 2005; de Graaf *et al.* 2006). The reference and field strains examined were from both clinically and subclinically infected hives (Genersch *et al.* 2006).

The results of the ERIC-PCR based genotyping led to the conclusion that the distinction between AFB and powdery scale disease caused by *Paenibacillus larvae* subsp. *larvae* and *P. larvae* subsp. *pulvifaciens* respectively was no longer valid (Genersch *et al.* 2006). Accordingly, these pathogens have been reclassified as one species (*P. larvae*) eliminating subspecies designations (Ashiralieva and Genersch 2006; de Graaf *et al.* 2006; Genersch *et al.* 2006).

However, from applying ERIC-PCR primers, Genersch *et al.* (2005) divided *P. larvae* into four different genotypes, *AB*, *Ab*, *ab* and *a*β. These were subsequently renamed as ERIC I, II, III, and IV (Genersch *et al.* 2006).

A recent account by Genersch (2010) explained the genotype nomenclature by equating the former subspecies *P. larvae larvae* as comprising the genotypes ERIC I and II. The former subspecies *P. larvae pulvifaciens* was designated as comprising *P. larvae* genotypes ERIC III and IV.

However, not all researchers have adopted the nomenclature described by Genersch (2010). For instance, *P. larvae* isolates from Italy were genotyped using ERIC-PCR and named ERIC A, B, C and D, whereas Bulgarian apiaries showing clinical signs of AFB revealed two genotypes that were named ab and AB. The prevailing genotype causing disease, ab, was detected in 78% of the Bulgarian strains (Rusenova *et al.* 2013). By extrapolating from the work carried out by Alippi *et al.* (2004), the investigators of the Bulgarian *P. larvae* strains concluded that their strains were indicative of, and could be referred to as ERIC I and II.

However, Rusenova *et al.* (2013) cautioned that precise comparison between results of ERIC genotypes in different studies is not possible. This is due to genetic variance in ERIC sequences of repetitive elements between strains. *P. larvae* strains may differ from published sequences and the primers used may not work across studies.

Loncaric *et al.* (2009) studying Austrian *P. larvae* strains reported two new genotypes, which had not been described in any other studies applying the same molecular genotyping techniques as Rusenova *et al.* (2013) carried out.

In an attempt to standardise laboratories and results across different studies, Morrissey *et al.* (2014) described multilocus sequence typing (MLST) that further differentiates *P. larvae* strains beyond the four ERIC genotypes. MLST utilises genes identified as evolving at a slow consistent pace across all strains of *P. alvei*. Consequently, Morrissey *et al.* (2014) describe 21 different *P. larvae* sequence types (ST). However, if MLST becomes widely accepted, further sequence types may be discovered since the full extent of ST diversity is not known.

The reporting of different genotypes of *P. larvae* is increasing because of the use of new molecular techniques. However, comparing the various genotypes of *P. larvae* discovered across studies is not possible because of genetic variance in ERIC sequences. Morrissey *et al.* (2014) report another molecular technique that further enhances differentiation beyond the four ERIC genotypes into 21 different sequence types. New techniques with increased discriminatory power like Next Generation Sequencing (NGS) are likely to enhance this differentiation further in the future.

In conclusion, there is currently no standard laboratory method or consistent naming principle of the reported genotypes.

19.1.2. OIE list

American foulbrood of honey bees is listed.

19.1.3. New Zealand status

American foulbrood is present and under official control (a National Pest Management Plan under the Biosecurity Act 1993).

Paenibacillus larvae larvae is listed as a reportable unwanted organism.

P. larvae that causes AFB is present in New Zealand and probably mostly caused by ERIC I since this genotype is considered the classical *P. larvae* causing AFB outbreaks worldwide. In early 2011, *P. larvae* strains of ERIC I and ERIC II genotypes were isolated from New Zealand samples of honey that were kept in stores (gifts of honey brought by visitors from New Zealand over a number of years) at the Institute for Bee Research at Hohen Neuendorf in Berlin (Genersch 2011).

Schäfer *et al.* (2014) also report finding ERIC II in New Zealand honey samples, and researchers at the Victoria University of Wellington have identified *P. larvae* ERIC I and II genotypes in New Zealand honey bees and bee larvae (Graham 2015).

Further, using a new typing system (MLST), Morrissey *et al.* (2014) report finding one genotype (ST3) that is unique to New Zealand.

19.1.4. Epidemiology

In accordance with the scope of this supplementary risk analysis, this Section examines the newly described molecular genotypes of *P. larvae*. Additionally, the most recent assessment of the risk of transmitting AFB through traded honey is presented.

The majority of publications in the field of AFB genotypes are by Genersch *et al.* who report four genotypes of *P. larvae*, namely ERIC I, II, III, and IV. Of these, *P. larvae* ERIC I and II are the two most important genotypes recognised to cause disease. Further, there have been no

outbreaks of AFB caused by ERIC III and IV reported (Genersch 2010). Funfhaus *et al*. (2009) quoted that "genotypes ERIC III and IV only exist as historical isolates in culture collections".

Rauch *et al*. (2009) conclude that the faster a *P. larvae* isolate is killing infected larvae, the less virulent it will be on the colony level. The extremely fast-killing strains may have caused the premature death of the infected larvae, allowing a too-efficient social immune response (nurse bee removing infected larvae and thus spores) and thereby impairing disease transmission. Hence, these two genotypes may represent an evolutionary blind alley for *P. larvae*, where the evolution of *P. larvae* virulence on the larval level came to its limitation on the colony level.

De Pinto *et al.* (2011) reported four genotypes, naming them ERIC A, B, C and D. All the genotypes originated from brood combs with clinical signs of AFB. In particular, ERIC A and B were the most common genotypes detected. Unlike the results reported by Genersch, De Pinto *et al*'s findings suggest that maybe all genotypes are capable of causing clinical disease.

However, De Pinto *et al.* (2011) explain that an accurate comparison with the results of Genersch (2010) is not possible because of differences in methodology related to the ERIC-PCR.

To explore virulence differences among genotypes experimentally, an infectious diet containing *P. larvae* subsp. *larvae* was fed to larvae 12 hours of age (most susceptible age). Generch *et al*. (2005) reported differences in the virulence of the strains. Larvae infected with genotype *AB* did not survive longer than 10 days postinfection with a mean of 7.8 days until 100% larval mortality. A mean time of about 11 days was required for 100% mortality of the larvae exposed to the other three strains.

