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Department of Agriculture, Fisheries and Forestry

Importation of queen honey bees

Final policy review

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Contents

Acronyms and glossary

Acronyms

AFB	American foulbrood
AHBIC	Australian Honey Bee Industry Council
ALOP	appropriate level of protection
APV	acute paralysis virus
AQIS	Australian Quarantine and Inspection Service
AQPM	Animal Quarantine Policy Memorandum
AUSVETPLAN	Australian Veterinary Emergency Plan
BAPM	Biosecurity Australia Policy Memorandum
CCD	colony collapse disorder
DAFF	Australian Government Department of Agriculture, Fisheries and Forestry
DNA	deoxyribonucleic acid
DWV	deformed wing virus
EPPRD	Emergency Plant Pest Response Deed
EFB	European foulbrood
IAPV	Israeli acute paralysis virus
IATA	International Air Transport Association
NSW	New South Wales
NT	Northern Territory
OIE	World Organisation for Animal Health
PAQ	post-arrival quarantine
PCR	polymerase chain reaction
PEQ	pre-entry quarantine
PNG	Papua New Guinea
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SA	South Australia
SBV	sacbrood virus
SHB	small hive beetle
SPV	slow paralysis virus

SPS Agreement	World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures
USA	United States of America
WA	Western Australia

Glossary

Africanised honey bees	honey bees of the sub-species *Apis mellifera scutellata* or hybrids between this sub-species and others. Usually denotes this sub-species when found in the Americas
Africanisation	the process of hybridisation of the sub-species *Apis mellifera scutellata* with other sub-species of *A*. *mellifera*
anthropogenic	caused or produced by humans
anthropomorphic	processes or materials that are derived from human activities
apiary	colonies, hives, and other equipment assembled in one location for beekeeping operations
apiculture	the science and art of raising honey bees
Apis cerana	scientific name of the Asian or eastern honey bee, which is now established in Australia in a limited area
Apis dorsata	scientific name of the major species of giant honey bee found in Asia
Apis florea	scientific name of the dwarf honey bee found in Asia and some parts of Africa and the Middle East
Apis mellifera	scientific name of the European or western honey bee that is naturalised throughout Australia
arrhenotokous	a form of parthenogenesis in which unfertilised eggs develop into haploid males
brood	immature honey bees not yet emerged from their cells: eggs, larvae, and pupae
buzz pollination	a pollination technique used by some bees. Their flight muscles are rapidly moved, causing the flower and anthers of the plant to vibrate and release pollen, which makes pollination more efficient
brood chamber	the part of the hive in which the brood is reared; may include one or more hive bodies and the combs

Capensis	honey bees of the sub-species *Apis mellifera capensis*
capped brood	pupae whose cells have been sealed with a porous cover by mature honey bees to isolate them during their non-feeding pupal period; also called sealed brood
chromosome	an organised structure of DNA and protein found in cells containing genes and other elements
cleptoparasite	an animal that takes food from another animal that has caught, collected or stored food. The term is also used to describe the stealing of nest material or other inanimate objects from one animal by another
colony	the aggregate of worker honey bees, drones, queen and developing brood living together as a family unit in a hive or other dwelling
diploid	containing two complete sets of chromosomes, one from each parent
DNA	is a nucleic acid (also called deoxyribonucleic acid) that contains the genetic instructions used in the development and functioning of living organisms
drone	a male honey bee
ecotone	transition area between two adjacent but different plant communities, such as forest and grassland
escorts	nurse honey bees that accompany the queen honey bee in the queen cage and feed her royal jelly
feral colonies	wild honey bee colonies, not under management
genotype	the genetic makeup of a cell, an organism, or an individual
grafting	the practice of removing worker larvae from its cell and adding it into an artificial queen cup meant for rearing the larvae into a queen honey bee
haploid	having a single set of unpaired chromosomes
haplotype	a group of genes which is inherited together by an organism from a single parent
hive/beehive	a box or receptacle with movable frames, used for housing a colony of honey bees

honey flow	a time when nectar is plentiful and honey bees produce and store surplus honey
larva (plural is larvae)	immature honey bee life-stage prior to pupation: white, legless, soft and grub-like
microsatellites	repeating sequences of base pairs of DNA used as molecular markers in genetics, for kinship, population and other studies
migratory beekeeping	the moving of colonies of honey bees from one locality to another during a single season to take advantage of two or more honey flows
mitochondria	an organelle found in large numbers in most cells, in which the biochemical processes of respiration and energy production occur
morphometrics	concerned with studying variation and change in the form (size and shape) of organisms
nurse honey bee	a worker honey bee whose role is to feed and care for brood
ovarioles	the tubes of which the ovaries of most insects are composed
parthenogenesis	is a form of asexual reproduction found in females, where growth and development of embryos occurs without fertilization by a male
phoretic	a relationship in which one organism transports another organism of a different species
pollination	the transfer of pollen from the anthers to the stigma of flowers
pupa (plural is pupae)	bee life-stage following the larval stage and takes place within the sealed brood cell. During this stage the adult structures of the honey bee are formed
queen honey bee	a fertile female honey bee, larger and longer than a worker honey bee; able to lay fertilised eggs
RNA	is a nucleic acid (also called ribonucleic acid) that is involved in protein synthesis and in the transmission of genetic information
Scutellata	honey bees of the sub-species *Apis mellifera scutellata* or hybrids between this sub-species and others
slumgum	residue of the beeswax rendering process
spermatheca	an organ of the female reproductive tract in insects. Its purpose is to receive and store sperm from the male

spores/endospores	a dormant, tough, and temporarily non-reproductive structure produced by certain bacteria and fungi
swarm	a large number of honey bees that leave a hive *en masse*
thelytokous	a form of parthenogenesis in which females are produced from unfertilised eggs
transhumance	seasonal movement of people with their livestock over relatively short distances
vector	an organism, typically a biting insect, mite or tick, that transmits a disease or parasite from one animal or plant to another
worker honey bee	a sterile female honey bee that builds, provisions and cleans the hive and feeds the larvae

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Summary

Australia has a favourable honey bee health status and has not had the honey bee colony losses that have been reported in many other parts of the world. To maintain this favourable status, Australia adopts a risk based approach to the management of honey bee imports.

Previous biosecurity policy and risk management measures for the importation of live honey bees (*Apis mellifera*) is contained in Australian Quarantine Policy Memorandum 1996/42 *Conditions for the Importation of Honey Bees* (see Appendix A).

In February 2006, importation from the United States of America (USA) under these conditions was suspended because of the inability to determine whether honey bees from that country contained genes from the Africanised honey bee, *Apis mellifera scutellata*, and its hybrids. Importation from all sources was suspended in August 2008 because of concerns about the international spread of colony collapse disorder.

There has been continuing interest from the honey bee industry to import diverse new genetic material into Australia that may assist in developing disease resistance against various pathogens. In response, the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) has conducted this review of the previous animal health conditions for the importation of honey bees.

This review assesses the biosecurity risks to Australia of the importation of queen honey bees (*Apis mellifera*, the European or western honey bee). All disease agents, pests and species of concern are assessed, including those that have emerged since the original policy for the importation of honey bees was developed in the 1990s. It examines risk management options to reduce identified risks to a level consistent with Australia's appropriate level of protection (ALOP).

This review recommends that imports be restricted in the first instance, to those countries that can provide a satisfactory level of assurance for certifying to Australia's biosecurity requirements: Canada, the European Union, Japan, New Zealand and the USA.

This review concludes that the importation of queen honey bees from these countries does not achieve Australia's ALOP with respect to the following hazards:

- Africanised honey bees (*A. m. scutellata* and its hybrids)
- varroosis
- acarapisosis (tracheal mite)
- *Tropilaelaps*

For these hazards, risk management measures are required and depending on the disease agent, a number of measures are recommended. However, all imported honey bees will be required to undergo post-arrival quarantine at a government approved quarantine facility where a colony will be propagated, derived from the imported honey bees and only larvae grafted from this colony will be released from quarantine.

In addition the following recommendations are made:

- For an additional country to be approved for the importation of queen honey bees, its competent authority would need to provide information for assessment on its animal health status and certification controls for honey bees.
- Honey bees should only be transported to Australia as air cargo and not via mail or as hand luggage.
- The importer is to supply worker honey bees as escorts and a nucleus hive as well as the required frames and hive boxes. These will no longer be supplied from a hive permanently resident on the quarantine station (see Appendix A).

DAFF acknowledges that there may be other measures that provide an equivalent level of protection against hazards identified as being of biosecurity concern. Submissions supporting equivalence measures will be evaluated on a case-by-case basis.

1 Introduction

Australia's biosecurity policy

Australia's biosecurity policies aim to protect Australia against risks that may arise from exotic diseases and pests entering, establishing or spreading, thereby threatening Australia's unique flora and fauna, as well as those agricultural industries that are relatively free from serious diseases and pests.

The Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) is responsible for developing and reviewing biosecurity policy for the import of animals and their products. It does this through a science-based risk analysis process. At the completion of the process, recommendations are provided to Australia's Director of Animal and Plant Quarantine (the Secretary of DAFF), who is responsible for determining whether or not imports can be permitted under the *Quarantine Act 1908*, and if so, under what conditions. DAFF is responsible for implementing the import protocol, including any risk management measures.

Australia's science-based risk analysis process is consistent with Australian Government policy and Australia's rights and obligations under the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement).

Australia implements a risk-based approach to biosecurity management. This approach is expressed in terms of Australia's appropriate level of protection (ALOP), which reflects community expectations through government policy and is currently aimed at reducing these risks to a very low level, but not to zero.

If the risks exceed Australia's ALOP, risk management measures are proposed to reduce the risks to an appropriate level. However, if it is not possible to reduce the risks to an appropriate level, then no trade will be allowed.

Background

This review of the biosecurity risks associated with the importation into Australia of queen honey bees has been undertaken in response to the suspension of the previous conditions for the importation of honey bees (see Appendix A) in August 2008 because of concerns regarding the emergence internationally of a potentially new disease affecting honey bees: colony collapse disorder.

As the Australian honey bee industry requires imported honey bee genetics for improvement programs in commercial honey bee breeding enterprises, this review is restricted to the importation of queen honey bees.

Scope

This review considers the biosecurity risks that may be associated with the importation into Australia of queen honey bees of the species *A. mellifera* (the European or western honey bee). The review includes an assessment of all the potential diseases and pests that may be introduced to Australia via the importation of these honey bees.

The previous conditions contained a list of countries (see Appendix A) from which importation is permitted. DAFF considered this list to be outdated.

Detailed information has been gathered in support of this assessment through review of the scientific literature and existing policy, and is provided in Chapter 4. This review also documents the risk assessment and proposes risk management measures for the importation of queen honey bees.

For the purposes of this review, 'hazard', 'pathogenic agent' or 'disease agent' can refer to infectious organisms, internal or external parasites, syndromes of unknown aetiology or detrimental species, sub-species or hybrids.

Previous import policy for live honey bees

Genetic material has been imported into Australia via the importation of live honey bees, most recently under the conditions in the Australian Quarantine Policy Memorandum (AQPM) 1996/42 *Conditions for the Importation of Honey Bees* which provide for queen honey bees to be imported and propagated under secure quarantine conditions with subsequent release of grafted queen cells to importers (see Appendix A).

Importation from the USA under these conditions was suspended in February 2006 as it was not possible to determine whether honey bees sourced from that country contained genes from the African honey bee, *Apis mellifera scutellata*.

The *Conditions for the Importation of Honey Bees* (AQPM 1996/42) was suspended completely in August 2008 because of concerns about the international spread of colony collapse disorder.

Approved countries

Australia takes into account the following criteria when considering the approval of conditions to export animals and their products to Australia:

- the animal health status of the country
- the effectiveness of the veterinary service and other relevant certifying authorities
- legislative controls over animal health, including biosecurity policies and practices
- the standard of reporting to the World Organisation for Animal Health (OIE) of major contagious disease outbreaks
- effectiveness of veterinary laboratory services, including compliance with relevant international standards, and
- effectiveness of systems for the control over certification/documentation of products intended for export to Australia.

DAFF restricted the scope of this review to specified countries (Canada, the European Union, Japan, New Zealand and the USA) as this provides a satisfactory level of assurance of the exporting countries' capacity to certify to Australia's biosecurity requirements.

Domestic movement regulations

The Australian Government is responsible for regulating the movement of animals and their products into and out of Australia. The state and territory governments have primary responsibility for animal health controls within their jurisdictions. Legislation relating to resource management or animal health may be used by state and territory governments to control interstate movement of animals and their products.

There are certain interstate movement controls for honey bees and their products. In particular, Western Australia is free of European foulbrood and prohibits the importation of honey and other honey bee products unless pasteurised or otherwise treated.

The honey bee industry in Australia

The honey bee

Honey bees are classified as the tribe Apini within the family Apidae. There is only one genus of honey bee, *Apis*, which includes eight known species of Asian honey bees as well as *Apis mellifera*—the European or western honey bee (Oldroyd and Wongsiri 2006). *A. mellifera* originated in Africa and has expanded into Eurasia at least twice (Whitfield et al. 2006). These expansions resulted in the four major lineages of honey bee diversity: the African lineage (A), the Eastern European lineage (C), the Western European lineage (M) and the Middle Eastern lineage (O) (Franck et al. 1998; Franck et al. 2001; Whitfield et al. 2006).

Within each of these lineages, there are various named subspecies which roughly correspond to their region of origin. In the A lineage, for example, there are more than seven named subspecies: *A*. *m*. *adansonii*, *A*. *m*. *capensis*, *A*. *m*. *intermissa, A*. *m*. *lamarkii, A*. *m*. *litorea*, *A*. *m*. *scutellata* and *A*. *m*. *unicolor*.

Modern beekeeping is mainly based on honey bees of the C lineage, particularly *A*. *m*. *carnica* and *A*. *m*. *ligustica*. *A*. *m*. *caucasica*, of the O lineage, is also popular. The original honey bees introduced into Australia from England and Spain were of the M lineage: *A*. *m*. *iberica* and *A*. *m*. *mellifera*. Descendents of these original imports make up the majority of existing feral honey bee populations in Australia (Oldroyd et al. 1992; Oldroyd et al.1995; Chapman et al. 2008). As far as can be determined, honey bees of the A lineage were never introduced into Australia, though there is one report of an import from Syria (Goodacre 1935). Honey bees of *A*. *m*. *ligustica* origin (the so-called ‘Italian’ bees) are now the major commercial subspecies in Australia.

Structure of the industry

Managed honey bees are found in all Australian states and territories. A survey of the industry carried out by the Rural Industries Research and Development Corporation (RIRDC) in 2007 estimated that, based on registration, there were around 10 000 beekeepers operating approximately 600 000 hives (RIRDC 2007). The largest concentration of hives was in New South Wales (NSW) and about 75 per cent of those hives were operated by beekeepers with a minimum of 200 hives and were considered to represent the commercial beekeeping industry (see Figures 1 and 2). The situation is similar in other states.

Migratory beekeeping involves moving hives from one source of flowering plants to another, and it is practised by virtually all commercial beekeeping operations. In addition, hives are being increasingly used for paid pollination services—this entails the movement of large numbers of hives from many sources, often over long distances, to concentrated points where pollination is required.