With regard to capping the cells containing larvae, about 5% of the larvae infected with *AB* survived until after capping. For the other strains, about 20-26% of the larvae died after capping. Although all strains were pathogenic for larvae, the impact on hives infected with the different strains in the field is not known. Genersch *et al.* (2005) hypothesise that the most virulent strains at the individual larval stage may in fact be less virulent at the colony level. This is because larvae infected with *AB* strain die rapidly and therefore mostly prior to capping. This allows nursing bees to remove dead larva leading to fewer bacterial spores contaminating the hive. On the other hand, the slower-acting strains that allow the bees to cap the cells before the larvae die may cause greater spore contamination of the hive.

Despite the results of Genersch *et al.* (2005), it is notable that the methodology of the experiment was highly artificial. This is because honey is not naturally fed to larvae less than three days after egg hatch, and larvae older than 53 hours are no longer susceptible to AFB. Therefore, the risk of transmitting AFB through honey is considered small (Mutinelli 2011).

Sturtevant (1932) concluded that although *P. larvae* may be present in honey, in most instances it is at a level that is not capable of initiating infection.

Moreover, the principal method of disease transmission is the interchange of honey bee equipment between hives. Honey is imported for human consumption and not as a feed for honey bees. The high purchase and shipping costs preclude the use of honey as feed for honey bees (Shimanuki and Knox 1997; Mutinelli 2011).

Therefore, the risk of transmitting AFB through trade in honey is minimal (Sturtevant 1932; Shimanuki and Knox 1997; Mutinelli 2011).

The OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* chapter on AFB of honey bees (2013) (adopted in 2008) has no prescribed or alternative diagnostic tests for international trade. The *Manual* describes PCR methodologies for confirmation of clinical AFB and for the analysis of spore solutions to identify the agent. The PCR methodologies outlined in the *Manual* do not differentiate *P. larvae* into genotypes or further break these down into sequence types.

There is new information on quantitative PCR methods being developed which, unlike traditional PCR methods, allow quantification of pathogen load. The following is based on a review carried out by Rivière *et al.* (2013) on molecular methods for foulbrood diagnosis.

It is predicted that quantitative PCR values could possibly be correlated to spore counts in honey or other products. Thus, indicating the likelihood of infectious dose and disease transmission based on whether the spore count exceeds a particular threshold.

Nevertheless, the actual spore threshold number for disease emergence or for its ability to spread is not known. Further, having a so-called "spore threshold number" is unlikely to be independent of other factors such as strain virulence, natural host resistance and environmental factors. That is, beyond the presence of the organism, disease expression and organism transmissibility may have multifactorial influences determining the epidemiology of the disease.

Forsgen and Laugen (2014) show that high spore loads on adult honey bees are indicative of clinical disease. Even if the larvae are not fed spore contaminated honey, the spore levels in the hive would increase with a higher infection pressure on the colony. There may not be a particular volume of contaminated honey that would initiate infection in every hive (i.e.400g of contaminated honey). It is more likely there would be a wide variability on the volume of honey required dependent on chance and the susceptibility of individuals (Forsgren 2016).

Additionally, there is no consensus on what gene is best targeted for amplification when using quantative PCR. There is further genetic research required on the *P. larvae* genome to study how many copies of a particular target gene are found in the pathogens entire genome. For instance, there may be numerous gene repetitions of a given target gene which could invalidate any attempt to correlate PCR quantification to a spore count.

A further consideration is determining the best method for DNA extraction (Forsgren and Laugen 2014).

Moreover, the quantitative PCR method requires sophisticated laboratories and there is no method validated according to international standards.

In summary, a good beginning in new molecular technologies involving genomics and quantitative PCR has been made. However, further research is required before quantitative PCR results could accurately be related to spore counts. Culture-based methods are currently more accurate and related to actual clinical disease (Forsgren and Laugen 2014).

19.1.5. Hazard identification conclusion

Sophisticated laboratories using state of the art molecular technology have the ability to discern the genetic structure of *P. larvae* into ERIC genotypes, and further break these down into sequence types (Morissey *et al*. 2014).

ERIC I and II genotypes of *P. larvae* are present in New Zealand (Genersch 2011; Graham 2015; Morrissey *et al.* 2014; Schäfer *et al.* 2014).

Although endemic, the genotypes capable of causing disease (ERIC I and II) are identified as a hazard in honey bee products since there is a domestic pest management strategy in place. ERIC III and IV are not found in the field and probably exist only as laboratory strains (Funfhaus *et al*. 2009; Forsgren 2016). Accordingly, ERIC III and IV genotypes are not identified as a hazard in honey bee products.

19.2. RISK ASSESSMENT

19.2.1. Entry assessment

In honey, spores of *P. larvae* are commonly detected. Sturtevant (1932) concluded that although *P. larvae* spores may be present in honey, in most instances it is at a level that is not capable of initiating infection.

Nevertheless, since imported honey may contain spores at a level that is capable of initiating infection, or at least would increase the spore levels in the hive (if exposed to such honey), entry is assessed to be non-negligible.

There is no new information that alters the previous entry assessment conclusions reached in the risk analysis of 2004 for bee venom, pollen, royal jelly, beeswax, propolis and beeswax foundation. That is, the likelihood of entry is non-negligible for pollen, royal jelly and propolis, but negligible for beeswax foundation and bee venom.

19.2.2. Exposure assessment

Although *P. larvae* spores are found in honey, the actual spore threshold number that causes AFB is not conclusively known. Moreover, the required spore count to allow transmission to honey bees from consuming contaminated honey is also not known (Rivière *et al.* 2013).

Additionally, having a so called “spore threshold number” is unlikely to be independent of other factors that would have a direct effect on the transmissibility of infection. For example, variations in strain virulence of genotypes are known to occur. In addition, natural host resistance or susceptibility of the honey bee and environmental factors are also likely to influence the number of spores required to cause disease.

In summary, it is not possible to accurately correlate spore counts in honey or other bee products to infectious dose and disease transmission.

Nevertheless, the most likely means of exposure that would initiate disease would be worker honey bees that have access to honey that contains spores, which is subsequently fed to susceptible young larvae. However, honey is not naturally fed to larvae less than three days after egg hatch, and larvae older than 53 hours are no longer susceptible to AFB. Therefore, in reality, the likelihood of transmitting AFB through honey is small (Mutinelli 2011). Forsgren

(2016) notes that in a hive with a high spore load the worker honey bees may transmit the bacteria to larvae through contaminated mouth parts.

Mutinelli (2011) argues that considering the reported average spore concentration in honey (Hansen 1984) and the estimated hive spore threshold of 50 million (Sturtevant 1932), even if contaminated honey were directly exposed to a susceptible hive, at least 400g of honey would need to be fed to initiate an AFB infection. However, in the context of imported honey, this practice would be an unlikely event since beekeepers do not generally feed honey of an unknown source to their hives.

The principal method of disease transmission of AFB is apicultural practices such as the interchange of honey bee equipment between hives. Honey is imported for human consumption and not as a feed for honey bees. The high purchase and shipping costs preclude the use of honey as feed for honey bees (Shimanuki and Knox 1997; Mutinelli 2011). Therefore, the likelihood of transmitting AFB through trade in honey is minimal (Sturtevant 1932; Shimanuki and Knox 1997; Mutinelli 2011).

In view of the above, the likelihood that imported honey would be directly exposed to hives and result in a disease outbreak in New Zealand is assessed to be low.

There is no new information that alters the previous exposure assessment conclusions reached in the risk analysis of 2004 for pollen, royal jelly, beeswax and propolis. That is, the likelihood of exposure is non-negligible for these commodities except for propolis which is considered to be negligible.

19.2.3. Consequence assessment

Consequences would be in proportion to the frequency and number of hives directly exposed to imported honey or other bee products that are capable of initiating infection. Any additional costs incurred as a result of infection would be in destroying the infected hive, lost productivity and the cost of hive replacement.

Assuming a direct exposure pathway of New Zealand hives to imported honey bee products, there could be consequences from introducing *P. larvae* spores in honey, pollen, royal jelly or beeswax.

Accordingly, the consequences are assessed to be non-negligible.

19.2.4. Risk estimate

For honey, pollen, royal jelly and beeswax, the likelihood of entry and exposure and the consequences are assessed to be non-negligible.

As the risk estimate is therefore non-negligible for honey, pollen, royal jelly and beeswax, the following risk management measures have been identified for risk managers to consider.

Since the entry assessment is negligible for bee venom, and the exposure assessment negligible for propolis, the risk estimate is negligible for these commodities.

Accordingly, no risk management measures are justified for propolis or bee venom.

19.3. RISK MANAGEMENT

The following points were considered when drafting risk management options for *P. larvae* in honey, royal jelly, beeswax and pollen.

- There is an official control program for AFB under the New Zealand National Pest Management Plan (NPMP). The acceptable level of risk is signalled by the rules of the control program. The relevant rules are 29 (1) and 31 (1) which prohibit the sale or use of bee products from hives known or suspected to be clinically affected by AFB. Under the principle of non-discrimination covered in article 2.3 of the WTO Sanitary and Phytosanitary agreement, the measures imposed on imported bee products must not be greater than those achieved under the rules of the official control program. Therefore, the same level of protection that is being applied to New Zealand honey bee products could be applied to imported bee products.

- An equivalent level of protection under the New Zealand NPMP for AFB could be achieved by requiring official veterinary certification from the country or zone of origin that the bee products were not derived from hives that were known or suspected to be clinically affected by AFB. For equivalence to the NPMP, such certification would require the Competent Authority to have surveillance in place, knowledge of, and authority over, all domesticated apiaries existing in the country or zone. However, few countries have either control programmes for American foulbrood or tracing systems to allow certification of the origin of honey bee products.

- Equivalence to the NPMP could theoretically be achieved if the baseline spore concentration in New Zealand honey were known. In that case, imported honey could be required to have no higher spore concentration than domestically produced honey.

- For countries officially free of AFB in accordance with the *Code's* requirements, recommendations are made for importing honey, pollen, beeswax, propolis and royal jelly. This is for use in apiculture and for human consumption whereby the commodity has been processed to ensure destruction of both vegetative and spore forms of *P. larvae* by irradiation with 10 kGy or any procedure of equivalent efficacy recognised by the Veterinary Authority of the importing and exporting countries.

  N.B. If recommendations for the destruction of *P. larvae* in these commodities are developed into an international standard, these will apply only to importing countries that are officially free from AFB. Therefore, these measures will not be applicable for New Zealand.

- In view of the new information on spore counts of *P. larvae*, it is not possible to accurately correlate spore counts in honey bee products to infectious dose and disease transmission (Rivière *et al.* 2013). Accordingly, the sanitary measure in the 2004 risk analysis where honey bee products have been tested and found to have a spore count of less than 500,000 per litre, is no longer considered to be scientifically justifiable.

- The *Code* recommends an option whereby honey bee products for use in apiculture or for human consumption have been tested and found free from spores of *P. larvae* by a test method described in the *Manual*.

- For comb wax, treatment in boiling water for 20 minutes removed the vast majority of spores. The viability of remaining spores was reduced to about 0.001% of the viability of unheated spores (Gochnauer 1981). This level of reduction in germination capability is similar to that produced by gamma irradiation at 0.2 Mrad (i.e. 2 kGy) (Gochnauer and Hamilton 1970). However, to ensure complete inactivation of spores, wax must be melted at a temperature of 120°C or higher and held at that temperature for 24 hours (Smirnov and Tsivilev 1969, cited in Matheson and Reid 1992).

19.3.1. Risk management options

For honey, pollen, royal jelly, and beeswax

One or a combination of the following measures could be considered to effectively manage the risk:

Each consignment could be either:

(1) from apiaries situated in a country or zone free from American foulbrood

or

(2) from hives that were inspected for American foulbrood within the previous 12 months, by a person certified as competent to diagnose the disease, and found not to be clinically infected or suspected to be clinically affected by American foulbrood.

or

(3) irradiated with 10 kGy

or

(4) heated to 120°C and then held at that temperature for 24 hours

or

(5) have been found free from spores of *P. larvae* by a test method described in the *Manual*.

N.B. There are no prescribed tests for international trade in honey bee products.

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20. *Melissococcus plutonius*

20.1. HAZARD IDENTIFICATION

20.1.1. Aetiological agent

European foulbrood (EFB) is a bacterial infection caused by *Melissococcus plutonius*. This is the sole species described in the *Melissococcus* genus. Isolates of *M. plutonius* have been regarded as remarkably homogeneous based on morphological, physiological, immunological as well as genetic studies (Forsgren 2010).