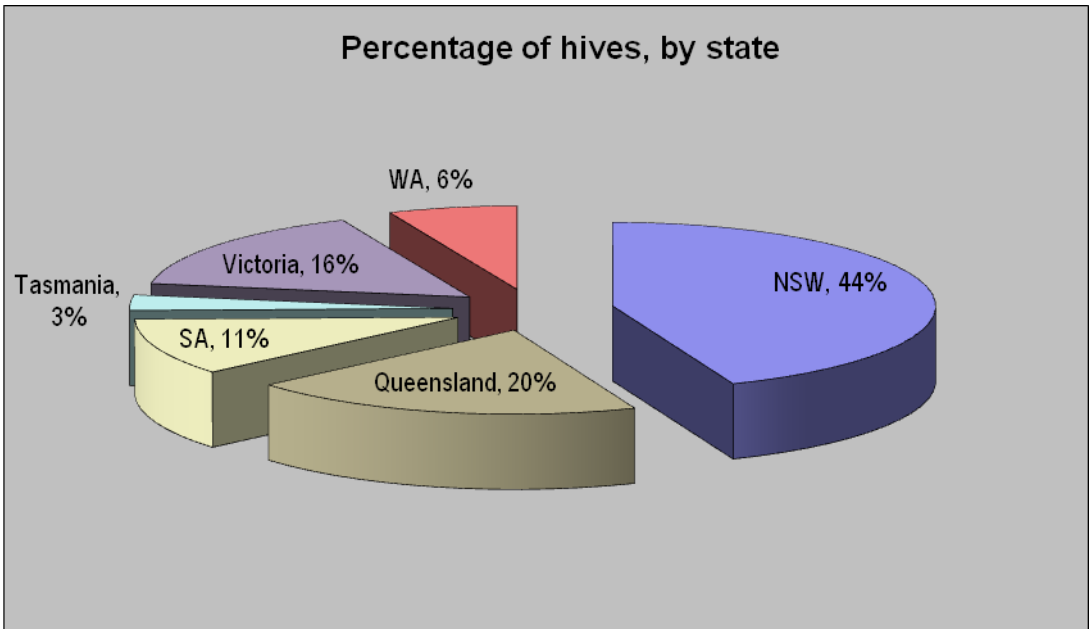

Figure 1. Beekeeping by state (from RIRDC 2007)

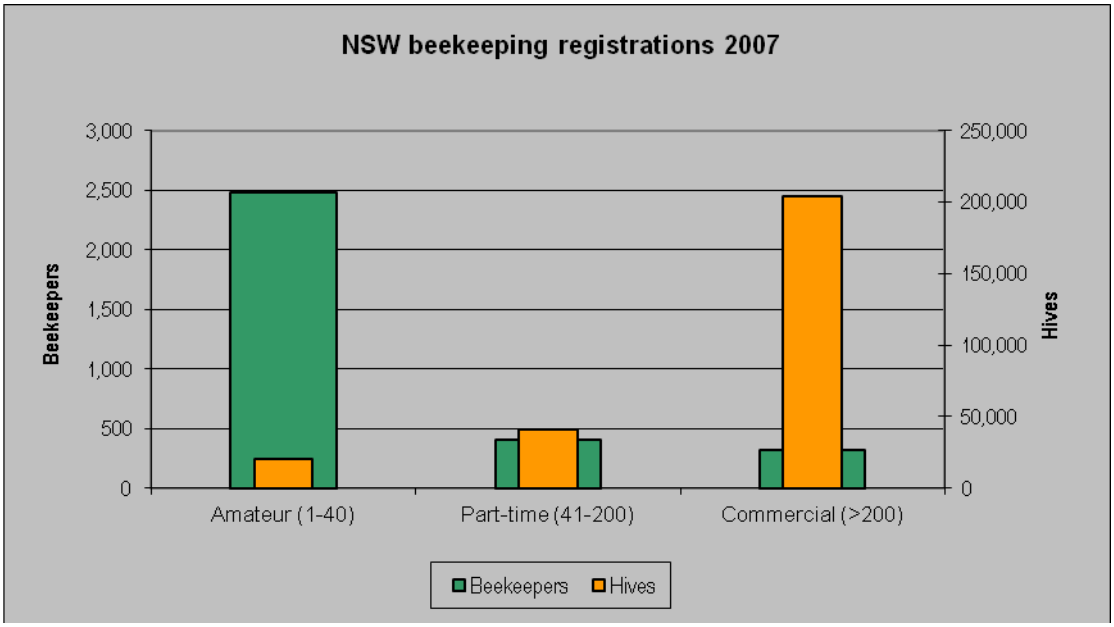

Figure 2. Type of beekeeping in NSW (from RIRDC 2007)

Industry bodies

There are associations of both commercial and amateur beekeepers in all states, composed of regional branches and a central body. The Federal Council of Australian Apiarists' Associations represents the state associations. A national body, the Australian Honey Bee Industry Council (AHBIC), represents the whole industry including the state associations, honey packers and marketers, queen honey bee breeders and representatives of the National Council of Pollination Associations. The AHBIC is voluntarily funded by contributions based on numbers of hives and on the amount of honey and queen honey bees sold.

Honey bee health

International

Diseases of Apidae, as listed by the OIE (OIE 2011) are:

- Acarapisosis (tracheal mite)
- American foulbrood
- European foulbrood
- Small hive beetle infestation (*Aethina tumida*)
- Tropilaelaps infestation
- Varroosis

A comprehensive survey of world honey bee health was published in 1993 (Matheson 1993) and updated in 1995 and 1996 (Matheson 1996). It showed the worldwide distribution of 11 honey bee diseases and parasites—American foulbrood, amoeba disease, *Braula* fly, chalkbrood, European foulbrood, Kashmir bee virus, nosemosis, sacbrood virus, tracheal mite, *Tropilaelaps* and *Varroa*.

Data have been collected from many sources to show the worldwide distribution of 12 viruses in *A. mellifera* (Allen and Ball 1996). Arkansas bee virus and Berkeley bee virus were not included in the survey as they appeared to have a strictly limited distribution. In addition, limited surveys were carried out on the other major *Apis* species. Reports on *A. cerana* from throughout Asia were collated for *Apis* iridescent virus, deformed wing virus, Kashmir bee virus and Thai sacbrood virus. Thai sacbrood virus has also been identified in both *A. dorsata* and *A. florea* in India, and black queen cell virus has been identified in *A. florea* in Iran (Allen and Ball 1996).

A review of the taxonomy of *Varroa* and a survey of its distribution on *A. cerana* and *A. mellifera* was carried out in the late 1990s (Anderson 2000) and, as a result, the species formerly known as *Varroa jacobsoni* was split into two—*V. destructor* and *V. jacobsoni*.

A similar review of the taxonomy of *Tropilaelaps* and a survey of its distribution in Asia was carried out in 2007 (Anderson 2007). As a consequence, the species formerly known as *Tropilaelaps clareae* was split into *T. clareae* and *T. mercedesae* and a new species *T. thaii* was identified.

Australia

Of the OIE listed diseases, American foulbrood is endemic throughout Australia and European foulbrood is present across the eastern seaboard. Western Australia (WA) remains free of European foulbrood.

Small hive beetle is present in the eastern states and in the north-west of WA but the south-west of WA, South Australia (SA) and Tasmania remain free.

Australia remains free of the three listed acarine mites—tracheal mites (*Acarapis woodi* or acarapisosis), *Tropilaelaps* and *V. destructor*. *V. jacobsoni* is found only on the Asian or eastern hive bees (*A. cerana*) on islands in the Torres Strait, adjacent to Papua New Guinea (PNG). *V. jacobsoni* was, until recently, not thought to be a pathogen of *A. mellifera*, but investigations in PNG have found a new form of *V. jacobsoni* that is deleterious to *A. mellifera* (Anderson 2008).

The OIE listed diseases are nationally notifiable in Australia and additional agents such as *Braula* fly, chalkbrood and nosemosis are notifiable in some jurisdictions (see Table 6). NSW, SA, Tasmania, Victoria and WA also require the notification of Africanised honey bees. These are honey bees of the sub-species *Apis mellifera scutellata* and its hybrids—the so-called 'killer bees' that have spread rapidly through the Americas over the past 50 years. Africanised honey bees remain exotic to Australia, as does another sub-species seen as detrimental to managed hives, *Apis mellifera capensis*—the Cape honey bee.

Australian emergency disease plans

The Australian Veterinary Emergency Plan (AUSVETPLAN) is Australia's national plan for responding in a consistent manner to an outbreak, or suspected outbreak, of an emergency animal disease. The AUSVETPLAN *Disease Strategy: Bee diseases and pests* (Animal Health Australia 2010) provides a framework for responding to the introduction of Africanised honey bees, Asian honey bees, *Braula* fly, tracheal mite, *Tropilaelaps* and *Varroa*. The emergency management of honey bee diseases and pests is in the process of being incorporated into the Australian Emergency Plant Pest Response Deed (EPPRD), a cost sharing agreement between government and industry, and the Australian Emergency Plant Pest Response Plan (PLANTPLAN), which are administered by Plant Health Australia.

Horticulture

The horticultural and honey bee industries are linked by the use, either deliberately or inadvertently, of honey bees as pollinators. Paid pollination is an increasing source of income for beekeepers in Australia. In recognition of the interdependence of the two industries, Pollination Australia was formed. It consists of representatives from the honey bee industry, pollination-dependent industries, research and development corporations and the Australian Government.

Some horticultural crops such as almonds set very little fruit without insect pollination. Others such as cucurbits and strawberries also require effective pollination by honey bees for fruit quality (shape and size).

Native bees

There are more than 1500 described species of bees native to Australia. Australian native bees are from different genera, subfamilies or families to honey bees.

There are approximately 15 species of the social stingless bees in the tribe Meliponini, represented by two genera, *Austroplebia* and *Tetragonula*. These are small, 3–8 mm, and are the only native species to make and store honey. However, the manner in which they do this differs significantly from *Apis* species—they mass provision the cells, an egg is then laid and sealed. The storage cells constructed are usually in clusters of small resinous pots near the extremities of the nest; the arrangement of the cells differs from species to species. These social stingless bees range from northern Australia and down the east and west coasts; on the east coast they do not survive in the wild further south than the NSW South Coast. Some species also live in central Australia. They are potential competitors with *A*. *mellifera* for floral resources in some instances, particularly where their ranges strongly overlap; however where *A*. *mellifera* do not thrive (in central and the very northern areas), there is no interaction.

The vast majority of the native bee species are solitary and include, for example, blue-banded bees, carpenter bees, *Homilictus* bees, leafcutter bees, masked bees, reed bees, resin bees and teddy-bear bees. A few species have a broad distribution across Australia however; in general, most species have a fairly limited distribution and are often dependant on a very narrow range of flowers.They range in size from a few millimetres to more than 20 mm in length. The nests vary from burrows cut into timber (the carpenter bees) to tunnels underground. In most species, all the work and provisioning of the nests is undertaken by a single individual female. In some exceptional circumstances cooperation between individuals is exhibited. These species do not store honey but collect tiny amounts of nectar to feed to their young. All native bees have a role in pollination but the buzz pollinators such as blue-banded bees (*Amegilla* species) have been identified as a potential effective substitute for bumblebees (*Bombus* species) inside greenhouses (Australian Native Bee Research Centre 2010).

The hazards considered in this review are pests or diseases of *A. mellifera* or *Apis* species, although some honey bee viruses may also be found in *Bombus* species. Australian native bees were determined not to be susceptible to any hazards considered in this review because of the significant biological and behavioural differences.

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2 Method

Overview

DAFF is responsible for developing and reviewing biosecurity policy for the import of animals and plants and their products. It does this through a science-based risk analysis process.

This review was conducted according to the principles outlined in Chapter 2.1 of the OIE *Terrestrial Animal Health Code* (OIE 2011c) for undertaking risk analysis. The components of risk analysis are:

- hazard identification
- risk assessment, incorporating
    - release assessment
    - exposure assessment
    - consequence assessment
    - risk estimation
- risk management
- risk communication.

At the completion of the process, a recommendation for a policy determination is made to Australia's Director of Animal and Plant Quarantine. This determination is taken into account by DAFF when considering import applications.

Australia's science-based risk analysis process is consistent with Australian Government policy and Australia's rights and obligations under the SPS Agreement.

Australia has a long-standing risk-based approach to biosecurity risk. The level of risk Australia is prepared to accept is known as Australia's ALOP and is expressed as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

Australia takes a consistent approach to addressing risks. Those risks that are 'very low' or less meet Australia's ALOP and no risk management measures are required. For those biosecurity risks that exceed Australia's ALOP, i.e. those risks that are greater than 'very low', risk management measures are recommended to reduce the level of risk in order to achieve the ALOP.

Hazard identification

Hazard is defined by the OIE as 'a biological, chemical or physical agent in, or a condition of, an animal or animal product with the potential to cause an adverse health effect' (OIE 2011b).

Hazard identification is described in the OIE Code 2.1 (OIE 2011c) as the process of identifying the hazards (also known as pathogenic agents) that could potentially produce adverse consequences if introduced in an imported commodity.

The OIE Code states that to be identified as a potential hazard in this review, an agent:

- should be appropriate to the animal species to be imported, or from which the commodity is derived

- should be OIE listed, emerging and/or capable of producing adverse consequences in the importing country
- may be present in the exporting country and
- should not be present in the importing country. If present, the hazard should be associated with a notifiable disease or be subject to an official control or eradication program.

In this review, hazard identification was initiated by generating a preliminary list of potential hazards with reference to the OIE disease list (OIE 2011a), the *Draft generic import risk analysis (IRA) for honeybee semen Technical Issues Paper*, *August 2002* (BAPM 2002/40), *Progress report on the import risk analysis of honeybee semen* (BAPM 2006/08), the *United States Department of Agriculture Diagnosis of Honey Bee Diseases* (Shimanuki and Knox 2000) and relevant scientific literature. The list was refined by applying the criteria stated above to each potential hazard. If reasons for the inclusion or exclusion of particular agents were not clear-cut, they were retained in the list and examined further in the risk assessment.

Risk assessment

Risk assessment is defined in the OIE Code as 'the evaluation of the likelihood and the biological and economic consequences of entry, establishment and spread of a hazard within the territory of an importing country'.

The OIE Code notes that 'the principal aim of import risk analysis is to provide importing countries with an objective and defensible method of assessing the disease risks associated with the importation of animals … ' and further 'provides recommendations and principles for conducting transparent, objective and defensible risk analyses for international trade'.

In accordance with the OIE Code, the 'release assessment describes the probability of the 'release' of each of the potential hazards (the pathogenic agents)' in an importing country and 'exposure assessment consists of describing the biological pathway(s) necessary for exposure of animals … and estimating the probability of the exposure(s) occurring'. The consequence assessment describes the potential consequences of a given exposure and estimates the probability of them occurring. The risk assessment for an identified disease agent concludes with risk estimation—the combination of the likelihood of release and exposure, and likely consequences of establishment and/or spread—and yields the unrestricted risk estimate. These steps are illustrated diagrammatically in Figure 3.

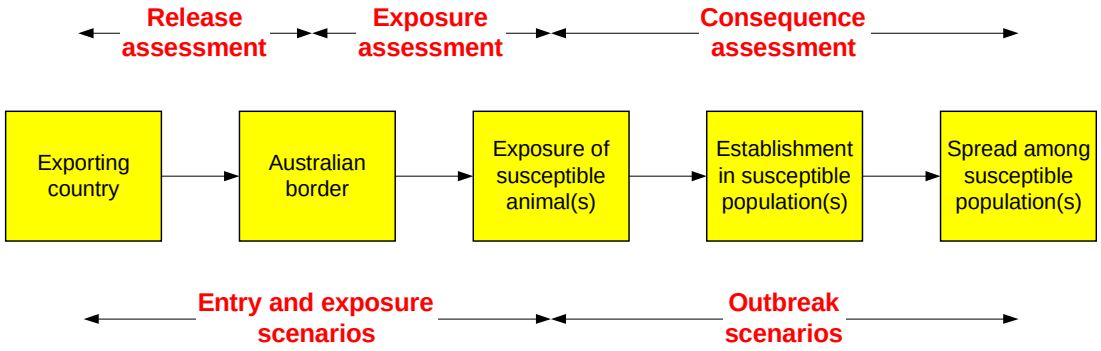

Figure 3. Components of the risk assessment

Evaluating and reporting likelihood

In this assessment, DAFF used available data sources, including information on related disease agents and host species. The assessment was conducted using a qualitative approach. The likelihood (or probability) that an event will occur was evaluated and reported qualitatively, using qualitative likelihood descriptors for the release and exposure assessment, and the outbreak scenario (Table 1).

Table 1. Nomenclature for qualitative likelihoods

Likelihood	Descriptive definition
High	The event would be very likely to occur
Moderate	The event would occur with an even probability
Low	The event would be unlikely to occur
Very low	The event would be very unlikely to occur
Extremely low	The event would be extremely unlikely to occur
Negligible	The event would almost certainly not occur

Risk assessment framework

Apis mellifera is not native to Australia and the first introduction dates back to the early 19th century (Warhurst and Goebel 2005). The species has spread widely throughout the continent in both managed and feral colonies and has only been limited by unfavourable climatic conditions. The evaluation of disease risks involved estimating the likelihood of susceptible honey bees in Australia becoming exposed to a disease agent (hazard) and the likely consequences of such exposure.

In evaluating the likelihood of susceptible honey bees in Australia becoming exposed to a hazard, the following factors were considered:

- the likelihood of the hazard being released into Australia via imported queen honey bees (release assessment) and
- the likelihood of susceptible honey bees becoming exposed to the hazard via imported queen honey bees (exposure assessment).

The determination of likely consequences required:

- the likelihood of the hazard being released into Australia via imported queen honey bees (release assessment)
- the identification of the most likely outbreak scenario that could follow exposure to a hazard. Possible outbreak scenarios can range from no infection occurring to the hazard establishing and spreading throughout local managed and feral honey bee colonies with further spread to other susceptible populations. Only the most likely outbreak scenario relating to the establishment and/or spread for each hazard was assessed
- estimation of the likelihood of establishment and/or spread for that outbreak scenario
- the effects (health, environmental and socioeconomic) associated with that outbreak scenario.

Likelihoods were assigned to release, exposure and establishment and/or spread (outbreak) scenarios.

The risk assessment considered the likelihood of entry and exposure of a disease agent over a period of one year.

This review did not consider Australia's previous risk management measures for imported live honey bees when estimating risk. The risk assessment thus concluded with an unrestricted risk for each hazard. If the unrestricted risk did not achieve Australia's ALOP, then risk management measures were recommended to reduce the risk in order to achieve the ALOP.

The outbreak scenario resulting from the exposure of susceptible animals was considered in a single pathway resulting in infection and establishment. Detailed disease considerations are presented in Chapter 4.