Nevertheless, due to recent advances in molecular technology, multilocus sequence typing of *M. plutonius* reveals many different isolates, which have been grouped into 3 clonal complexes (Budge *et al*. 2014; Takamatsu *et al*. 2015). Further, atypical strains that are distinct from typical *M. plutonius* have also been recently reported (Takamatsu *et al.* 2015).

20.1.2. OIE list

European foulbrood of honey bees is a listed disease.

20.1.3. New Zealand status

Melissococcus plutonius is an exotic and notifiable organism.

20.1.4. Epidemiology

European foulbrood is found throughout the world where apiculture is practised except for New Zealand (Alippi 2014).

European foulbrood is an intestinal infection of honey bee larvae (Takamatsu *et al*. 2015). The infectious cycle begins when a larva eats brood food contaminated with the causative agent, *M. plutonius*. Bacteria multiply vigorously in the midgut of the honey bee larvae.

Infected larva that survive excrete the organism with their faeces when pupating and contaminate the comb. As adult worker honey bees, they act as carriers of the bacterium not only within the colony but also between colonies and apiaries. If the infected larvae die before they pupate, most of the bacteria in them are eliminated from the colony when they are cleaned out by adult honey bees (Forsgren 2010). Outbreaks generally are self-limiting (Bailey 1960). Clinical signs of EFB are only likely when the ratio of nurse bees to diseased larvae decreases for some reason, such as when nurse bees are recruited away from larval feeding by the demands of a high nectar flow. When this imbalance occurs, infected larvae that have a higher than normal demand for food are not removed and visual signs of the disease in the form of diseased larvae in combs begin to appear (Alippi 1999). Once sufficient nurse bees are again able to clean out dead larvae, the disease usually subsides (Bailey and Ball 1991). Therefore, honey bee colonies are usually more seriously affected during the spring and early summer (Tarr 1938; White 1920).

Outbreaks of the disease appear to be linked to colony stress conditions such as lack of food or water. Genetic factors, weather and geography may also play a role. Most larvae die within

a brief period usually around midsummer and sudden outbreaks of the disease followed by a spontaneous recovery a few weeks later has been observed (Forsgren 2010).

M. plutonius is a homogeneous bacteria and the sole species described in the *Melissococcus* genus. However, multilocus sequence typing of international isolates revealed the presence of 3 clonal complexes in the *M. plutonius* population (Budge *et al*. 2014; Takamatsu *et al*. 2015). Further, atypical strains that are phenotypically (can grow under conditions where typical *M. plutonius* cannot) and genotypically distinct from typical *M. plutonius* have been reported (Takamatsu *et al.* 2015).

Haynes *et al.* (2013) using next-generation sequencing identified highly polymorphic regions of the *M. plutonius* genome (in an otherwise genetically homogenous organism) and used these loci to create a modified sequence typing scheme. The results of the metagenomic technique showed that the global distribution of *M. plutonius* variants is not uniform.

As far as variation in virulence of *Melissococcus plutonius* is concerned, there appears to be some recent evidence that suggests different strains exist across the globe, possibly with variations in pathogenicity. Using sophisticated molecular technology, differences in sequences differing by only one point mutation can be detected (Rivière *et al.* 2013). The different strains identified have been grouped into so-called sequence types (Arai *et al.* 2014; Budge *et al*. 2014; Takamatsu *et al.* 2014). Using multilocus sequence typing, Budge *et al*. (2014) typed 15 *M. plutonius* isolates from outbreaks in England and Wales that were grouped into three clonal complexes. It was noted that clonal complexes appeared to vary in pathogenicity since infection caused by the more pathogenic variants was more likely to lead to honey bee colony destruction (Budge *et al*. 2014).

The following is based on a review carried out by Rivière *et al.* (2013) on molecular methods for detecting *M. plutonius*. It is predicted that by using quantitative PCR values, these could possibly be correlated to pathogen load in honey or other bee products. The conjecture is that knowing the pathogen load may indicate the likelihood of infectious dose and disease transmission based on whether the pathogen load exceeds a particular threshold.

Nevertheless, the actual gene copy threshold number for disease emergence or for its ability to spread is not known. Further, having a so-called "gene copy threshold number" is unlikely to be independent of other factors such as strain virulence, natural host resistance and environmental factors. That is, beyond the presence of the organism, disease expression and organism transmissibility may have multifactorial influences determining the epidemiology of the disease.

Additionally, there is no consensus on what gene is best targeted for amplification when using quantitative PCR. There is further genetic research required on the *M. plutonius* genome to study how many copies of a particular target gene are found in the pathogens entire genome. For instance, there may be numerous gene repetitions of a given target gene which could invalidate any attempt to correlate PCR quantification to a pathogen load.

Moreover, the quantitative PCR method requires sophisticated laboratories and there is no method validated according to international standards.

In summary, a good beginning in new molecular technologies involving genomics and quantitative PCR has been made. However, further research is required before quantitative PCR results could accurately be related to pathogen load in honey bee products.

In the field, EFB diagnosis is based on visual inspection and detection of diseased larvae. Laboratory diagnostics include culturing *M. plutonius*, but this is difficult due to bacterial overgrowth and fastidious growth requirements. However, PCR assays are available that readily identify *M. plutonius*.

Nevertheless, for PCR, the target sequences in the genes of typical *M. plutonius* are identical to those of the corresponding genes of atypical *M. plutonius*. Therefore, the PCR probes and primers currently in use are unable to differentiate the two types. Aria *et al*. (2014) developed a novel *M. plutonius*-specific PCR which detects typical and atypical *M. plutonius*.

M. plutonius can be transmitted via honey bee products such as honey, royal jelly and bee pollen that has not undergone heat treatment (Alippi 2014).

20.1.5. Hazard identification conclusion

Sensitive genetic discernment using genomic analysis of isolates of *Melissococcus plutonius* has identified different isolates around the world, including atypical isolates. However, all isolates of *M. plutonius* are exotic organisms (no matter what the particular genetic strain designations thereof may be).

Accordingly, since *M. plutonius* is exotic and listed as an unwanted and notifiable organism, all isolates are identified as a hazard in honey bee products.

20.2. RISK ASSESSMENT

20.2.1. Entry assessment

European foulbrood is an intestinal infection of honey bee larvae and there is no evidence that this fastidious anaerobic organism grows anywhere other than in the lumen of the larval midgut (Bailey 1959; Takamatsu *et al*. 2015). Erler *et al*. (2014) demonstrated that although *M. plutonius* may be present in honey, growth of *M. plutonius* is completely inhibited.

It is generally accepted that *M. plutonius* can be present and can survive in honey, pollen, beeswax, propolis and royal jelly that has not undergone heat treatment (Alippi 2014).

Honey

M. plutonius can be found in honey from infected hives, albeit in relatively low numbers. In one study, 6% of bulk honey samples from endemic areas were culture positive for *M. pluton* (Hornitzky and Smith 1998). The concentration of *M. plutonius* reported in honey under natural conditions is up to 3.3×10^3 organisms per ml (Wootton *et al*. 1981).

McKee *et al.* (2003) has demonstrated that the polymerase chain reaction (PCR) assay is a more sensitive tool for the detection of *M. plutonius* in honey than culture. In a study of 80 honey samples from different states in Australia 22/80 (27.5%) were positive by culture whereas 57/80 (71.3%) were positive using PCR. This work demonstrates that *M. plutonius* is more common in honey than previously demonstrated.

The likelihood of entry in honey is therefore assessed to be non-negligible.

Royal jelly

M. plutonius can be found in royal jelly, although there is little indication from the literature of the likelihood of this occurring. Royal jelly imported into New Zealand was routinely tested for the presence of *M. plutonius* until December 2001 (Giacon and Malone 1995), when imports were suspended following the isolation of *M. plutonius* in quarantine from three out of 10 samples of freeze-dried, bulk royal jelly imported from China (Jamaludin *et al*. 2002). The likelihood of entry in royal jelly is therefore assessed to be non-negligible.

Pollen

Bee-collected pollen does not appear to have been tested for the presence of *M. plutonius*. However, in view of the ability of the organism to survive on the walls of cells that have contained infected larvae (Bailey 1959), it appears reasonable to assume that bee-collected pollen is likely to be contaminated with the organism when collected by bees from infected colonies. The likelihood of entry in pollen is therefore assessed to be non-negligible.

Propolis

There are no reports of propolis being contaminated with *M. plutonius*, and the antimicrobial properties of propolis (Ghisalberti 1979; Grange and Davey 1990) may indeed limit the survival of this organism. However, since *M. plutonius* is thought to be able to overwinter on the sides of cell walls or in faeces and wax debris on the bottom of the hive (Shimanuki 1997), and since propolis, whether from scrapings or propolis mats, often has a wax component, the likelihood of *M. plutonius* being present in propolis is assessed to be non-negligible.

Beeswax

Although wax has not been tested for *M. plutonius*, since the organism is known to persist on the walls of cells, it is reasonable to assume that wax may be contaminated by the organism, at least at the time of its formation. Therefore, the likelihood of *M. plutonius* being present in raw beeswax cappings and comb wax is assessed to be non-negligible. The likelihood of *M. plutonius* being present in processed beeswax as defined in section 4.4 or in beeswax foundation will depend on the time/temperature parameters of the manufacturing process and it is therefore reasonable to consider the likelihood to be non-negligible unless a critical time/temperature has been achieved that destroys *M. plutonius*.

Bee venom

There is no evidence that bee venom can harbour *M. plutonius* and, considering the collection methods for bee venom, and the anaerobic conditions required for growth of the organism, there is no good reason to assume that significant contamination of bee venom is likely. The likelihood of the organism being present in venom is therefore assessed to be negligible.

Entry assessment conclusion

The likelihood of entry of *M. plutonius* is assessed to be non-negligible for honey, propolis, pollen, royal jelly and beeswax, but it is assessed to be negligible for bee venom.

20.2.2. Exposure assessment

For *M. plutonius* bacteria in imported bee products to come into contact with susceptible species in New Zealand, these commodities would have to be harvested by worker bees and taken back to hives and fed to young larvae, or be fed directly to the colony by beekeepers.

Experimentally, at least 100 bacteria were able to initiate infection in exposed larva (Bailey 1960). McKee *et al.* (2004) transmitted EFB to healthy larva by providing excess contaminated food at a minimum concentration of 200 bacterial cells per millilitre. Clinical signs of EFB are only likely when the ratio of nurse bees to diseased larvae decreases for some reason, such as when nurse bees are recruited away from larval feeding by the demands of a high nectar flow. When this imbalance occurs, infected larvae that have a higher than normal demand for food are not removed and visual signs of the disease in the form of diseased larvae in combs begin to appear (Alippi 1999).

Therefore, it is important to note that in the experiments of McKee *et al*. (2004) and Bailey (1960) the natural housekeeping effect of adult bees in the hive was absent.

Therefore, it is not possible to extrapolate from these experimental results to natural conditions, except to say that the infectious dose of *M. plutonius* is likely to be considerably higher under natural conditions.

Honey, pollen and some forms of royal jelly are considered to be attractive to bees. Royal jelly is traded mainly for use in the cosmetic industry and in the human health food market (OIE 2015) and in this form it is unlikley to be exposed to honey bees in a form that could transmit infection.

Propolis and beeswax, in the forms that are internationally traded, are not considered to be attractive to bees. This notwithstanding, if wax is made into foundation or applied to plastic frames, it may be put into direct contact with honey bees regardless of its attractiveness to bees. Similarly, beekeepers may feed pollen to bees or added to protein supplement feeds to increase attractiveness, and royal jelly may be used to prime queen cell cups when producing queen bees.

Moreover, if any unattractive bee products are mixed with honey, they will become attractive to bees.

Therefore, the likelihood of exposure to susceptible species in New Zealand is assessed to be non-negligible for honey, pollen and royal jelly. In addition, since beeswax may be made into foundation or applied to plastic frames, the likelihood of exposure is assessed to be non-negligible. For propolis the likelihood of exposure is assessed to be negligible.

20.2.3. Consequence assessment

Except for New Zealand, EFB occurs worldwide wherever apiculture is practised and appears to be benign in some parts of the world and yet more severe in others. In general, EFB is self-limiting and is not considered a serious disease by most beekeepers (Bailey 1960; Alippi 2014).

However, under certain conditions, as is being currently reported in Switzerland, EFB may cause severe losses in brood, resulting in lower yields of honey (Alippi 2014).