Release assessment

The release assessment considered a single release scenario defined as the period from pre-export inspection, through transport to Australia from the importing country, up to the arrival in Australia. A number of factors were taken into account in determining the likelihood of a disease agent entering Australia in a queen honey bee such as:

- exposure of a honey bee or honey bee colonies
- transmission of the disease agent
- prevalence of the hazard in the exporting country, zone or compartment
- the age of honey bees to be imported
- agent predilection sites
- the effect of storage and transport
- whether the hazard is detected at an Australian port of entry.

No risk management measures were considered in the unrestricted release scenario, except basic evaluation of health and fitness to travel by the certifying authority in the country of origin.

The final outcome of the release assessment is the likelihood of entry of a potential hazard into Australia.

Exposure assessment

The exposure assessment describes the process that was used to estimate the likelihood that a susceptible honey bee in Australia will be exposed to an infected honey bee. It takes into account the groups of bees most likely to be affected as well as the possible pathways by which exposure of these groups of animals could occur.

The recognised exposure groups in this review were:

Group 1 – managed *A. mellifera* colonies and

Group 2 – feral *A. mellifera* colonies.

As these groups are so closely related, they were combined to form one exposure group. Non-susceptible animals were not considered.

The potential for transmission by different pathways is shown in Figure 4. For each agent, the final outcome of the exposure assessment was an estimate of the likelihood that susceptible honey bees would be exposed to the disease agent (i.e. the likelihood of exposure). The likelihood estimation of the exposure assessment did not consider Australia's previous risk management options for imported queen honey bees.

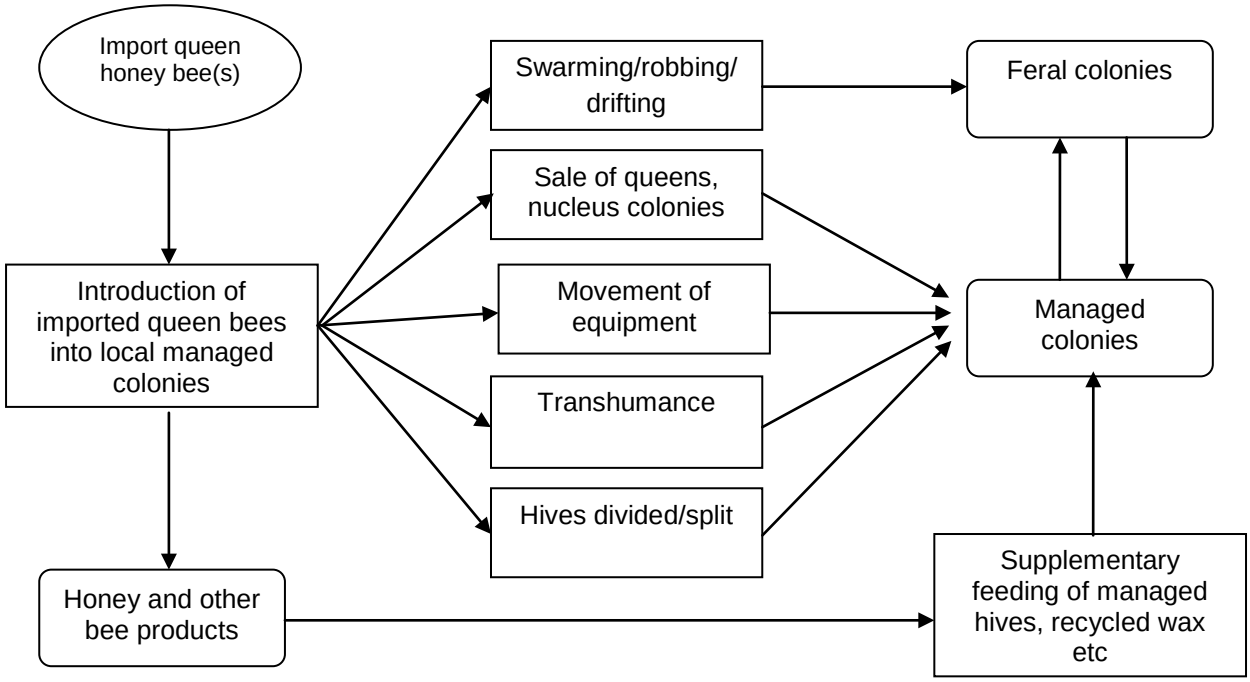

Figure 4. Possible transmission pathways

Estimation of the likelihood of release and exposure

The likelihood of release and exposure was the estimated likelihood that there was at least one exposure event during an average year for the expected number of queen honey bees imported from countries where the hazard being assessed was endemic.

The likelihood of release and exposure was estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix shown in Table 2. The basis for combining qualitative likelihoods using a matrix is described by Standards Australia and Standards New Zealand (Standards Australia 2005).

Table 2. Matrix for combining qualitative likelihoods

	High	**Moderate**	**Low**	**Very low**	**Extremely low**	**Negligible**
High	High	Moderate	Low	Very low	Extremely low	Negligible
Moderate	Moderate	Low	Low	Very low	Extremely low	Negligible
Low	Low	Low	Very low	Very low	Extremely low	Negligible
Very low	Very low	Very low	Very low	Extremely low	Extremely low	Negligible
Extremely low	Extremely low	Extremely low	Extremely low	Extremely low	Negligible	Negligible
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible

Consequence assessment

Criteria for assessing consequences associated with a pest or disease incursion are outlined in relevant Australian legislation and international agreements, and in the standards prepared by the OIE. In particular:

- the *Quarantine Act 1908* requires decision makers to take into account the probability of harm being caused (to humans, animals, plants, other aspects of the environment, or economic activities) and the probable extent of the harm (Section 5D)
- the SPS Agreement states that 'Members shall take into account as relevant economic factors: the potential damage in terms of loss of production or sales in the event of the entry, establishment or spread of a pest or disease; the costs of control or eradication in the territory of the importing Member; and the relative cost-effectiveness of alternative approaches to limiting risks'
- the OIE Code expands the 'relevant economic factors' described in the SPS Agreement and provides examples of factors that will typically be relevant. In each case, consequence assessments do not extend to considering the benefits or otherwise of trade in a given commodity, nor to the effect of import competition on industries or consumers in the importing country.

The OIE Code also states that a consequence assessment 'describes the potential consequences of a given exposure and estimates the probability of them occurring'. This

approach is reflected in the *Quarantine Proclamation 1998*, which requires that the 'level of quarantine risk' is considered when deciding whether to grant a permit for importation into Australia (Section 70).

In this review, likely consequences are considered for those outcomes attributable to the most likely outbreak scenario. These were addressed in terms of direct and indirect effects on animal and plant life and health on a national scale, including adverse health, environmental and socioeconomic effects (as detailed below), and separately in terms of consequences to human life or health. The latter is dealt with separately because primary responsibility for matters of human life or health rests with the Australian Government Department of Health and Ageing.

The following sequence of steps was taken in determining the likely consequences associated with an outbreak scenario:

1. identification of the most likely outbreak scenario (detailed in the relevant disease chapter) that may occur as a result of release of a hazard and exposure to susceptible honey bee populations

2. estimation of the likelihood of the outbreak scenario occurring to obtain a likelihood of establishment and/or spread

3. determination of the effects (health, environment and socioeconomic) resulting from the outbreak scenario

4. combination of the likelihood of establishment and/or spread for the outbreak scenario with the corresponding effect to obtain an estimation of likely consequences.

Identification of an outbreak scenario

Once exposure of a susceptible honey bee population has occurred, a number of possible outbreak scenarios could follow, representing a continuum ranging from no spread to widespread establishment. For risk assessment purposes, two outbreak scenarios were considered based on the epidemiology of each hazard for the single exposure group identified.

Outbreak scenario 1 – disease agent does not establish or is not recognised within the directly exposed population

Outbreak scenario 2 – disease agent establishes in directly exposed population, spreads and becomes endemic in Australia.

It was assumed that eradication of any exotic honey bee pest or disease using legislated control measures would occur based on:

- notification in Australia
- inclusion in an cost sharing agreement between government and industry
- there being a single peak body representing potentially susceptible honey bee populations (AHBIC).

Whether or not an exotic honey bee pest or disease becomes endemic or is eradicated by natural causes (e.g. lack of competent vectors) will depend on the nature of the pathogenic agent under consideration.

For each disease agent, the likelihood of establishment and/or spread, and the associated overall effect for the outbreak scenario was determined. The likely consequences were determined using the matrix shown in Table 2.

Likelihood of establishment and/or spread associated with the outbreak scenario

When estimating the likelihood of establishment and/or spread associated with the outbreak scenario, qualitative descriptors were used as detailed in Table 2.

Determination of the effects resulting from the outbreak scenario

Potential effects of establishment and/or spread associated with the outbreak scenario may be direct or indirect.

Direct effects:

- life or health (including production effects) of susceptible animals, including public health consequences
- the living environment, including life and health of wildlife, and any effects on the non-living environment.

Indirect effects:

- new or modified eradication, control, monitoring or surveillance and compensation strategies or programs
- domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries
- international trade, including loss of markets, meeting new technical requirements to enter or maintain markets and changes in international consumer demand
- the environment, including biodiversity, endangered species and the integrity of ecosystems
- communities, including reduced tourism, reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures.

An effect was not assessed more than once and direct effects were considered separately from indirect effects.

The overall effect of establishment and/or spread associated with the outbreak scenario took into account the geographic level of these effects:

- local—restricted to a single locality or town
- regional—a recognised geographic area such as far north Queensland
- state or territory
- national

and the magnitude of these effects:

- indiscernible—not usually distinguishable from normal day-to-day variation
- minor significance—recognisable, but minor and reversible
- significant—serious and substantive, but reversible and unlikely to have permanent economic effects
- highly significant—extremely serious and irreversible and likely to have permanent economic effects.

Based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread was determined using the rules described in Table 3.

Table 3. Rules for determining the overall effect of establishment and/or spread

Extreme	The effect is likely to be highly significant at the national level. Implies that economic stability, societal values or social well-being would be seriously affected.
High	The effect is likely to be significant at the national level and highly significant within affected zones. Implies that the effect would be of national concern. However, serious effects on economic stability, societal values or social well-being would be limited to a given zone.
Moderate	The effect is likely to be recognised on a national level and significant within affected zones. The effect is likely to be highly significant to directly affected parties.
Low	The effect is likely to be recognised within affected zones and significant to directly affected parties. It is not likely that the effect will be recognised at the national level.
Very low	The effect is likely to be minor to directly affected parties. The effect is unlikely to be discernable at any other level.
Negligible	The effect is unlikely to be recognised at any level within Australia.

Derivation of likely consequences

The likely consequences were estimated by combining the likelihood of establishment and/or spread (associated with the outbreak scenario) with the overall effect of establishment and/or spread using the matrix shown in Table 4.

Table 4. Likely consequences: a combination of the likelihood and overall effect of establishment and/or spread

Likelihood of establishment and/or spread						
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Very low	Low	Moderate	High	Extreme
Low	Negligible	Negligible	Very low	Low	Moderate	High
Very low	Negligible	Negligible	Negligible	Very low	Low	Moderate
Extremely low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low
	Negligible	*Very low*	*Low*	*Moderate*	*High*	*Extreme*

Overall effect of establishment and spread

Risk estimation

Risk estimation is the integration of likelihood of release and exposure, and likely consequences of establishment and/or spread. This derives the unrestricted risk associated with release, exposure, establishment and/or spread of a hazard introduced by the importation of queen honey bees into Australia.

Estimation of risk of release, exposure, establishment and/or spread

The risk is estimated by:

- determining the likelihood of release and exposure
- combining the likelihood of release and exposure with the estimate of likely consequences of establishment and/or spread.

Combining the likelihood of release and exposure and likely consequences of establishment and/or spread was undertaken using the rules shown in the risk estimation matrix in Table 5.

Table 5. Risk estimation matrix

Likelihood of release and exposure	Likely consequences of establishment and/or spread					
High likelihood	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate likelihood	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low likelihood	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low likelihood	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Extremely low likelihood	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible likelihood	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk
	Negligible effect	*Very low effect*	*Low effect*	*Moderate effect*	*High effect*	*Extreme effect*

Evaluation of unrestricted risk

Risk evaluation is described in the OIE Code as the process of comparing the estimated risk with a country's ALOP.

A risk estimation that was either 'very low' or 'negligible' was considered sufficient to achieve Australia's ALOP. This provided a benchmark for evaluating risk and determining whether risk management was required.

The use of a benchmark for evaluating risks for each disease agent is illustrated in the process outlined below:

- if the unrestricted risk was 'negligible' or 'very low', then it achieved Australia's ALOP and risk management was not required
- if the unrestricted risk was 'low', 'moderate', 'high' or 'extreme', risk management measures were required.

This was considered the final output of the risk assessment.

Risk management

Risk management options considered in this review aim to reduce the likelihood that the imported queen honey bees would lead to the release, exposure, establishment and/or spread of honey bee pests and diseases of biosecurity concern in Australia. Risk management options included measures relevant to reducing the likelihood of release and/or exposure to achieve Australia's ALOP. They are described in detail in Chapter 5.

In general, risk management can be implemented by reducing the likelihood of:

- disease agents being released into Australia in infected imported honey bees by imposing risk management measures, such as pre-entry measures and post-arrival quarantine, that reduce the likelihood of release

- exposure of susceptible honey bee populations in Australia by infected imported queen honey bees by imposing risk management measures that reduce the likelihood of exposure.

If a disease agent is already present in Australia, Article 2.1.2 of the OIE Code states that import measures are not to be more trade restrictive than those applied within the country.

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3 Hazard identification

Hazard list

In this review, hazard identification (as defined in Chapter 2) was initiated by generating a preliminary list of pests and disease agents of relevance to honey bees that were considered to be potential hazards, with reference to DAFF policy documents, the OIE listed diseases and scientific literature. This list was refined by applying the criteria stated in Chapter 2. The hazard identification decision tree is shown in Figure 5.

The potential hazard list is set out in Table 6. Of the disease agents considered as being potentially of biosecurity concern, 13 were retained for further risk assessment (see Chapters 4 and 5). Substantiating decisions to retain or reject pests and disease agents as hazards for further consideration in the review are provided in the following section.

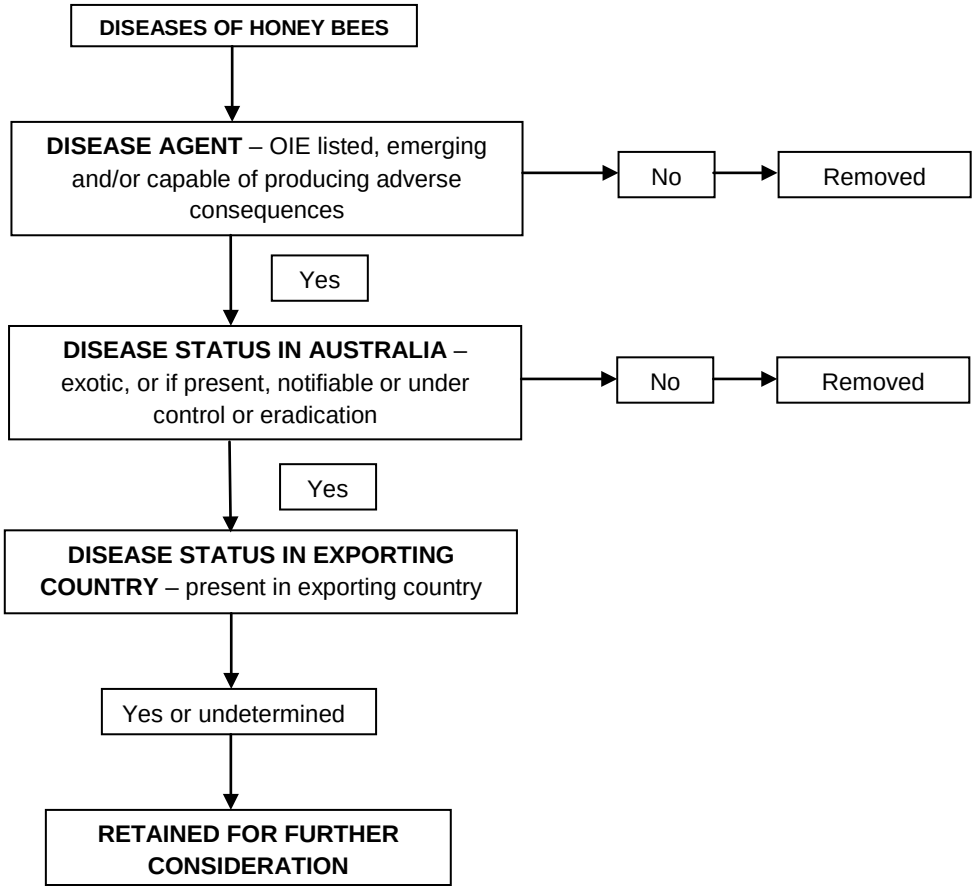

Figure 5. Hazard identification and refinement decision tree

Table 6. Hazard identification and refinement

Hazard	Present in Australia	Controls within Australia	Distribution outside Australia	Adverse consequences	Retain for further consideration (Yes / No)
Bacteria					
American foulbrood (*Paenibacillus larvae*)	Yes	Notifiable disease	Worldwide	Yes	**Yes, retained as it is notifiable within Australia**
European foulbrood (*Melissococcus plutonius*)	Yes except in WA	Notifiable disease	Worldwide but not present in New Zealand	Yes	**Yes, retained as it is notifiable within Australia**
Paenibacillus alvei	Yes	No control measures	Worldwide	No	No, removed. It is widespread in Australia and causes secondary infections only
Powdery scale	No	No control measures	Nil	No	No, removed. The putative causal agent– *Paenibacillus larvae* var *pulvifaciens*– has now been shown to be identical to *Paenibacillus larvae* var *larvae* (Genersch et al. 2006)
Fungi					
Chalkbrood (*Ascosphaera apis*)	Yes	Notifiable disease in Victoria	Worldwide	Yes	No, removed as it is now endemic in Australia
Nosema apis	Yes	Notifiable in NSW and Victoria	Worldwide	Yes	No, removed as it is present in Australia with no control measures
Nosema ceranae	Yes except in WA	Notifiable in NSW	Asia, Europe and the USA	Yes	No, removed as it is present in Australia with no control measures
Stonebrood (*Aspergillus* species)	Yes	No control measures	Worldwide	Yes	No, removed as it is present in Australia with no control measures
Pests and parasites					
Braula fly	Tasmania only	Notifiable in NT, SA, Victoria, WA	Worldwide	Yes	**Yes, retained as it is notifiable within Australia**
Phorid fly *Apocephalus borealis*	No	No	USA	Yes	**Yes, retained as it has not been identified within Australia**
External acariasis (*Acarapis dorsalis, A. externus*, *A. vagans*)	Yes	No control measures	Worldwide	No	No, removed as it does not have adverse consequences and there are no control measures in Australia. *A. vagans* is now considered to be a synonym of *A. externus* (García Fernández 1999)

Hazard	Present in Australia	Controls within Australia	Distribution outside Australia	Adverse consequences	Retain for risk assessment (Yes / No)
Pests and parasites cont'd					
Small hive beetle (SHB)	Yes: NSW, Qld, Victoria, WA	Notifiable disease	Africa, North America	Yes	**Yes, retained as there are control measures in Australia and SHB is notifiable in WA**
Acarapisosis (*Acarapis woodi*)	No	Notifiable disease	Europe, North America	Yes	**Yes, retained as it is notifiable within Australia**
Tropilaelaps (*Tropilaelaps* species)	No	Notifiable disease	Asia	Yes	**Yes, retained as it is notifiable within Australia**
Varroosis (*Varroa* species)	No	Notifiable disease	Worldwide	Yes	**Yes, retained as it is notifiable within Australia**
Protozoa					
Amoeba disease (*Malpighamoeba mellificae*)	Yes	No control measures	Worldwide	Yes	No, removed as it is present in Australia with no control measures
Gregarine disease (Gregarinidae)	Yes	No control measures	Worldwide	No	No, removed as it is present in Australia with no control measures
Viruses					
Acute paralysis virus	No	No control measures	Worldwide	Yes	**Yes, retained as it has not been confirmed to be present in Australia**
Apis iridescent virus	Unknown	No control measures	Worldwide	No	No, removed as it has only been isolated from *Apis cerana,* and there are no known adverse consequences in *A. mellifera* (Allen and Ball 1996)
Arkansas (Berkeley) bee virus	No	No control measures	USA	No	No, removed as there are no known adverse consequences
Bee virus X	Yes	No control measures	Europe, New Zealand	Yes	No, removed as it is present in Australia with no control measures
Bee virus Y	Yes	No control measures	Worldwide	Yes	No, removed as it is present in Australia with no control measures
Black queen cell virus	Yes	No control measures	Worldwide	Yes	No, removed as it is present in Australia with no control measures
Chronic paralysis virus	Yes	No control measures	Worldwide	Yes	No, removed as it is present in Australia with no control measures

Hazard	Present in Australia	Controls within Australia	Distribution outside Australia	Adverse consequences	Retain for risk assessment (Yes / No)
Viruses cont'd					
Cloudy wing virus	Yes	No control measures	Worldwide	Yes	No, removed as it is present in Australia with no control measures
Deformed wing virus	No	No control measures	Worldwide	Yes	**Yes, retained as it has not been confirmed to be present in Australia**
Egypt bee virus	No	No control measures	Egypt	No	No, removed as there are no known adverse consequences (Bailey et al. 1979; de Miranda 2008)
Filamentous virus	Yes	No control measures	Worldwide	No	No, removed as it is present in Australia with no control measures
Israeli acute paralysis virus	Yes	No control measures	Israel, North America	Yes	No, removed as it is present in Australia with no control measures
Kakugo virus	No	No control measures	Worldwide	Yes	No, removed as it is classified as deformed wing virus (Carter and Generesch 2008)
Kashmir bee virus	Yes	No control measures	Worldwide	Yes	No, removed as it is present in Australia with no control measures
Sacbrood virus	Yes	No control measures	Worldwide	Yes	No, removed as it is present in Australia with no control measures
Slow paralysis virus	No	No control measures	Britain, Fiji, Switzerland, Western Samoa (de Miranda et al. 2010)	Yes	**Yes, retained as it has not been confirmed to be present in Australia**
Thai sacbrood virus	Unknown	No control measures	Asia	No	No, removed as there are no known adverse consequences in *Apis mellifera* (Allen and Ball 1996)
Varroa destructor virus 1	Unknown	No control measures	Worldwide	No	No, removed as it is genetically closely related to deformed wing virus, and there are no known adverse consequences in *Apis mellifera* (de Miranda 2008)

Hazard	Present in Australia	Controls within Australia	Distribution outside Australia	Adverse consequences	Reason for removal or retention (Yes / No)
Other					
Africanised honey bee (*Apis mellifera scutellata* and its hybrids)	No	Notifiable in NSW, SA, Tasmania, Victoria and WA	Southern Africa, South and Central America, southern and western states of the USA	Yes	**Yes, retained as it is notifiable within Australia**
Apis species other than *A. mellifera*	Yes–an incursion of *A. cerana* has been present in far north Queensland since 2007	*A. cerana* notifiable in NSW, SA and WA Dwarf and giant honey bees notifiable in NSW	Various distributions in Africa, Asia, Middle East, Pacific Islands and Papua New Guinea according to species	Yes	There is reproductive isolation between *A. mellifera* and other *Apis* species (Koeniger and Koeniger 2000). For *Apis cerana* Interspecific matings are possible, though viable offspring do not occur (Ben Oldroyd *pers com* 2012)
Cape honeybee (*Apis mellifera capensis*)	No	No control measures	Southern Africa	Yes	**Yes, retained as it is not present in Australia, and there are adverse consequences in *A. mellifera***
Colony collapse disorder	No	No control measures	Europe and the USA	Yes	**Yes, retained as the syndrome has not been reported in Australia**
Half-moon disorder	No	No control measures	New Zealand?	Unknown	No, removed as the syndrome is of unknown aetiology and it is not now recognised (Alippi 1999)

Conclusion

On the basis of the hazard refinement process, the following pathogenic agents were retained for risk assessment:

Bacteria

- American foulbrood
- European foulbrood

Pests and parasites

- Acarapisosis (tracheal mite)
- *Braula* fly
- Phorid fly *(Apocephalus borealis)*
- Small hive beetle
- *Tropilaelaps*
- Varroosis

Viruses

- Acute paralysis virus
- Deformed wing virus
- Slow paralysis virus

Other

- Africanised honey bee (*A. m. scutellata* and its hybrids)
- Cape honey bee (*A. m. capensis*)
- Colony collapse disorder

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4 Risk assessments

4.1 American foulbrood

Technical information

Background

American foulbrood (AFB) is a severe bacterial brood disease of the European honey bee *Apis mellifera*. It can cause substantial economic losses.

The agent responsible for AFB was first isolated and identified in 1906 as *Bacillus larvae*. It has since undergone a number of taxonomic reclassifications and is now classified as *Paenibacillus larvae* (Genersch et al. 2006).

P. larvae is distributed worldwide. It is present throughout Australia where it is nationally notifiable (DAFF 2011). AFB is an OIE listed disease (OIE 2011b).

OIE requirements

The OIE Code recommendations (OIE 2011a) for the importation of live queen honey bees, worker honey bees and drones require the presentation of an international veterinary certificate attesting that the honey bees come from a country or zone/compartment officially free from AFB.

Agent characteristics

P. larvae is a rod-shaped, facultative anaerobic or micro-aerophilic, gram positive, spore-forming bacterium. The endospores are extremely hardy; they are resistant to desiccation and heat and have been known to survive in scales (the remnants of diseased larvae), soil and food for up to 35 years (Hansen and Brødsgaard 1999).

Epidemiology and pathogenesis

The spores are the only infectious form of *P. larvae* and only honey bee brood is susceptible. Larvae up to three days of age become infected by ingesting spores that are present in their food. Larvae less than 24 hours old are the most susceptible; the LD_{50}[1] for 24-hour-old larvae has been estimated to be only 35 spores (Shimanuki 1997). Resistance to infection increases rapidly with age, with larvae over 48 hours being relatively resistant and adult honey bees completely resistant. Spores can infect the larvae of all honey bee castes with the observed variability in resistance being dependant on food received. For example, queen larvae get the least pollen and are least resistant, while drone larvae get the most pollen and are therefore, the most resistant.

The spores germinate in the gastrointestinal tract of the larva. The vegetative form of the bacteria then starts to grow, using the larva as a source of nutrients. Infected larvae normally

[1] LD_{50} is the dilution of each preparation that would have killed half of the bees in a group

die after their cell is sealed; the proteases produced by the bacteria causing the larval host to disintegrate after death, leaving an indigestible scale. The vegetative form of *P. larvae* also dies but not before it has produced many millions of spores. A single decaying larva may form approximately 2.5 x 10^9 spores. These spores are then distributed by cleaning honey bees that, although resistant to disease, can carry spores in their gastrointestinal tract for up to two months after infection (Hansen and Brødsgaard 1999; Genersch 2010).

The natural spread of AFB between colonies is low and has generally been ascribed to the robbing of spore-containing honey from infected hives. Honey bees drifting between adjacent colonies are only a minor cause of disease spread (Hornitzky 1998). The role of queen honey bees in spreading AFB from colony to colony has been examined, with the consensus being that although they may carry spores in their gastrointestinal tracts, the spores are in numbers too low to pass on the infection (Wilson and Alzubaidy 1975; Greer 2007).

Fries et al. (2006) demonstrated that *P. larvae* spores can be transferred in swarms from sub-clinically infected primary colonies, and that swarms from colonies showing AFB disease can quickly lose the infection and not develop clinical disease. They concluded that AFB was not easily transmitted through vertical transmission (in this case, transmission from mother colonies to daughter swarms) in a natural system. This conclusion, together with the low levels of *P. larvae* generally found in feral colonies (Hornitzky et al. 1996), supports the view that AFB is more a disease of beekeeping than a disease of honey bees. The transfer of infection by the movement of beekeeping equipment or through the feeding of infected honey or pollen, are the most common and effective ways that AFB is spread.

Clinical signs

In colonies with clinical signs of AFB, the dead brood is found in the late larval or pupal stages. The affected combs have a patchy brood pattern consisting of healthy, capped brood, uncapped cells containing the remains of diseased larvae and empty cells. This is referred to as the characteristic ‘pepperbox’ appearance. The capping over the infected cells is dark, sunken and with irregular holes.

The dead larvae first decay to a brown, viscous mass that can be drawn out to a dark, ropy thread exceeding two centimetres in length. The larvae progressively dry into black scales that adhere tightly to the bottom of the cells. If death occurs in the pupal stage, there is a characteristic protrusion of the mouth parts, the ‘tongue’, which point upwards (Alippi 1999).

Combs containing diseased larvae can have a sour odour which gives the disease its common name.

Diagnosis

A preliminary diagnosis may be made on the clinical signs of disease (see Clinical signs above).

Methods for diagnostic procedures are detailed in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE 2008) and by various authors such as Alippi (1999). A brief description of some of these tests will be presented here for completeness.

Routine laboratory diagnosis of AFB was traditionally based on a sample of brood. The Holst milk test, for example, depends on the proteolytic action of enzymes liberated by *P. larvae*

on a milk solution. However, this test is not always reliable as it will not detect AFB infections before dead larvae reach the ropy stage (Shimanuki 1997; Williams 2000).

Standard bacteriological methods are used to examine samples from dead brood. Cultures using selective agar media followed by colony morphology and Gram staining of vegetative bacteria or phase contrast microscopy of the spore form can identify *P. larvae*. The vegetative cells are slender, Gram positive rods with a tendency to form chains of variable length. The spores are ellipsoidal (Alippi 1999). The organism is also catalase positive (Hansen and Brødsgaard 1999).

A culture technique for detecting spores in adult honey bees has been developed (Hornitzky and Karlovskis 1989). The honey bees are homogenised before being filtered, centrifuged and then heat shocked. The sample is then plated onto a culture medium supplemented with nalidixic acid.

A number of methods for the isolation and culture of *P. larvae* spores from honey have also been developed (see Alippi 1997, for example).

Control

Treatment with antibiotics, principally oxytetracycline, has been widely used; tylosin is a suitable alternative (Alippi 1999). However, antibiotic therapy is ineffective against the spore form of *P. larvae*, which means it can mask infection and disease. As a consequence, a number of European jurisdictions have banned its use (European Medicines Agency 2010). There is growing prevalence of resistance to tetracyclines—a survey carried out by the United States Department of Agriculture in western areas of the USA in 2005 showed that 27 per cent of *P. larvae* colonies isolated were resistant (Cox et al. 2005).

In addition, antibiotic residues in honey may reduce its quality and fitness for human consumption and there is some evidence that antibiotic therapy may reduce the viability of brood and the longevity of adult honey bees (Genersch 2010). Antibiotics are not permitted for the control of AFB in mainland Australia (Oldroyd et al. 1989).

Research into the use of non-chemical treatments including breeding for resistance, bio-control through antagonistic bacteria and the use of natural anti-bacterial substances, is continuing. Genersch (2010) noted that, despite considerable efforts devoted to non-chemical treatments, ‘little progress is evident’.

The use of the ‘shook swarm’ technique—the transfer of adult honey bees by shaking them from an infected hive onto disease free comb and equipment—has also been used for AFB control. To date, the experimental results have not been conclusive—Hornitzky and White (2001) had a high proportion of shaken hives subsequently dying, while Pernal (2008) observed a reduction in disease symptoms and spore concentrations, and an increase in colony viability.

Resistance to AFB has a genetic component and selective breeding for disease resistance has long been pursued (Shimanuki 1997). A number of breeding programs have concentrated on the ‘hygienic’ behaviour of honey bee lines—how well and how quickly workers remove infected brood (Spivak and Reuter 2001; Brødsgaard and Hansen 2003). Some of the variability in response to infection has been attributed to the variability in virulence of different strains and genotypes of *P. larvae* (Genersch 2005).

Control of AFB infection often relies on the complete destruction of infected hives, and hence destruction of the colony, by burning or sterilisation by irradiation. These methods are successful in minimising the incidence of AFB (Somerville 2010).

Conclusion

AFB is present in Australia and is likely to be present in exporting countries. DAFF concluded that based on all the available information, further assessment of AFB was not required.

Although AFB is nationally notifiable in Australia, the agent is endemic throughout the country. Consequently, besides a general requirement that honey bees for export should come from hives or colonies without obvious signs of disease, there should be no restrictions placed on importation due to AFB.

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4.2 European foulbrood

Technical information

Background

European foulbrood (EFB) is a contagious bacterial disease of the larvae of honey bees and can cause extensive losses in both amateur and commercial apiaries. EFB is primarily a disease of *Apis mellifera*; however, there have been a few reports of infections in other *Apis* species (Bailey and Collins 1982; Oldroyd and Wongsiri 2006).

The disease has a worldwide distribution with the notable exception of New Zealand (Matheson 1993; Matheson 1996). The identification of the disease in Australia was confirmed in 1977 but it was suspected to be present earlier (Tham 1978). WA is the only state or territory in Australia that is EFB free. Geographically diverse Australian isolates of EFB exhibit minimal genotypic diversity (Djordjevic et al. 1999).

EFB is an OIE listed disease (OIE 2011a) and is nationally notifiable in Australia (DAFF 2011).

OIE requirements

The OIE Code recommendations (OIE 2011b) for the importation of live queen honey bees, worker honey bees and drones are certification that the honey bees come from a country or zone/compartment officially free from EFB.

Agent characteristics

The causative agent of EFB was first identified in 1912 and named as *Bacillus pluton*. The agent was renamed *Streptococcus pluton* on the basis of Gram reaction and morphology. It was later reclassified as *Melissococcus pluton* (Bailey and Collins 1982; Shimanuki 1997) and is now known as *M. plutonius*.

M. *plutonius* is a Gram positive, non-spore forming lanceolate coccus that occurs singly, in pairs or in short chains. The organism can be difficult to isolate due to its growth requirements—it is microaerophillic to anaerobic and requires carbon dioxide—and competition from secondary bacteria which are usually present in collected samples (Alippi 1999).