Apart from the presence of the organism, the difference in severity of disease around the world may be due to other factors such as climate, variation in nutritional quality of food and genetic variations in host susceptibility.

Historically, since *M. plutonius* has been regarded as genetically homogeneous, differences in virulence between isolates found worldwide could have been considered to have a negligible effect. Nevertheless, based on new molecular technology, it appears there are different isolates found worldwide. However, it is not known which isolates have the ability to express virulence factors that would make them more pathogenic.

Consequences of introduction and establishment in New Zealand would be in proportion to whether benign or severe clinical signs are caused by infection. EFB could have a consequence for bee colonies used for pollination (Shimanuki 1997), since more than 70,000 colonies in New Zealand are used for kiwifruit pollination and for the many thousands of colonies used for pollinating pip and stone fruits, berry fruits and small seeds.

Beekeepers in Australia and elsewhere find it necessary to feed antibiotics to control EFB, and this may be necessary if the disease were to be introduced to New Zealand. The feeding of antibiotics to honey bees has implications for the American Foulbrood National Pest Management Strategy, which relies on beekeepers being able to diagnose clinical signs of American foulbrood. Feeding antibiotics has been reported to mask American foulbrood disease signs, thus making it more difficult to detect and control (Oldroyd *et al*. 1989).

Although the presence of EFB would probably not result in restrictions being placed on the export of bees and bee products from New Zealand, assuming the feeding of antibiotics to honey bees were necessary, then there could be a minor negative effect on honey exports. This is because some importing countries may require New Zealand honey to be tested to ensure it does not contain antibiotic residues.

European foulbrood is unlikely to have any effects on New Zealand native insects since this fastidious anaerobic organism is restricted to honey bees and it appears able to grow only in the honey bee larval midgut. EFB causes problems in commercial beekeeping because of the social nature of *A. mellifera*, which forms colonies comprising many thousands of individuals in close contact with one another, quite unlike native bees which are solitary insects and where there is no contact between generations (Donovan 1980; Donovan *et al*. 1984; Matheson 1997).

Accordingly, the consequences are assessed to be non-negligible.

20.2.4. Risk estimate

For honey, pollen, royal jelly and beeswax the likelihood of entry and exposure, and the consequences are assessed to be non-negligible.

As a result, the risk estimate is non-negligible and *M. plutonius* is assessed to be a risk in honey, pollen, royal jelly and beeswax. The following risk management measures have been identified for risk managers to consider.

Since the entry assessment is negligible for bee venom, and the exposure assessment negligible for propolis, the risk estimate is negligible for these commodities.

Accordingly, no risk management measures are justified for propolis or bee venom

20.3. RISK MANAGEMENT

The following points could be considered when drafting risk management options for *M. plutonius* in honey, royal jelly, beeswax and pollen.

- Irradiation at 10 – 15 kGy eliminated *M. plutonius* from honey (Hornitzky 1981). In honey that had a starting concentration of 1.23×10^5 organisms per ml, no organisms survived 14 kGy, and this appears to be a generally recommended treatment level (Hornitzky 1994).

- The *Code* recommends honey, honey bee-collected pollen, beeswax, propolis and royal jelly (that comes from apiaries not in a country or zone free from EFB) be irradiated with 15kGy or any procedure of equivalent efficacy.

- However, the *Code* does not specify what treatments are of equivalent efficacy to irradiation. The *Code* recommends that an equivalent measure is to be recognised by the Veterinary Authorities of the importing and exporting country.

- Thermal inactivation of *M. plutonius* has been studied only in honey. It is assumed thermal inactivation would be similar for all honey bee products. Utilising the results of two thermal inactivation studies for *M. plutonius* in honey (Wootton *et al.* 1981; Ball *et al.* 2001), a model was developed that predicts the time and temperature combinations required to achieve a 6D reduction (99.9999%) in organism number[B]. Adopting the time temperature combinations from this model would provide a very high level of assurance that *M. plutonius* would be inactivated and that this could be considered equivalent to irradiation.

- Additional to treatment, the *Code* also provides an option where the commodity can be found free of *M. plutonius* by a test method described in the *Manual*.

- The *Manual* describes culture methods and a PCR for the detection of nucleic acid of *M. plutonius* in honey and pollen.

- In regards testing for the presence of *M. plutonius* in honey bee products, the organism is relatively difficult to culture in the laboratory, as it is a fastidious anaerobe. Nevertheless, cultivation has been used to detect the organism in honey bee products (Giacon and Mallone 1995; Hornitzky and Smith 1998).

- The PCR described in the *Manual* can be used for the detection of *M. plutonius* in honey and pollen (Djordjevic *et al*. 1998; Govan *et al*. 1998; McKee *et al*. 2003). However, the use of PCR for testing honey or pollen that has been heat-treated to inactivate *M. plutonius* may cause false positive test results. This is due to the high sensitivity of PCR whereby nucleic acid could still be detected although heat treatment has inactivated the organism.

[B] The details of the model and predicted results can be found in MAF's 2004 honey bee products risk analysis (MAF 2004).

20.3.1. Risk management options

For venom and propolis

No sanitary measures are required.

For honey, pollen, royal jelly, and beeswax

One or a combination of the following measures could be considered to effectively manage the risk:

Each consignment could be either:

(1) from apiaries situated in a country or zone free from European foulbrood

or

(2) irradiated with 15 kGy

or

(3) heated in accordance with the table below to achieve a 6D reduction in organism number

Time-temperature treatments required to reduce the number of *M. plutonius* by 99.9999%.

Temperature (°C)	Time
50	54 h
60	10 h
70	1 h 48 min
80	22 min
90	5 min
100	5 min

Note : intermediate temperatures can be considered in discussion with MPI using the predictive model developed.

An alternative to the above measures, for royal jelly and pollen only, is to import the pollen in a form that is not considered to be attractive to bees, such as consumer-ready capsules or tablets packaged for direct retail sale.

or

(4) have been found free from *M. plutonius* by a test method described in the *Manual*

N.B. There are no prescribed tests for international trade in honey bee products. The *Manual* presents culture and PCR testing methodologies. However, PCR is unable to differentiate viable from inactivated organisms.

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21. *Apocephalus borealis*

21.1. HAZARD IDENTIFICATION

21.1.1. Aetiological agent

Apocephalus borealis is a species of phorid fly (Order Diptera, Family *Phoridae*). This fly belongs to the parasitic subgenus *Mesophora* (Brown 1993).