M. *plutonius* itself is usually only detectable early in the infection cycle and the odour associated with EFB and the consistency of the dead larvae is associated with secondary infections from one or more of four other species of bacteria (Forsgren 2010). The role of these organisms in EFB is not fully understood.

- *Achromobacter euridice* (formerly *Lactobacillus eurydice* and *Bacterium eurydice*) is frequently found in larvae with EFB but it is also a normal inhabitant of the gastrointestinal tract of adult honey bees and healthy larvae. It has not been reported in Australia (Djordjevic et al. 1998)

- *Paenibacillus alvei* (formerly *Bacillus alvei*) was initially thought to be the causative agent of EFB due to its isolation from affected larvae. The presence of *P*. *alvei* is used as an indicator of EFB as its growth produces a characteristic odour
- *Enterococcus faecalis* produces a sour smell. In contrast to *M*. *plutonius*, it grows quickly and easily on nutrient agar
- *Brevibacillus laterosporus* is only occasionally found in EFB.

Alippi (1999) also mentions *Paenibacillus apiarius* but with the caveat that it is rarely found and may not truly be associated with EFB.

Epidemiology and pathogenesis

Larvae become infected through ingestion of contaminated brood food supplied by the nurse honey bees; as few as 100 bacteria can cause infection and colonisation of the larval gastrointestinal tract (Forsgren 2010). While larvae of all castes of honey bees are susceptible to infection at any age, the effects of infection are less in older larvae (Bailey 1981; DEEDI 2008).

The bacteria multiply rapidly in the larval gastrointestinal tract. By five days after hatching, the gastrointestinal tract may be almost entirely occupied by the bacteria (Alippi 1999). There are a number of possible fates for the infected larvae, usually depending on the species of bacteria involved (Bailey 1981; Hornitzky and Anderson 2003). These are:

- sudden death and ejection of the larva by the nurse honey bees. This typically occurs four to five days after hatching. *M*. *plutonius* is the dominant organism involved
- death and secondary infection—the dead larva remains in the unsealed cell and forms a scale. A variety of secondary organisms can be involved, but most commonly it is *E*. *faecalis* or *P*. *alvei*
- the cell is capped but the larva fails to pupate and dies. Secondary infections, usually *P*. *alvei*, can occur and the dead larva will contain large numbers of this organism
- the larva pupates and an adult honey bee emerges but it may be undersized due to insufficient food.

It has been suggested that larvae starve to death as the bacterial mass consumes the available nutrition (Bailey 1981). However, when experimentally supplied with excess food, infected larvae still died, thereby suggesting that other pathogenic mechanisms involving penetration of the host's tissues are involved (McKee et al. 2004). To date, the factors initiating this tissue invasion and damage remain unknown (Forsgren 2010).

Infected larvae that survive and pupate excrete *M*. *plutonius* in their faeces and contaminate the walls and capping of their cells. In turn, the honey bees that clean out infected cells become contaminated with the bacteria and then act as vectors to contaminate the larval food.

Adult worker honey bees act as carriers of the bacteria not only within the colony, but also between colonies and apiaries (Belloy et al. 2007; McKee et al. 2003 in Forsgren 2010). The activities of beekeepers also spread EFB between colonies.

Apparently healthy apiaries have been shown to contain adult honey bees infected with *M*. *plutonius* (Belloy et al. 2007; Roetschi et al. 2009). A colony infected with *M*. *plutonius*

may have a balance between some larvae reproducing and spreading the bacteria and the nurse honey bees removing others. The infection is maintained but disease is not evident.

Clinical EFB, in the form of diseased larvae in combs, tends to be seasonal and will appear when nectar flow increases. At this time, the colony can produce proportionally more brood but the nurse honey bees are unable to feed all the brood and remove dead larvae, and as a result, the characteristic signs of foulbrood appear. Any imbalance in the amount of pollen needed to produce the brood food, the numbers of nurse honey bees or the amount of brood will affect the appearance and/or the course of the disease.

EFB can resolve spontaneously when the colony's nurse honey bees are numerous enough or the amount of new brood decreases sufficiently to enable the nurse honey bees to remove the infected larvae before clinical signs of the disease appear (Bailey 1981; Alippi 1999).

Clinical signs

The death of 4–5 day-old larvae is an indication of EFB. Healthy pearly white larvae turn yellow and then brown after death, and become displaced within their cells. The dead larvae are often twisted into unnatural positions (Hornitzky and Anderson 2003) and dry to a rubbery, brown scale that is easier to remove than the scales generated by American foulbrood. Also, dead larvae do not exhibit the 'ropiness' that is one of the characteristics of American foulbrood (Alippi 1999). If a high enough proportion of larvae are affected and die, EFB infected combs can show a 'pepperbox' appearance.

A foul, sometimes sourish, odour, that gives the disease its name, can be emitted if secondary bacterial infection occurs (Hornitzky and Anderson 2003).

Diagnosis

The clinical signs may be sufficient to give a tentative diagnosis. If larvae are dissected before decomposition and scale formation, the masses of bacteria may be seen as opaque white clumps within the gastrointestinal tract (Bailey 1981). Microscopy of stained smears of sick or dead larvae can confirm the diagnosis (Hornitzky and Smith 1998).

There are detailed descriptions of the growth and appearance of *M*. *plutonius* and the secondary bacteria associated with EFB (for example, Shimanuki and Knox 2000). However, the culture technique for *M*. *plutonius* is insensitive—only about 1 in 500 organisms are recovered (McKee et al. 2003).

The presence of *M*. *plutonius* in asymptomatic colonies can be demonstrated by the use of an enzyme-linked immunosorbent assay (Alippi 1999). The development of polymerase chain reaction technology (McKee et al. 2003; Roetschi et al. 2009) has enabled identification of *M*. *plutonius* in larvae, adult honey bees, pollen and honey in both diseased and apparently healthy colonies.

A hand-held diagnostic device using monoclonal antibodies to provoke a colour change when *M*. *plutonius* is present (similar to commercial pregnancy tests) has been developed in Britain to give accurate diagnosis of EFB in the field (Tomkies et al. 2009).

Control

Colonies can naturally overcome the infection (Bailey 1981; Shimanuki 1997).

A number of management techniques can be employed to prevent or reduce the effects of EFB (Waite 2003; Somerville 2010) including:

- ensuring adequate nutrition, particularly the supply of pollen. The disease becomes a problem in colonies deficient in protein and therefore with low quality brood food (Alippi 1999)
- reducing stress, particularly when moving honey bees
- maintaining hive hygiene—regular replacement of brood combs will reduce the amount of bacterial contamination within hives
- requeening—with potentially more 'resistant' queen honey bees; but will also give a break in brood production enabling the nurse honey bees to remove diseased brood
- burning severely affected hives.

The antibiotic oxytetracycline is widely used in Australia as a treatment for clinical EFB or prophylactically in colonies with a history of EFB before anticipated honey flows (Somerville 2010). Unlike American foulbrood, there have not been significant resistance issues—surveys undertaken in Australia in 1999 (Hornitzky and Smith 1999) and in Britain in 2003 (Waite et al. 2003a) found all isolates of *M. plutonius* to be sensitive to the levels of oxytetracycline used for control.

A method of managerial treatment known as 'shook swarm' is also used in many beekeeping areas. This technique involves the transfer of adult honey bees from the infected colony to a new hive, leaving all the brood behind in the infected hive, which is destroyed. Waite et al. (2003b) concluded that the use of 'shook swarm' in conjunction with oxytetracycline therapy was significantly superior to oxytetracycline therapy alone in reducing the re-infection rate.

Conclusion

EFB is present throughout large areas of Australia and is likely to be present in some exporting countries. DAFF concluded that based on all the available information, further assessment of EFB was not required.

Other than Western Australia, EFB is endemic throughout the country. Consequently, besides a general requirement that honey bees for export should come from hives or colonies without obvious signs of disease, there should be no restrictions placed on importation due to EFB.

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4.3 Acarapisosis (tracheal mite)

Technical information

Background

Acarapisosis (or acariosis or acarine disease) is a disease of adult *Apis mellifera* and other *Apis* species caused by the mite *Acarapis woodi* (OIE 2008). It is also known as the tracheal mite due to its lifecycle, which is spent almost entirely within the tracheae of the adult honey bee.

Tracheal mites are found throughout the world—Africa, North, Central and South America, Europe, India and Thailand are all reported as being infested (Matheson 1996).

Tracheal mites have not been detected in Australia, and they are nationally notifiable (DAFF 2011). Acarapisosis of honey bees is an OIE listed disease (OIE 2011b).

OIE requirements

The OIE Code recommendations (OIE 2011a) for the importation of live queen honey bees, worker honey bees and drones with or without associated brood combs are the presentation of an international veterinary certificate attesting that the honey bees come from a country or zone/compartment officially free from acarapisosis.

Epidemiology and pathogenesis

Tracheal mites are an internal parasite of the respiratory system of the adult honey bee, living and reproducing mainly in the large prothoracic tracheae, and feeding on the host's haemolymph (OIE 2008). Mortality rates for infested honey bees range from moderate to high (OIE 2008) and the importance of the disease worldwide also varies—it is a serious problem in areas such as North America (García Fernández 1999).

While all stages of the tracheal mite—adults, larvae, nymphs and eggs—live exclusively within the respiratory system of the honey bee (García Fernández 1999), infestation is spread by direct contact (OIE 2008). Mated females may do one of two things: firstly, they may leave the trachea and migrate to the tip of the honey bee's body hair to await hair contact by another passing honey bee (García Fernández 1999). These free females survive only a few hours, depending on temperature, humidity and mite nourishment (García Fernández 1999). Alternatively, the mated female mite may move to the spiracles and into the trachea where it lays 5–7 eggs after two days. Maturation after hatching takes 11–12 days for male mites and 14–15 days for females (García Fernández 1999), with 2–4 times more female mites being produced than males (OIE 2008).

Tracheal mites have a preference for drones and honey bees under four days of age are the most susceptible to infestation (Gary et al. 1989). Due to their longevity, queen honey bees may serve as reservoirs for mites (Wilson et al. 1997).

Rapid dispersal of the tracheal mite is believed to be due to movements of migratory beekeepers and the trade of colonies that are infested. Within apiaries, spread is thought to

be due to individual honey bees drifting between neighbouring colonies (García Fernández 1999). Spread after its introduction into the USA was rapid (Delfinado-Baker 1985).

Pathogenic effects depend on the number of mites present in the trachea and are due to mechanical injury and physiological disorders such as obstruction of the air ducts, tracheal wall lesions and haemolymph depletion (OIE 2008).

Disruption of honey bee thermoregulation by tracheal mites during winter conditions has been suggested as the critical mechanism involved in the mite's ability to kill colonies (McMullan 2010).

Clinical signs

Clinical signs of tracheal mite infestation are non-specific. Affected honey bees crawl around in front of the hive and are unable to fly. Dysentery may also be present (OIE 2008).

Infestations are not usually noticed in the early stages—a slow decrease in colony size may be the only indication—becoming apparent only when the infestation is heavy. In the Northern Hemisphere where there is seasonal variation in honey bee reproduction, the decrease in colony size usually occurs in early spring following winter clustering when the tracheal mites have multiplied undisturbed (OIE 2008).

Gary and Page (1989) found no significant differences between infested and non-infested colonies in terms of number and frequency of foraging trips, round trip times, frequency of pollen collection and time between foraging trips. However, Eischen et al. (1989) found correlations between infestation levels and honey production in *A*. *mellifera* colonies. Differences exist between the over-wintering capability of infested and non-infested colonies (FAO 2006).

Diagnosis

Tracheal mites are the smallest of the parasitic mites of honey bees—adult females measure 143–174 µm long and 77–81 µm wide, while adult males are smaller (125–136 µm long and 60–77 µm wide) (Wilson et al. 1997). Consequently, they are very difficult to detect and identify (Shimanuki and Knox 2000). There is no reliable method for detecting very low levels of infestation macroscopically (OIE 2008).

Tracheal mites can only be detected using laboratory methods. They may be observed within the tracheae, or removed and observed, microscopically. Tracheal walls of infested honey bees become opaque and discoloured with blotchy black areas (OIE 2008).

Techniques for detection include dissection, grinding and staining. Maceration is the simplest and most reliable technique for diagnosis, allowing the detection of early and light infestations using a dissecting microscope (OIE 2008). An enzyme-linked immunosorbent assay test for tracheal mites has also been developed but may produce false-positive results and thus, is only recommended for survey examinations (OIE 2008).

Control

It is not possible to eradicate tracheal mite from an infested colony (Wilson et al. 1997). Contact acaricides (pesticides used to kill ticks and mites) used in *Varroa* control are generally ineffective against tracheal mites due to their inability to produce sufficiently high

enough amounts inside the honey bee tracheal system to kill the mites (Scott-Dupree and Otis 1992; Eischen 1998).

Tracheal mite levels can be kept under control by using menthol crystals or oil patties made with vegetable oil and white granulated sugar. Infested colonies may also be treated with formic acid (OIE 2008). Formic acid gel formulations are more effective than menthol treatment; controlled exposure of formic acid for 24 hours caused 97 per cent tracheal mite mortality whereas menthol produced 70 per cent mortality (Baxter et al. 2000). However, fumes from formic acid and menthol can disrupt honey bee behaviour (Wilson et al. 1997).

Inherited resistance to tracheal mite infestation is well recognised (Danka and Villa 2000; Nasr et al. 2001) and is used in a number of honey bee breeding programs.

Tracheal mite-free colonies can be established by removing sealed brood from affected colonies, ensuring all adhering adult honey bees have been removed from sealed brood combs (Wilson et al. 1997).

Conclusion

Tracheal mites are not present in Australia and are likely to be present in some exporting countries. The OIE Code recommendations (OIE 2011a) for the importation of live queen honey bees, worker honey bees and drones with or without associated brood combs are the presentation of an international veterinary certificate attesting that the honey bees come from a country or zone/compartment officially free from tracheal mites. Australia's previous biosecurity requirements differ from those in the OIE Code. Therefore, DAFF concluded that further risk assessment was required.

Risk assessment

For details of the method used in this risk assessment see Chapter 2. A summary of the risk assessment is shown in Figure 6.

Release assessment

The following factors were considered relevant to the estimate of the likelihood of tracheal mites being present in imported queen honey bees:

- tracheal mites have not been detected in Australia but are prevalent in many other significant honey bee keeping countries, including North America, Europe and Japan (Matheson 1996)
- tracheal mites are an internal parasite of the respiratory system of the adult honey bee (OIE 2008). Free mites survive only a few hours, depending on temperature, relative humidity and mite nourishment (García Fernández 1999)
- queen honey bees may serve as a reservoir for tracheal mites (Wilson et al. 1997)
- early infestations are not usually noticed (OIE 2008)
- tracheal mites are difficult to detect and identify due to their small size and internal location.

Conclusion: based on this information, the likelihood of release of tracheal mites associated with imported queen honey bees was estimated to be **high**.

Exposure assessment

The exposure group considered is managed and feral *A*. *mellifera* colonies, and the most likely exposure pathway is the introduction of infested imported adult honey bees to a managed honey bee colony.

The following factors were considered relevant to the estimate of the likelihood of susceptible honey bees being exposed to tracheal mites via imported queen honey bees:

- infestation is spread by direct contact between adult honey bees (OIE 2008). Mated female tracheal mites leave the tracheae and migrate to the tip of the honey bee's body hair to await hair contact by another passing honey bee (García Fernández 1999). These free females survive only a few hours, depending on temperature, relative humidity and mite nourishment (García Fernández 1999)
- rapid dispersal of the tracheal mite believed to be due to movements of migratory beekeepers and the selling of infested honey bees and queen honey bees; within apiaries, spread to neighbouring colonies is thought to be due to drifting honey bees (García Fernández 1999).

Conclusion: based on these considerations, DAFF considered the likelihood of susceptible honey bees being exposed to tracheal mites via infested imported queen honey bees to be **moderate**.

Estimation of the likelihood of release and exposure

The likelihood of release and exposure is estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (Table 2). With the likelihood of release estimated to be 'high' combined with the likelihood of exposure estimated to be 'moderate', the likelihood of release and exposure was estimated to be **moderate**.

Consequence assessment

The consequence assessment describes the potential consequences associated with hazard entry and exposure, and estimates the likelihood of them occurring. This involves estimating the likelihood of establishment and/or spread of the hazard for the most likely outbreak scenario, and determining the direct or indirect effects (health, environment and socioeconomic) should this outbreak scenario occur. Combining the likelihood of establishment and/or spread for this outbreak scenario with the corresponding overall effect gives an estimation of likely consequences.

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred, a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario was determined by describing the likely extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to tracheal mites is considered to be establishment and/or spread to populations of susceptible honey bees within a state/territory through direct contact.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of susceptible honey bees to tracheal mites:

- early infestations are not usually noticed—a slow decrease in colony size may be the only indication—becoming apparent when infestation is heavy (OIE 2008)
- rapid dispersal of the tracheal mite is believed to be due to movements of migratory beekeepers and the trade in infested honey bees; within apiaries, spread to neighbouring colonies is thought to be due to drifting honey bees (García Fernández 1999)
- spread after its introduction into the USA was rapid (Delfinado-Baker 1985).