21.1.2. OIE list

Phorid flies are not listed.

21.1.3. New Zealand status

Apocephalus borealis has not been reported and it is not listed as an unwanted organism.

21.1.4. Epidemiology

Apocephalus borealis is native to North America and widely distributed there. *A. borealis* has historically been known to parasitise bumblebees and wasps but not honey bees.

However, Core *et al.* (2012) recently described the first report of *A. borealis* parasitising honey bees in the San Francisco bay region. The authors suggested that phorid fly may be a possible cause of colony collapse disorder (CCD). Runckel *et al.* (2011) also detected phorid fly RNA in a survey of honey bee samples taken from colonies moved around Mississippi, South Dakota and California. However, *A. borealis* parasitism of honey bees is an uncommon phenomenon (Runckel *et al.* 2011) and so unlikely to a have role in CCD.

Most recently, Ravoet *et al.* (2013) provided molecular evidence for the presence of parasitic phorid fly in honey bees collected in Belgium. However, phorid fly was not associated with any winter losses in the affected bees' colonies. The detection of phorid fly RNA was unexpected and it is the first description of *A. borealis* (or a genetically similar phorid fly) parasitising honey bees outside of the United States of America.

The life cycle of *A. borealis* involves female flies pursuing host honey bees until they land on their abdomens and insert the ovipositor and inject eggs. As the larvae develop in the bee, they cause bees to exhibit neurological signs such as disorientation, walking in circles and an inability to stand. The honey bees also remain inactive during the daytime until the growing larvae eventually cause the death of their host. The parasitised bees are also known to fly at night and show other unusual behaviors such as abandoning or being ejected from their hives to die some distance from the hive. Consequently, no phorid fly adults are found in the hives (Core *et al.* 2012).

In the parasitised honey bee, larvae take about a week to develop. Mature fly larvae then typically emerge from the host bee between the head and thorax and pupate away from the dead honey bee's body (Core *et al.* 2012).

21.1.5. Hazard identification conclusion

Phorid fly larvae are uncommonly associated with honey bees and adult flies have not been found in hives (Runckel *et al.* 2011; Core *et al.* 2012).

There is no evidence to show that phorid flies or any part of their life cycle are associated with honey bee products. Further, there are no reports of phorid fly transmission via honey bee products and it is not an OIE listed disease of honey bees.

In view of the above, phorid flies are not identified as a hazard in honey bee products.

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22. *Apicystis bombi*

22.1. HAZARD IDENTIFICATION

22.1.1. Aetiological agent

Apicystis bombi is a protozoal parasite (phylum Apicomplexa) of bumble bees that has been described in honey bees.

22.1.2. OIE list

Apicystis bombi is not listed.

22.1.3. New Zealand status

No bumble bees infected with *A. bombi* were detected during studies carried out on 30 samples between 1974-1977 (Macfarlane *et al.* 1995). Accordingly, this provides some evidence that New Zealand may be free from *A. bombi*. However, sensitive molecular detection methods are now available.

A. bombi is not listed as an unwanted organism and there have been no surveys carried out to detect *A. bombi* in *Apis mellifera*.

22.1.4. Epidemiology

Apicystis bombi is a low prevalence protozoan parasite principally of bumble bees (Lipa and Triggiani 1996; Plischuk *et al.* 2011). This protozoan has been reported from North America, France, Italy and Switzerland but could occur universally where bumble bees are present (Macfarlane *et al.* 1995; Lipa and Triggiani 1996). However, in China, a small survey of five honey bee colonies using molecular detection methods did not detect *A. bombi* DNA (Yang *et al.* 2013).

The sporozoites of *A. bombi* emerge in the intestine of bumble bees from ingested oocysts which penetrate through the midgut wall into the body cavity and infect the fat body cells. In these fat cells, the spores develop and multiply. A description of the pathology caused by infection is lacking but apparently heavy spore accumulations in the fat body of queen bumble bees may cause premature death (Macfarlane *et al.* 1995).

Macfarlane *et al.* (1995) suggested that perhaps *A. bombi* may be able to infect honey bees as well as bumble bees since the host specificity of *A. bombi* and *A. mellifera* was not known.

Lipa and Triggiani (1996) reported that *A. bombi* was a cosmopolitan parasite of *Bombus* and *Apis* species since 10 bumble bee species in Europe and North America were found to be infected. In regard *A. mellifera*, the first report of the parasite in one bee in Finland in 1990 was documented.

This observation indicates that *A. bombi* may also infect *A. mellifera*. However, the researchers Lipa and Triggiani (1996) included the caveat that the single observation of *A. bombi* in a honey bee requires confirmation. This is because identification of the parasite was

based on oocyst morphology and there could be other species of protozoa involved. It is not known whether the *A. bombi* discovered in bees are the same strain as those found in bumble bees and whether interspecies transmission is possible. Graystock *et al*. (2015) suggest that flowers are likely hotspots for the transmission of pollinator parasites.

Subsequently, Plischuk *et al.* (2011) provided only the second report showing molecular evidence that *A. bombi* was present in the adipose tissue of *A. mellifera* but at a lower level of infection than in bumble bees. There were no clinical signs in the parasitised honey bees.

Most recently, molecular surveys to detect DNA of *A. bombi* in honey bees have been carried out in Japan, Europe, Argentina and Mexico (Morimoto *et al.* 2013; Ravoet *et al.* 2013; Maharramov *et al.* 2013). The general conclusion from these studies is that *A. bombi* is distributed widely but at a low prevalence in honey bees compared with other parasites and viruses of honey bees.

Although it has been shown through molecular detection methods that *A. bombi* may be present in honey bees, its significance is not known. Ravoet *et al.* (2013) reports that the presence of this protozoan in honey bee colonies had no effect on winter colony losses.

The pathology caused by infection in bumble bees has not been well investigated or described. No clinical signs have been described in honey bees infected with *A. bombi*.

Moreover, although *A. bombi* may be present in honey bees, it is not known if they are dead-end hosts or true hosts in which the life cycle could be completed.

22.1.5. Hazard identification conclusion

It is not conclusively known if *A. bombi* is exotic to New Zealand. There is weak evidence to support a claim of country freedom. However, it appears *A. bombi* could be present since overseas studies using molecular detection methods have identified its ubiquitous nature everywhere bumble bees are found (Maharramov *et al.* 2013; Morimoto *et al.* 2013; Ravoet *et al.* 2013).