Conclusion: based on these considerations for the identified outbreak scenario, the likelihood of establishment and/or spread of tracheal mites was estimated to be **high**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect impacts of tracheal mites were estimated at the national, state or territory, district/region and local levels. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria.

The following factors were considered relevant to a conclusion on the effects of the establishment and/or spread of tracheal mites:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- correlations have been found between infestation levels and honey production in *A. mellifera* colonies (Eischen et al. 1989). Differences also exist between the over-wintering capability of infested and non-infested colonies (FAO 2006)
- pathogenic effects depend on the number of mites present in the tracheae and are due to mechanical injury and physiological disorders as a result of air duct obstruction, tracheal wall lesions and haemolymph depletion (OIE 2008)
- honey bee mortality rates range from moderate to high (OIE 2008)
- the importance of the disease is not the same worldwide, it is a serious problem in areas such as North America (García Fernández 1999).

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- the presence of tracheal mites in managed and feral honey bee colonies is not considered to negatively impact on pollination of native plant species as native flora is not dependent on *A. mellifera*
- the susceptibility of Australian native bees to tracheal mites is unknown.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- tracheal mites are notifiable in Australia (DAFF 2011)

- if tracheal mites were identified in Australia, the response policy as outlined in the AUSVETPLAN *Disease Strategy: Bee Pests and Diseases* (Animal Health Australia 2010a) would be determined by how early the incursion was detected, the extent of the incursion and the location of affected hives. Control and eradication of tracheal mites using stamping out is the default policy (Animal Health Australia 2010a)[2]
- controls over honey bee movements, products and equipment would be imposed on managed apiaries within designated areas until further decisions were made (Animal Health Australia 2010a)
- movement controls could affect commercial interests of the apiarist and the health of honey bee colonies (Animal Health Australia 2010a)
- if the decision was made not to attempt eradication but to recommend that control practices be initiated, state/territory and/or industry-based control measures would be initiated, which may include encouraging industry to develop its own long-term policies and procedures (Animal Health Australia 2010a).

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- as a result of the detection of tracheal mites, movement restrictions would be imposed until tracing and surveillance were completed and results analysed (Animal Health Australia 2010a)
- if eradication was not attempted, control measures may include interstate movement controls (Animal Health Australia 2010a)
- the presence of tracheal mites in managed and feral honey bee colonies may negatively impact on pollination services to horticultural and agricultural crops. The loss of honey bee pollination from agricultural production has been estimated to result in a flow-on loss of A$2 billion and 11 000 jobs (Gordon and Davis 2003)
- prices of products from honey bees may increase, thereby reducing consumer demand.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- tracheal mites are found throughout the world but have not been detected in Australia (Matheson 1996)
- loss of Australia's tracheal mite–free status may reduce international consumer demand for Australian honey bees and bee products
- if tracheal mites were to become established, renegotiations of trade conditions may become necessary.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- if used, the application of chemicals to control tracheal mites may have an effect on a range of arthropod species and disrupt the food source of wildlife, lead to environmental contamination (including water sources) and increased resistance to the chemicals.

[2] The emergency management of honey bee diseases and pests are in the process of moving under the Australian Emergency Plant Pest Response Deed (EPPRD) and the Australian Emergency Plant Pest Response Plan (PLANTPLAN)

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- the cost of beekeeping would increase due to expenses associated with control of tracheal mites
- non-commercial and small-scale commercial beekeepers may become non-viable
- use of chemicals for tracheal mite control may lead to contamination of honey products (Wilson et al. 1997). Honey bee products may be declared unfit for human consumption and consumers may lose confidence in the domestic market.

Conclusion for overall direct and indirect effects: based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread for the outbreak scenario was estimated to be **moderate** from Table 3. The effect is likely to be recognised on a national level and significant within affected zones, and be highly significant to directly affected parties.

Derivation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario using Table 4 to obtain an estimation of likely consequences.

The likelihood of establishment and/or spread ('high') is combined with the estimate of the overall effect of establishment and/or spread ('moderate') which results in **moderate** likely consequences.

Unrestricted risk estimation

Risk estimation is the integration of likelihood of release and exposure, and likely consequences of establishment and/or spread to derive the risk associated with release, exposure, establishment and/or spread of tracheal mites introduced by imported queen honey bees into Australia.

Using Table 5, the likelihood of release and exposure ('moderate') is combined with the likely consequences of establishment and/or spread ('moderate'), resulting in a risk estimation of **moderate**.

Therefore, as the unrestricted risk associated with tracheal mites exceeds Australia's ALOP of 'very low', risk management is considered necessary for this agent.

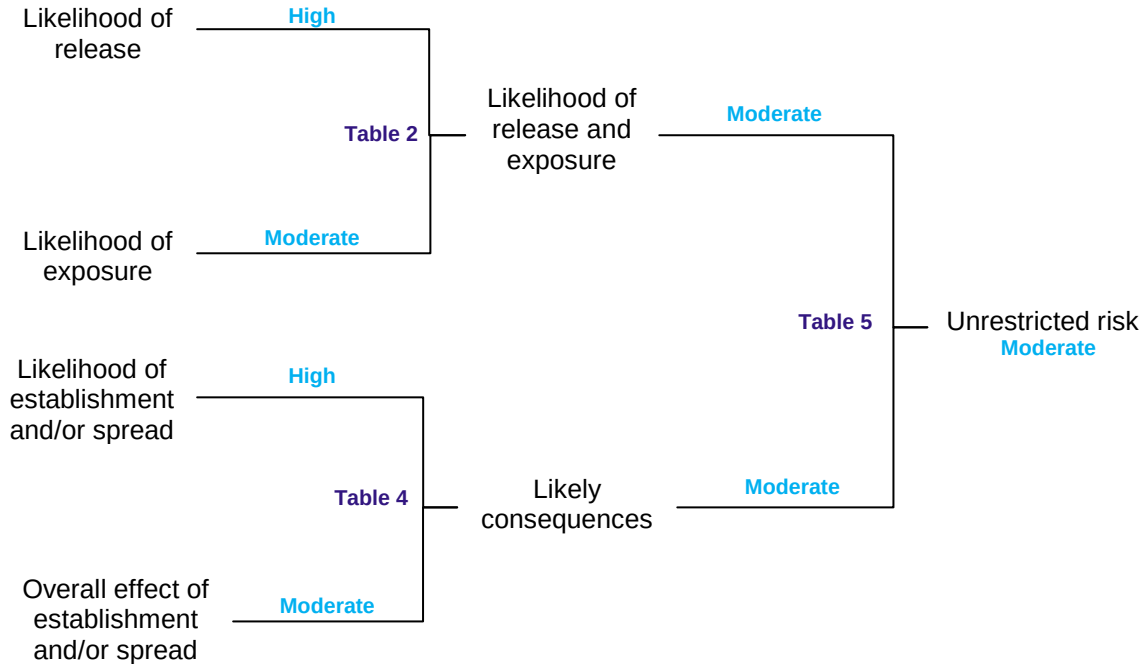

Figure 6. Summary of the risk assessment pathways for tracheal mites

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4.4 *Braula* fly

Technical information

Background

Braula fly is a wingless ectoparasite of adult honey bees; specifically *Apis mellifera*. It is also known as the 'bee louse'.

Braula fly belongs to the family Braulidae within the order Diptera, There are five species (*B. coecea*, *B. kohli*, *B. orientalis*, *B. pretoriensis* and *B. shmitzi*) which are found in Africa (*B. coeca*), Asia (*B. shmitzi*), Europe (*B. coeca*), and North and South America (*B. coeca*). They are not present in New Zealand (Ellis and Munn 2005) or on mainland Australia. *B. coeca* is present in Tasmania (Animal Health Australia 2010).

Braula flies are roughly the same size as, and can be confused with, *Varroa*. However, *Braula* have the six legs of insects rather than the eight of the arachnid mites (Shimanuki and Knox 2000).

Although not nationally notifiable, it is notifiable in the NT, SA, Victoria and WA.

OIE requirements

Braula fly is not an OIE listed disease (OIE 2011) and there are no OIE recommendations.

Epidemiology and pathogenesis

Braula species have not been seen on species of honey bees other than *A. mellifera* but two related species, *Megabraula onerosa* and *M. antecessor* are reported to infest *A. laboriosa* (Oldroyd and Wongsiri 2006a).

A study carried out in semi-arid conditions in Jordan found the prevalence of *Braula* fly infested honey bees within a hive to be between 15 to 24 per cent, with a distinctly seasonal variation in numbers, increasing through late summer and autumn as honey bee numbers in the colony decrease (Zaitoun and Al-Ghzawi 2008).

The female fly lays eggs beneath the wax capping on the walls of honey cells. Emerging larvae tunnel through the wax and eat honey and pollen grains within the wax. These tunnels appear as raised lines of wax debris. Adult flies emerge in three weeks and attach to adult honey bees of all castes on the thorax, abdomen and around the head. Queen honey bees in particular can be infested with large numbers, presumably because they are fed more often and live longer (Sammatara 1997; Zaitoun and Al-Ghzawi 2008). The flies feed on honey and pollen being eaten by the honey bee—feeding at the base of the honey bee's extended tongue (Ellis and Zettel Nalen 2010).

Braula flies are not known to survive without direct contact with adult honey bees (Somerville 2007, Animal Health Australia 2010). Swarming, drifting and robbing activities of infested honey bees spread the flies between colonies. The other major mode of spread is by the movement of hives and equipment and comb honey by beekeepers (Warhurst and Goebel 2005).

Clinical signs

Damage done to beeswax by migrating larvae is the major clinical sign seen in infested colonies (Sammatara 1997).

A severe infestation may decrease the efficiency of the queen honey bee but most infestations are considered to be harmless to the honey bees themselves (Bailey 1981).

Diagnosis

Diagnosis is usually by visual inspection of adult honey bees for the presence of adult flies and inspection of honey frames for larval tunnelling activity (Warhurst and Goebel 2005). The larvae themselves are small and difficult to see. Adult flies are approximately 1.5 mm long and reddish-brown in colour; they may be confused with adult *Varroa* (Zaitoun and Al-Ghzawi 2008; Ellis and Zettel Nalen 2010).

Treatment of infested hives with tobacco smoke to remove the flies has also been used as a diagnostic tool (Sammatara 1997, Warhurst and Goebel 2005).

Control

As previously mentioned, *Braula* fly is considered to be harmless; however, controls are recommended in most countries where it is endemic (Ellis and Zettel Nalen 2010). Suitable controls include: removal of wax capping during regular honey extraction to reduce the number of larval stages present; treatment of infested hives with tobacco smoke (Sammatara 1997; Warhurst and Goebel 2005), and application of the insecticide fluvalinate, which is also used for the treatment of *Varroa* infestation (Kulincevic et al. 1991). Cold treatment may also be used to kil the eggs and larvae of *Braula* fly.

Conclusion

Braula fly is not present on mainland Australia and is likely to be present in some exporting countries. It is a notifiable pest in NT, SA, Victoria and WA. There are no recommendations in the OIE Code. Therefore, DAFF concluded that further risk assessment was required.

Risk assessment

For details of the method used in this risk assessment see Chapter 2. A summary of the risk assessment is shown in Figure 7.

Release assessment

The following factors were considered relevant to the estimate of the likelihood of *Braula* fly being present on imported queen honey bees.

- *Braula* fly is distributed worldwide; New Zealand is the only major beekeeping country that is free from *Braula* fly
- the prevalence of *Braula* fly in endemic countries is unknown
- the prevalence of infested adult honey bees within a hive was found to be between 15 and 24 per cent in a study carried out in semi-arid conditions in Jordan (Zaitoun and Al-Ghzawi 2008)
- *Braula* fly may be present within hives with no clinical signs

- *Braula* fly is an ectoparasite of adult honey bees. Pre-export inspection of honey bees (independent of specifically applied risk management measures) is likely to detect the presence of *Braula* fly.

Conclusion: based on this information, the likelihood of release of *Braula* fly associated with the importation of queen honey bees was estimated to be **very low**.

Exposure assessment

The exposure group considered was managed and feral honey bee colonies and the most likely exposure pathway is the introduction of infested imported adult honey bees to a managed honey bee colony.

The following factors were considered relevant to the estimate of the likelihood of susceptible honey bees being exposed to *Braula* fly via imported queen honey bees:

- all castes of honey bees are susceptible (Sammatara 1997)
- the host range is restricted to *A*. *mellifera* (Oldroyd and Wongsiri 2006b)
- infestation is presumed to be by close contact between adult honey bees.

Conclusion: based on this information, the likelihood of susceptible honey bees being exposed to *Braula* fly via infested imported queen honey bees was estimated to be **high**.

Estimation of the likelihood of release and exposure

The likelihood of release and exposure is estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (Table 2). With the likelihood of release estimated to be ‘very low’ combined with the likelihood of exposure estimated to be ‘high’, the likelihood of release and exposure for *Braula* fly was estimated to be **very low**.

Consequence assessment

The consequence assessment describes the potential consequences associated with hazard entry and exposure, and estimates the likelihood of them occurring. This involves estimating the likelihood of establishment and/or spread of the hazard for the most likely outbreak scenario, and determining the direct or indirect effects (health, environment and socioeconomic) should this outbreak scenario occur. Combining the likelihood of establishment and/or spread for this outbreak scenario with the corresponding overall effect gives an estimation of likely consequences.

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred, a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario was determined by the extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to *Braula* fly was considered to be establishment and/or spread through direct contact to local populations of susceptible honey bees.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of susceptible honey bees to *Braula* fly:

- infestation is presumed to be by close contact between adult honey bees
- *Braula* fly is not known to survive without direct contact with adult honey bees (Somerville 2007, Animal Health Australia 2010).

Conclusion: based on these considerations, it was determined that the likelihood of establishment and spread of *Braula* fly for the exposure group was **low**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect impacts of *Braula* fly were estimated at the national, state or territory, district/region and local levels. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria.

The following factors were considered relevant to a conclusion on the effects of the establishment and/or spread of *Braula* fly.

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- *Braula* fly can infest adult honey bees of all castes
- queen honey bees can be infested with large numbers of *Braula* flies, which can reduce their efficiency
- the *Braula* fly larval stage burrows under the cappings of honey combs and it is the appearance of this burrowing activity that detracts from honey comb intended for retail sale
- as most honey is extracted mechanically, *Braula* fly does not pose a threat to regular liquid honey producers (Somerville 2007)
- the socioeconomic impact of treating an infested hive or apiary would be borne by individual beekeepers.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- feral honey bee colonies could be infested but with little deleterious effect
- the susceptibility of Australian native bees to *Braula* fly is unknown.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- *Braula* fly infestation is notifiable in NT, SA, Victoria and WA. It is endemic in Tasmania
- if *Braula* fly was identified on mainland Australia, the response policy as outlined in the AUSVETPLAN *Disease Strategy: Bee Pests and Diseases* (Animal Health Australia 2010) would be determined by how early the incursion was detected, the

extent of the incursion and the location of affected hives. Control and eradication using stamping out is the default policy (Animal Health Australia 2010)[3]
- if the decision was made not to attempt eradication but to recommend that control practices be instigated, state/territory and/or industry-based control measures would be initiated, which may include encouraging industry to develop its own long-term policies and procedures (Animal Health Australia 2010).

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- if *Braula* fly was detected, movement restrictions would be imposed until tracing and surveillance were completed and results analysed (Animal Health Australia 2010). These may turn into permanent restrictions
- infected apiaries could face increased costs in managing their hives for optimal performance
- there would be no effects on consumer demand.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- there would be minimal impacts on trade or industry
- if *Braula* fly was to become established, renegotiation of trade conditions with importing countries may become necessary.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- if used, application of chemicals to control *Braula* fly could have an effect on a range of arthropod species and disrupt the food source of wildlife. It could possibly lead to environmental contamination (including water sources) and contribute to increased resistance to the chemicals.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- the cost of beekeeping may increase due to expenses associated with control of *Braula* fly.

Conclusion for overall direct and indirect effects: based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread for the outbreak scenario was estimated to be **very low** from Table 3. The effect is likely to be **minor** to directly affected parties and it is **unlikely** to be discernable at any other level.

[3] The emergency management of honey bee diseases and pests are in the process of moving under the Australian Emergency Plant Pest Response Deed (EPPRD) and the Australian Emergency Plant Pest Response Plan (PLANTPLAN)

Derivation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario using Table 4 to obtain an estimation of likely consequences.

Therefore the likelihood of establishment and/or spread ('low') is combined with the estimate of the overall effect of establishment and/or spread ('very low') which resulted in **negligible** likely consequences.

Unrestricted risk estimation

Risk estimation is the integration of likelihood of release and exposure, and likely consequences of establishment and/or spread to derive the risk associated with release, exposure, establishment and/or spread of *Braula* fly introduced by imported queen honey bees into Australia.

Using Table 5, the likelihood of release and exposure ('very low') is combined with the likely consequences of establishment and/or spread ('negligible'), which results in a risk estimation of **negligible**.

Therefore as the unrestricted risk estimate achieves Australia's ALOP of 'very low', no specific risk management is considered necessary for this agent.

As *Braula* fly is a notifiable pest in some jurisdictions within Australia, DAFF concluded that honey bees and their escorts should not be sourced from colonies where active infestation is present. Therefore, certification requirements for colony inspection for freedom from *Braula* fly will be included in Australia's biosecurity measures.

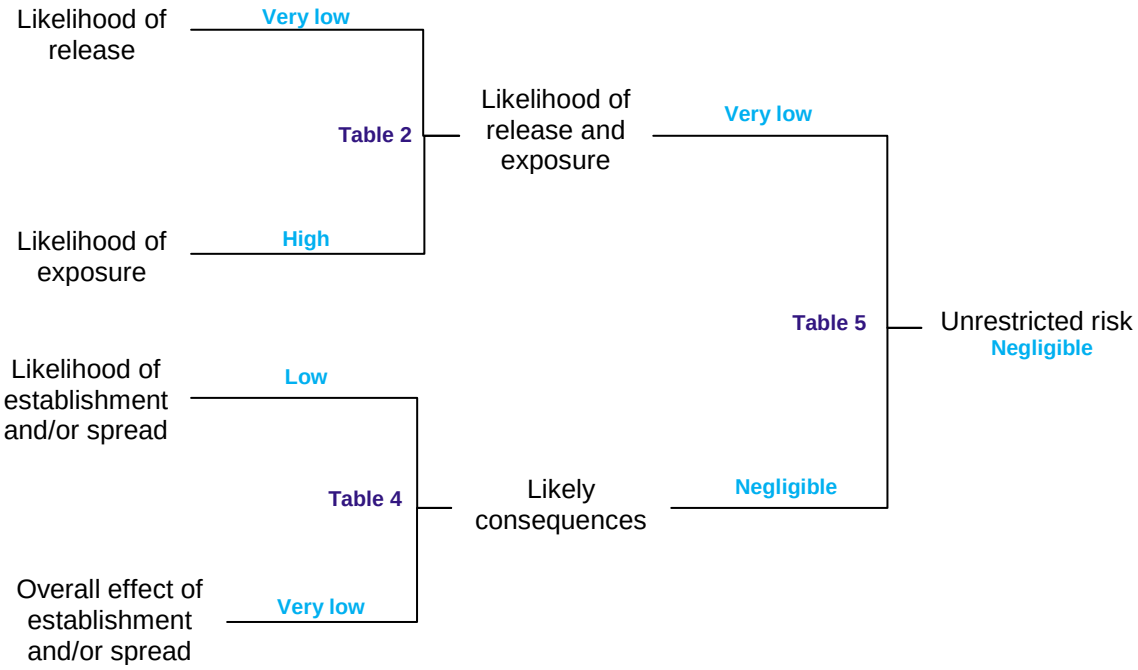

Figure 7. Summary of the risk assessment pathways for *Braula* fly

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4.5 Phorid fly (*Apocephalus borealis*)

Technical information

Background

Apocephalus borealis is a phorid fly which can infect and kill honey bees (Runckel et al 2011; Core 2012). It was previously known to parasitise bumble bees and paper wasps. *A. borealis* belongs to the subfamily Phorinae, subgenus Mesophora, within the order Diptera. The subgenus Mesophora contains species that attack a variety of hosts including ants, beetles, bumble bees, spiders and wasps (Brown 1994; Core 2012).

A. borealis is a relatively new parasite of honey bees having first been identified in 2011 (Core 2012). It is native to North America.

A. borealis infestation is not an OIE listed disease and is not notifiable in Australia (DAFF 2011).

OIE requirements

A. borealis infestation is not an OIE listed disease (OIE 2011) and there are no OIE recommendations.

Epidemiology and pathogenesis

A. borealis has only been detected in North America, specifically, the San Francisco area, other areas of California, and South Dakota (Runckel et al 2011).

A. borealis inserts eggs on or within the honey bee's body, probably while they are foraging at flowers (Otterstatter et al 2002). In laboratory infections, the flies were observed to attack honey bees and lay eggs within the honey bee's abdomen soon after they were placed with them (Core 2012). Mature *A. borealis* larvae usually developed within seven days and emerged from the junction between the head and thorax. Adult flies emerged around 28 days after pupation (Core 2012).

Multiple *A. borealis* larvae can develop in each honey bee host, and in the bumble bee they are known to feed primarily on the thoracic flight muscle (Otterstatter et al 2002). A recent study using honey bees from the San Francisco area in the USA, indicated there was widespread parasitism with 77 per cent of the sample sites being positive for *A. borealis* (Core 2012). Also, deformed wing virus (DWV) and *Nosema ceranae* were detected in some of the parasitised honey bees, as well as in *A. borealis* adults and larvae (Core 2012).

A. borealis has a negative effect on the behaviour of its host honey bees, causing them to abandon their hives at night. This abandonment behaviour is consistent with symptoms described as part of colony collapse disorder. In addition, the number of honey bees in the study hive declines, and *A. borealis* pupae and empty pupal casings can be observed among dead honey bees at the bottom of the hive, thereby indicating that *A. borealis* can multiply within a hive and infect the queen honey bee (Core 2012).

There is little information available on the specific effect of *A*. *borealis* on honey bees. However, bumble bees containing *A*. *borealis* larvae have considerably shorter residual life spans than unparasitised bees, surviving less than half as long as worker bumble bees in the field (Otterstatter et al 2002).

Clinical signs

Early infestations may go unnoticed until the number of honey bees in the hive start to decline, and pupae and casings are noticed. Changes in honey bee behaviour including increased night activity and hive abandonment at night, may also occur (Core 2012).

Diagnosis

Diagnosis can be based on identification of the larvae emerging from the honey bees or the presence of pupae and pupal casings with the hive. Although the larvae are readily visible due to their size compared with adult honey bees, they move away from the honey bee to pupate and thus, may not be seen (Core 2012).

Adult *A*. *borealis* may be seen within or around the hive.

Control

This is a newly discovered parasite of honey bees and no control methods have been described. *A. borealis* could potentially attack honey bees at any time, especially while they are foraging at flowers, thereby making hive control difficult.

Treatment of the soil outside the hives with an insecticide may be considered to target the pupal stage and emerging adult *A*. *borealis*.

Conclusion

A. borealis is not present in Australia. It is present in areas of North America and the possibility that it is present in other countries cannot be excluded. There are no recommendations in the OIE Code. Therefore, DAFF concluded that further risk assessment was required.

Risk assessment

For details of the method used in this risk assessment see Chapter 2. A summary of the risk assessment is shown in Figure 8.

Release assessment

The following factors were considered relevant to the estimate of the likelihood of *A*. *borealis* being present on imported queen honey bees.

- *A. borealis* is present in North America. Its exact distribution is unknown
- the prevalence of *A. borealis* in one study was estimated to be 77 per cent (Core 2012)
- *A*. *borealis* may be present within hives with no clinical signs
- *A*. *borealis* is an ectoparasite of adult honey bees. Pre-export inspection of honey bees (independent of specifically applied risk management measures) is likely to detect the presence of adult *A*. *borealis*

- Larval stages of *A*. *borealis* develop inside the honey bee. Emerged larvae are large in comparison with their honey bee hosts and are easily visible.

Conclusion: based on this information, the likelihood of release of *A*. *borealis* associated with the importation of queen honey bees was estimated to be **very low**.

Exposure assessment

The exposure groups considered were managed and feral honey bee colonies and the most likely exposure pathway is the introduction of infested imported adult honey bees to a managed honey bee colony.

The following factors were considered relevant to the estimate of the likelihood of susceptible honey bees being exposed to *A*. *borealis* via imported queen honey bees:

- all castes of honey bees are presumed to be susceptible
- infestation is only by close contact between individual adult honey bees and free-flying *A*. *borealis* flies.

Conclusion: based on this information, the likelihood of susceptible honey bees being exposed to *A*. *borealis* via infested imported queen honey bees was estimated to be **low**.

Estimation of the likelihood of release and exposure

The likelihood of release and exposure is estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (Table 2). With the likelihood of release estimated to be 'very low' combined with the likelihood of exposure estimated to be 'low', the likelihood of release and exposure for *A*. *borealis* was estimated to be **very low**.

Consequence assessment

The consequence assessment describes the potential consequences associated with hazard entry and exposure, and estimates the likelihood of them occurring. This involves estimating the likelihood of establishment and/or spread of the hazard for the most likely outbreak scenario, and determining the direct or indirect effects (health, environment and socioeconomic) should this outbreak scenario occur. Combining the likelihood of establishment and/or spread for this outbreak scenario with the corresponding overall effect gives an estimate of likely consequences.

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred, a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario was determined by the extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to *A*. *borealis* was considered to be establishment and/or spread through direct contact to local populations of susceptible honey bees.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of susceptible honey bees to *A*. *borealis*:

- infestation is only by close contact between individual adult honey bees and the free-flying *A*. *borealis* flies
- besides the bumble bees endemic in Tasmania, it is not known what other species, if any, *A*. *borealis* may parasitise in Australia.

Conclusion: based on these considerations, it was determined that the likelihood of establishment and spread of *A*. *borealis* for the exposure group was **very low**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect impacts of *A*. *borealis* were estimated at the national, state or territory, district/region and local levels. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria.

The following factors were considered relevant to a conclusion on the effects of the establishment and/or spread of *A*. *borealis*.

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- *A. borealis* can infest adult honey bees of all castes
- only individual honey bees are affected, there is no transmission from honey bee to honey bee
- the socioeconomic impact of treating an infested hive or apiary would be borne by individual beekeepers.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- feral honey bee colonies could be infested but with little known deleterious effect
- the susceptibility of Australian native bees to *A*. *borealis* is not known.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- *A. borealis* is not notifiable in Australia and there are no control, monitoring or surveillance programs in place.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries.

- if *A*. *borealis* was detected, movement restrictions may be imposed
- infected apiaries could face increased costs in managing their hives for optimal performance
- there would be no effects on consumer demand.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- there may be impacts on the export of live packaged honey bees
- if *A. borealis* was to become established, renegotiation of trade conditions with importing countries may become necessary.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- there are none known.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- the cost of beekeeping may increase due to expenses associated with control of *A. borealis.*

Conclusion for overall direct and indirect effects: based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread for the outbreak scenario was estimated to be **very low** from Table 3. The effect is likely to be minor to directly affected parties and it is unlikely to be discernable at any other level.

Derivation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario using Table 4 to obtain an estimation of likely consequences.

The likelihood of establishment and/or spread ('very low') is combined with the estimate of the overall effect of establishment and/or spread ('very low') which results in **negligible** likely consequences.

Unrestricted risk estimation

Risk estimation is the integration of likelihood of release and exposure, and likely consequences of establishment and/or spread to derive the risk associated with release, exposure, establishment and/or spread of *A. borealis* introduced by imported queen honey bees into Australia.

Using Table 5, the likelihood of release and exposure ('very low') is combined with the likely consequences of establishment and/or spread ('negligible'), which results in a risk estimation of **negligible**.

Therefore, as the unrestricted risk estimate achieves Australia's ALOP of 'very low', no specific risk management was considered necessary.

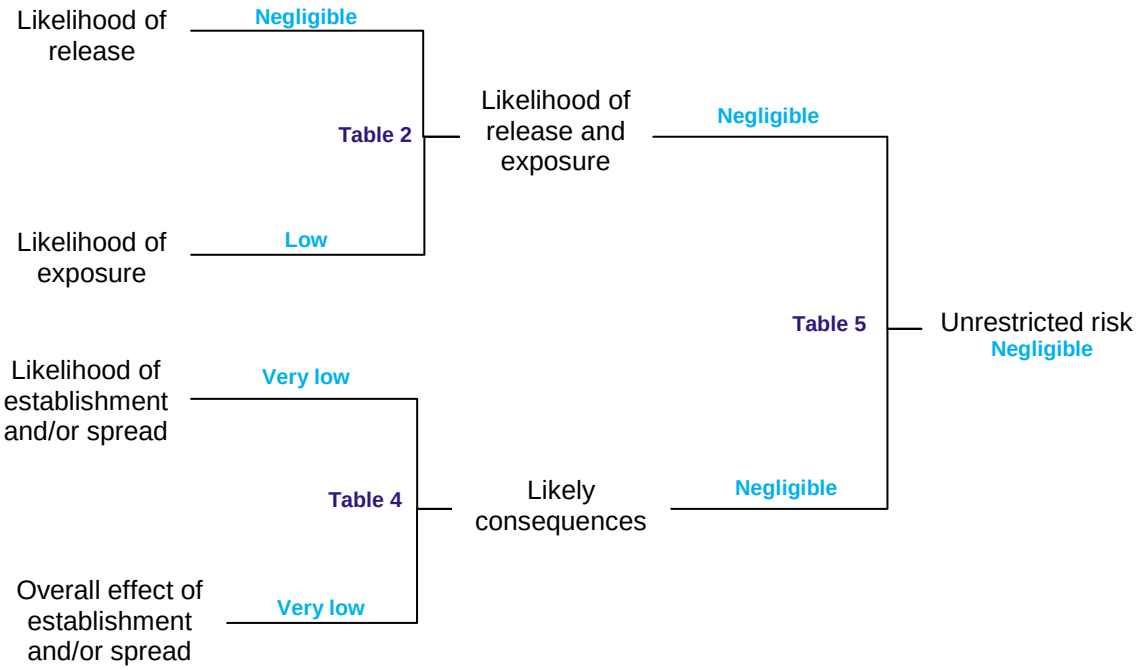

Figure 8. Summary of the risk assessment pathways for *A. borealis*

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4.6 Small hive beetle

Technical information

Background

The small hive beetle (*Aethina tumida*; SHB) is a cleptoparasite and scavenger of honey bee colonies. First described in 1867, *A. tumida* belongs to the family Nitidulidae within the order Coleoptera. The Nitidulidae are sap beetles, usually feeding on decayed vegetation, rotting fruit or sap.

Originally a native of sub-Saharan Africa, *A. tumida* has become an invasive pest in Australia and in North America. SHB was first described from samples sent from West Africa and the beetle has since been identified in most countries of Africa south of the Sahara (Neumann and Elzen 2004). Early studies of SHB were carried out in South Africa, where it is widespread and was not considered to be a major pest (Lundie 1940).

The first confirmed identification outside Africa was in Florida in the USA in June 1998 but earlier, unidentified specimens had been collected in South Carolina in 1996–97. SHB spread rapidly throughout the eastern and southern USA, reaching Texas by 2003 (Hood 2004) and have been reported by beekeepers in California (Neumann and Ellis 2008). Canada has had a number of incursions (Manitoba in 2002, Alberta and Manitoba in 2006 and Quebec in 2008–09) and Mexico reported its first incursion to the OIE in October 2007 (OIE 2008b).

A. tumida were first found in the western Sydney basin of NSW in July 2002 and subsequently throughout that state and in Queensland. It was estimated that SHB had been in Australia for at least six months before it was detected (Gillespie et al. 2003). It is now endemic across the eastern seaboard of Australia and, since 2007, in the far north-west of WA (Animal Health Australia 2010).

SHB infestation is an OIE listed disease (OIE 2011a) and is notifiable in Australia (DAFF 2011).

OIE requirements

The OIE Code recommendations (OIE 2011b) for the importation of individual consignments containing a single live queen honey bee, accompanied by a small number of associated attendants (a maximum of 20 attendants per queen honey bee) are:

- certification that the honey bees come from a country or zone officially free from SHB infestation, OR
- certification that
    - the honey bees come from hives or colonies which were inspected immediately prior to dispatch and show no signs or suspicion of the presence of *A. tumida* or its eggs, larvae or pupae; and

- the honey bees come from an area of at least 100 km radius where no apiary has been subject to any restrictions associated with the occurrence of *A*. *tumida* for the previous 6 months; and
- the honey bees and accompanying packaging presented for export have been thoroughly and individually inspected and do not contain *A*. *tumida* or its eggs, larvae or pupae; and
- the consignment of honey bees is covered with fine mesh through which a live beetle cannot enter.

Epidemiology and pathogenesis

SHB is a parasite of *A*. *mellifera* colonies but it has been shown experimentally to infest and reproduce in colonies of bumble bees (*Bombus* species) (Spiewok and Neumann 2006). Greco et al. (2010) noted that there was evidence that SHB could parasitise Australian native stigless bees but also showed that they were able to prevent SHB entering and reproducing in their colonies by rapidly mummifying adult SHB in a mixture of resin, wax and mud.

SHB lay clusters of eggs within the cracks and crevices around the honey bee hive and, if allowed to by the honey bees, they will lay directly in the brood area. The eggs hatch in approximately three days and the larval stage feeds within the hive for a period of between 1–4 weeks before leaving the hive and pupating, followed by three days in the soil. Mature larvae are attracted to light and move outside the hive to pupate in the surrounding soil by burrowing 10–20 cm deep. Pupation times vary with soil temperature: pupation in summer takes between 15 and 60 days with most adult SHB emerging between 21 and 28 days. Pupation rates vary with soil moisture—drier soils have lower rates—and this appears to be a determining factor in reproduction rates.