A. bombi in honey bees has not been reported to cause clinical disease and it is not known if honey bees are a true host for this parasite.

The life cycle of *A. bombi* is such that it is primarily associated with bumble bees, but also may infect honey bees. However, there are no reports of an association of this protozoan with honey bee products and there are no reports of transmission via honey bee products.

Apicystis bombi is not listed within the OIE list of honey bee diseases.

In view of the above, *A. bombi* is not identified as a hazard in honey bee products.

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23. *Crithidia mellificae* and *Lotmaria passim*

23.1. HAZARD IDENTIFICATION

23.1.1. Aetiological agent

Crithidia mellificae is a protozoan flagellate that belongs to the Order Kinetoplastea, family *Trypanosomatidae*.

In the past, honey bee trypanosomes have been classified as a single species, *Crithidia mellificae*. However, Schwarz *et al*. (2015) who carried out phylogenetic analysis of trypanosomes from honey bees, conclude that what previously had been classified as *Crithidia mellificae* may in fact be another species. Schwarz *et al*. (2015) propose the new species be named *Lotmaria passim*.

23.1.2. OIE list

Trypanosomes of honey bees are not listed.

23.1.3. New Zealand status

No specific surveys have been carried out to detect *C. mellificae* in *Apis mellifera* and it is not listed as an unwanted organism.

However, a recent investigation into hive mortality events in the Coromandel district using new diagnostic techniques identified *Lotmaria passim* for the first time in New Zealand (Borowick and Goodwin 2015; MPI 2015).

Subsequently, *L. passim* has been detected in beekeeper operations outside of the Coromandel district. *Lotmaria passim* is likely to have been present for some time. It has simply gone undetected until the advancement in diagnostic techniques enabled detection (MPI 2015).

23.1.4. Epidemiology

C. mellificae is a cosmopolitan trypanosome of honey bees reported from North and South America, Australia, China, France, Japan, Switzerland and Belgium (Langridge *et al.* 1967; Morimoto *et al.* 2013; Ravoet *et al.* 2011; Runckel *et al.* 2011; Yang *et al.* 2013). Recently, Schwarz *et al*. (2015) have concluded that their newly discovered species (based on genetic differences) *Lotmaria passium*, is likewise global and common in honey bees. Ravoet *et al*. (2015) developed a molecular diagnostic method to differentiate *C. mellificae* and *L. passim*.

The original description of *C. mellificae* isolated from *Apis mellifera* in apiaries of Victoria, Australia, was reported in 1967 (Langridge *et al.* 1967). Recent phylogenetic analysis on genetic data from a trypanosome isolated from a honey bee (thought most likely to be *C. mellificae*) was carried out by Schmid-Hempel and Tognazzo (2010). Their genetic analysis separated *C. mellificae* from *C. bombi*, a globally distributed trypanosome of bumble bees. This

genetic separation agreed with experimental results that show *A. mellifera* is not susceptible to *C. bombi* and vice versa (Ruiz-González and Brown 2006).

Little is known about *C. mellificae*, possibly due to its low pathogenicity (Ruiz-González and Brown 2006). The lifecycle, transmission and survivability of *C. mellificae* is very likely to be similar to the closely related *C. bombi* of bumble bees. *Crithidia bombi* is transmitted by the faecal-oral route. The parasite resides in the intestine of the hindgut of the bumble bee host where infective stages are passed with faeces (Ruiz-González and Brown 2006). These infective stages associated with the faeces can survive only a very short time outside the host (Schmid-Hempel and Tognazzo 2010). Infection with *C. bombi* is only of slight significance to bumble bee health and it is not a recognised pathogen (Macfarlane *et al.* 1995).

In the United States of America, Runckel *et al.* (2011) using molecular methods readily detected *C. mellificae* in honey bees. As a result, honey bee intestines were dissected and *C. mellificae* were visualised and isolated. However, the presence of the organism had no effect on colony health.

Further, the original description of *C. mellificae* isolated from *Apis mellifera* in apiaries of Victoria, Australia also involved experimentally examining whether there were any health effects for infected honey bees. No significant differences in mortality rates between the infected and non-infected honey bees was observed (Langridge *et al.* 1967).

From sampling 363 honey bees in Belgium, Ravoet *et al.* (2013) reported molecular evidence of *C. mellificae* being present in 70% of the samples. No clinical signs in honey bees infected with *C. mellificae* were reported. Nevertheless, a vague correlation between the presence of *C. mellificae* and colony collapse disorder (CCD) was suggested. It is explained that the proposed correlation is probably because of synergistic effects of a multifactorial nature whereby colonies are more vulnerable when also infected with *Nosema ceranae* and *Varroa destructor* (Ravoet *et al.* 2013; Runckel *et al.* 2014).

Also, Cox-Foster *et al.* (2007), Cornman *et al.* (2012) and vanEngelsdorp *et al.* (2009) investigated whether *C. mellificae* may be implicated as a cause of CCD. Results of these studies determined that *C. mellificae* was commonly found in both CCD-affected colonies and in healthy controls.

The presence of *C. mellificae* did not affect colony health and trypanosomes were not identified as a cause of CCD in any of these studies carried out.

Concerning the presence of *L. passim* in hive mortality events in the Coromandel district in New Zealand, an investigation did not show an association of the trypanosome with honey bee mortality (MPI 2015).

23.1.5. Hazard identification conclusion

It is not conclusively known if *C. mellificae* is exotic to New Zealand. It appears *C. mellificae* could be here since overseas studies using molecular detection methods have documented the ubiquitous nature of *C. mellificae* (Langridge *et al.* 1967; Morimoto *et al.* 2013; Ravoet *et al.* 2011; Runckel *et al.* 2011; Yang *et al.* 2013). *L. passim* has been detected in New Zealand (Borowick and Goodwin 2015; MPI 2015).

Both *C. mellificae* and *L. passim* infection of honey bees are not known to cause clinical disease.

C. mellificae is found within the hindgut of honey bees (Runckel *et al.* 2011) and like *C. bombi* of bumble bees, it probably survives only a very short time outside the host.

There are no reports associating *C. mellificae* or *L. passim* with honey bee products or reports of transmission via honey bee products.

C. mellificae and *L. passim* are not listed within the OIE list of honey bee diseases.

In view of the above, *C. mellificae* and *L. passim* are not identified as a hazard in honey bee products.

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