An adult SHB can live for 4–6 months and in that time may produce in excess of 1000 eggs (Wenning 2001, Hood 2004; Stedman 2006; OIE 2011b).

Although closely associated with honey bees, SHB has been shown to survive for prolonged periods away from honey bee colonies and on fruit diets, albeit with reduced reproductive efficiency (Ellis, Jr. et al. 2002). Increased infestations of SHB have been found in the proximity of honey rooms (Spiewok et al. 2007).

Adult SHB fly between honey bee colonies. Estimates of their range vary but flights of up to 13 km have been recorded (Somerville 2003; OIE 2011b). SHB are attracted to honey bee hives by odours given off by worker honey bees, pollen, unripe honey and slumgum (Suazo et al. 2003). It has been proposed that SHB can detect, and are attracted to, failing or disturbed honey bee hives (Wenning 2001). Some of the beetles' movement may be determined by aggregation pheromones, as have been demonstrated in other Nitidulid beetles (Neumann 2004). The major pathway for the spread of SHB is thought to have been through the transfer of eggs, larvae and adult SHB via the movement of packaged honey bees, managed hives and equipment (Hood 2004).

SHB has been regarded as a minor pest of managed hives and around honey houses in its native range; however, in Australia and the USA, it has become a major pest of the beekeeping industries. This is due in part to the lack of behavioural resistance mechanisms of the sub-species of European honey bees in these countries when compared to the African

sub-species (Neumann et al. 2001). The African sub-species will also more readily abscond in the face of a threat to the colony such as a heavy and damaging SHB infestation. Beekeeping practices, climate, and soil type and moisture may also alter the balance between the host bees and SHB (Hood 2004).

The primary damage to honey bee colonies and stored honey is through the feeding activity of the SHB larvae. Larvae tunnel through comb containing honey or pollen consuming the contents and fouling honey with their faeces. They are also predatory, feeding on honey bee brood and, by preference, the eggs (Elzen et al. 1999). This feeding activity causes fermentation of honey, either in the hive or in stored honey.

SHB can cause particularly severe damage in honey stored in the hive before processing when there are no honey bees to disrupt their activity (Gillespie et al. 2003). The fermented honey is not fit for human consumption or for feeding back to honey bees; it froths and weeps out of cells and an affected frame is said to be 'slimed' (Wenning 2001; Annand 2007). A heavy infestation may lead to loss of the honey bee colony (Eischen et al. 1998; Neumann and Elzen 2004).

Clinical signs

The presence of adult SHB may be the first sign of infestation and early infestations may go unnoticed (OIE 2008a). Within the hive, adult SHB or burrowing larvae may be observed with or without 'slimed' frames.

Infestation in a honey house may generate the characteristic odour of fermenting honey.

Diagnosis

Diagnosis is based on the identification of the various life stages of the beetles and clinical signs seen in the hive or in stored honey. The eggs are laid in clusters, are pearly white, elongate and approximately 1.4 mm long, while the larvae are white with 3 pairs of legs, dorsal spikes and are about 11 mm when fully grown. Adult SHB are oval in shape, 5–7 mm in length and 2.5–3.5 mm wide; the abdomen is largely covered by the elytra (wing cases) and they have a pair of rounded, clubbed antennae. An adult SHB is about one third the size of an adult honey bee (Lundie 1940; Annand 2007). Adult SHB can be observed hiding inside cells or in hive debris; they avoid light and scurry to darker locations when the hive is opened.

There are several other species of Nitidulid beetles that may be found in, or around, hives which must be distinguished from the SHB (Neumann and Ritter 2004; Stedman 2006). SHB larvae can be distinguished from wax moth larvae, for example, by examination of certain characteristics including differing numbers of prolegs and the cocooned pupation stage of the wax moth being within the hive (Stedman 2006).

Control

A variety of control methods for SHB have been used but the basis is good apiary management; that is, keeping colonies strong (specifically, maintaining a high honey bee to comb ratio), having good hygiene around the apiary and honey house and avoiding the use of contaminated equipment (Lundie 1940; Wenning 2001; Annand 2007).

Physical controls for SHB are also available. SHB adults or larvae may be removed by hand or by vacuum, and while this is effective, it is time-consuming and impractical for large

apiaries. A number of traps that exploit the SHBs' instinct to shelter in cracks or crevices have been described. SHB entering these traps are killed in a reservoir of oil, lime or diatomaceous earth (RIRDC 2005; Annand 2007).

Cold treatment has been used for the treatment of stored comb and equipment. All life-stages of SHB are susceptible to cold temperatures, adults more so than larvae. Boxes of infested comb need six hours at commercial freezer temperatures (–13ºC to –22ºC) and 12 days at refrigerator temperatures (1ºC to 9ºC) to kill all stages of SHB (RIRDC 2005).

Within-hive acaricides have been used to control SHB. Coumaphos has been reported to kill over 90 per cent of adult SHB (Elzen et al. 1999) but a later report found that a number of acaricides widely used against *Varroa*, including coumaphos, were relatively ineffective against adult SHB (RIRDC 2005). No acaricides are currently registered for use in honey bees in Australia.

The treatment of the soil outside the hives with an insecticide is used to target the pupal stage and emerging adult SHB. Permethrin, imidacloprid and chlorpyrifos have all been found to be effective (RIRDC 2005). The chemicals are applied either before hives arrive on the site or, if they are present, at a time when the honey bees are inactive. Fipronil is used in a harbourage placed on the bottom board of the hive. The entrance of the harbourage restricts the entry of bees but allows SHB to enter and contact the insecticide.

Conclusion

SHB is present throughout large areas of Australia and is likely to be present in some exporting countries. DAFF concluded that based on all the available information, further assessment of SHB was not required.

Although SHB is nationally notifiable in Australia, there are no mandatory control measures in place in the areas of the country where it is endemic. Consequently, there should be no restrictions placed on importation due to SHB.

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4.7 *Tropilaelaps*

Technical information

Background

Tropilaelaps species are acarine mites that infest the brood of honey bees (*Apis* species). Their primary hosts are the giant honey bees of Asia (*A. dorsata* and *A. laboriosa*). Four species of *Tropilaelaps* have been identified, of which two, *Tropilaelaps clareae* and *T. mercedesae* (previously mistaken for *T. clareae*), also parasitise *A. mellifera* (Anderson 2007).

Tropilaelaps are found throughout the range of the giant honey bee including mainland Asia, Indonesia and the Philippines. However, since infesting *A. mellifera*, *Tropilaelaps* has spread beyond the geographical range of its primary host to Afghanistan, Iran, Kenya, New Guinea and South Korea (Anderson 2007).

Tropilaelaps do not survive in areas where interruption of brood rearing occurs and have not become a serious problem in temperate zones except where continuous invasion of *Tropilaelaps* from tropical areas can occur (Woyke 1984). However, *Tropilaelaps* is considered an emerging threat to *A. mellifera* worldwide. Rising temperatures, causing *A. mellifera* to produce brood throughout the year, have been suggested as a mechanism that could lead to *Tropilaelaps* spreading into temperate regions (Anderson 2007).

Tropilaelaps has not been detected in Australia and *T. clareae* is notifiable in Australia (DAFF 2011).

Tropilaelaps infestation of honey bees caused by *T. clareae*, *T. koenigerum*, *T. mercedesae* and *T. thaii* is an OIE listed disease (OIE 2011a).

OIE requirements

The OIE Code recommendations (OIE 2011b) for the importation of live queen honey bees, worker honey bees and drones without associated brood combs are the presentation of an international veterinary certificate attesting that the honey bees have been held in isolation from brood and honey bees with access to brood, for a period of at least seven days.

Epidemiology and pathogenesis

Tropilaelaps are parasites of *A. mellifera* brood (OIE 2011b). One or more female *Tropilaelaps* enters the brood cell just before it is capped so multiple *Tropilaelaps* can be found within a single cell. Each female lays up to four eggs and the progeny (usually one male and several females) feed on the honey bee brood, causing serious damage.

Development takes approximately one week and the mature *Tropilaelaps*, including the original female, emerge from the cell along with the hatching honey bee and search for new hosts, preferably drone larvae (OIE 2008). Mating is thought to occur outside the brood cell (Oldroyd 2006).

Tropilaelaps has only a short and phoretic stage on the adult honey bee because it lacks the ability to feed on the mature honey bee (OIE 2008). They will survive only a short time if confined solely to adult honey bees—estimates of this period vary from as little as two days to as long as ten days (Woyke 1984; Rinderer 1994; OIE 2008). *Tropilaelaps* return to brood cells within 1.3 days (Oldroyd 2006) and gravid females die within two days if they do not deposit their eggs in a brood cell (OIE 2008).

Early signs of infestation usually go unnoticed. The *Tropilaelaps* population grows rapidly and can result in high hive mortality (OIE 2011b). Infestations can lead to rapid death of honey bee colonies and in Asia, *Tropilaelaps* is often considered to be more damaging to *A. mellifera* colonies than *Varroa destructor* (Anderson 2007).

Tropilaelaps spread when adult honey bees move between colonies through the natural processes of drifting, robbing and swarming. They can spread slowly over long distances in this way. Distribution of infested combs and honey bees through usual beekeeping practices allows spread within apiaries; however, the most rapid and main method of spread is through movement of infested colonies of *A. mellifera* to new areas by beekeepers (DEFRA 2005). There is documented evidence of human activity being responsible for spread (Anderson 2007).

Tropilaelaps can act as vectors for honey bee viruses (OIE 2011b). Deformed wing virus has been found in *T. mercedesae* in China, suggesting that this species may act as a viral vector in the same way as *V. destructor* (Forsgren 2009).

Clinical signs

Death of up to 50 per cent of honey bee larvae can occur with *Tropilaelaps* infestation (OIE 2008). An irregular brood pattern, cadavers partially protruding from cells and perforated cappings may be observed (OIE 2008). Honey bees that survive infestation during development can show physical or physiological damage, including shortened lifespan, reduced body weight, shrunken and deformed wings and legs, and they may be seen crawling at the hive entrance (DEFRA 2005).

Diagnosis

The OIE Manual (OIE 2008) describes methods of diagnosis of *Tropilaelaps* infestation by examination of adult honey bees, the colony and brood or hive debris.

Adult *Tropilaelaps* are red-brown and elongated and are less than one mm long (OIE 2008). Depending on species and gender, *Tropilaelaps* measure 600–1000 µm long and 400–550 µm wide (Anderson 2007).

For the examination of adult honey bees, the OIE Manual recommends shaking between 100 and 200 honey bees in ether, 70 per cent alcohol or soapy water or powdered sugar or flour (OIE 2008). The *Tropilaelaps* stick to the side of the container, are present in the strained liquid or can be shaken onto paper (OIE 2008). However, they are rarely found on adult honey bees (FAO 2006).

Examination of brood is conducted by uncapping drone and worker brood and observing the mites against the bodies of the brood (OIE 2008). *Tropilaelaps* are smaller than *Varroa* but can be seen under a magnifying glass or by using a dissecting microscope (Shimanuki 2000).

Collection and examination of hive debris is facilitated by placing sticky boards above the bottom boards and under a wire frame. Acaricides can be used to kill *Tropilaelaps* that subsequently detach from the honey bees and fall onto the sticky boards (OIE 2008).

Control

The main aim of *Tropilaelaps* control is to keep the population to a level where economic harm is unlikely (DEFRA 2005). Both chemical (e.g. acaricides) and non-chemical (e.g. husbandry) treatments can be used to control *Tropilaelaps*.

Many of the same chemicals used for *Varroa* control will kill *Tropilaelaps*, such as fluvalinate or formic acid (OIE 2008). As for control of *Varroa*, use of acaricides in honey bee colonies can leave chemical residues in honey bee products (Wallner 1999). Exposure of honey bees to these chemicals and their accumulation over time in wax have been implicated in colony loss syndromes (Johnson 2010; Le Conte 2010).

Given *Tropilaelaps*' inability to survive for long periods away from brood, husbandry techniques that create breaks in the brood, such as caging queen honey bees, use of artificial swarms and comb trapping, can be used to reduce its numbers (DEFRA 2005). Other non-chemical control methods use physical means alone to reduce the *Tropilaelaps* population, such as trapping of *Tropilaelaps* in combs of brood, which are then removed and destroyed (DEFRA 2005).

Conclusion

Tropilaelaps species are not present in Australia and may be present in some exporting countries. The OIE Code recommendations (OIE 2011b) for the importation of live queen honey bees, worker honey bees and drones without associated brood combs are the presentation of an international veterinary certificate attesting that the honey bees have been held in isolation from brood and honey bees with access to brood, for a period of at least seven days. Australia's previous biosecurity requirements differ from those in the OIE Code. Therefore, DAFF concluded that further risk assessment was required.

Risk assessment

For details of the method used in this risk assessment see Chapter 2. A summary of the risk assessment is shown in Figure 9.

Release assessment

The following factors were considered relevant to the estimate of the likelihood of *Tropilaelaps* being present on imported queen honey bees.

- the primary hosts of *Tropilaelaps* are the giant honey bees of Asia (*A. dorsata* and *A. laboriosa*) (Anderson 2007)
- *Tropilaelaps* have been found in mainland Asia, Indonesia and the Philippines. Since infesting *A. mellifera*, *Tropilaelaps* has spread beyond the geographical range of its primary host to Afghanistan, Iran, Kenya, New Guinea and South Korea (Anderson 2007)
- *Tropilaelaps* are parasites of *A. mellifera* brood (OIE 2011b)
- *Tropilaelaps* will survive only a short time if confined solely to adult honey bees—estimates of this period vary from as little as two days to as long as ten days (Woyke

1984; Rinderer 1994; OIE 2008). Therefore, if a consignment was infested, the *Tropilaelaps* would need to survive for the duration of transport
- early signs of infestation usually go unnoticed (OIE 2011b).

Conclusion: based on this information, the likelihood of release of *Tropilaelaps* associated with importation of queen honey bees was estimated to be **low**.

Exposure assessment

The exposure group considered was managed and feral honey bee colonies and the most likely exposure pathway is the introduction of infested imported adult honey bees to a managed honey bee colony.

The following factors were considered relevant to the estimate of the likelihood of susceptible honey bees being exposed to *Tropilaelaps* via imported queen honey bees:

- *Tropilaelaps* spread when adult honey bees move between colonies through the natural processes of drifting, robbing and swarming. They can spread slowly over long distances in this way. Distribution of infested combs and honey bees through usual beekeeping practices allows spread within apiaries; however, the most rapid and main method of spread is through movement of infested colonies of *A. mellifera* to new areas by beekeepers (DEFRA 2005)
- *Tropilaelaps* survives only a short time if confined solely to adult honey bees—estimates of this period vary from as little as two days to as long as ten days (Woyke 1984; Rinderer 1994; OIE 2008).

Conclusion: based on this information, the likelihood of susceptible honey bees being exposed to *Tropilaelaps* via infested imported queen honey bees was estimated to be **moderate**.

Estimation of the likelihood of release and exposure

The likelihood of release and exposure is estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (Table 2). With the likelihood of release estimated to be 'low' combined with the likelihood of exposure estimated to be 'moderate', the likelihood of release and exposure was estimated to be **low**.

Consequence assessment

The consequence assessment describes the potential consequences associated with hazard entry and exposure, and estimates the likelihood of them occurring. This involves estimating the likelihood of establishment and/or spread of the hazard for the most likely outbreak scenario, and determining the direct or indirect effects (health, environment and socioeconomic) should this outbreak scenario occur. Combining the likelihood of establishment and/or spread for this outbreak scenario with the corresponding overall effect gives an estimation of likely consequences.

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred, a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario was determined by describing the likely extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to *Tropilaelaps* is considered to be establishment and/or spread to populations of susceptible honey bees within a state/territory, confined to non-temperate regions, through direct contact.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of susceptible honey bees to *Tropilaelaps*.

- early signs of infestation usually go unnoticed (OIE 2011b)
- *Tropilaelaps* do not survive in areas where interruption of brood rearing occurs and have not become a serious problem in temperate zones except where continuous invasion of *Tropilaelaps* from tropical areas can occur (Woyke 1984)
- the most rapid and main method of spread is though movement of infested colonies of *A. mellifera* to new areas by beekeepers (DEFRA 2005).

Conclusion: based on these considerations, it was considered that the likelihood of establishment and/or spread of *Tropilaelaps* for the exposure group was **high**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect impacts of *Tropilaelaps* were estimated at the national, state or territory, district/region and local levels. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria.

The following factors were considered relevant to a conclusion on the effects of the establishment and/or spread of *Tropilaelaps*:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- infestations can lead to the rapid death of honey bee colonies and in Asia, *Tropilaelaps* is often considered to be more damaging than *V. destructor* (Anderson 2007)
- *Tropilaelaps* populations grow rapidly and can result in high hive mortality (OIE 2011b)
- death of up to 50 per cent of honey bee larvae can occur with *Tropilaelaps* infestation (OIE 2008)
- honey bees that survive infestation during development may show physical or physiological damage, including shortened lifespan, reduced body weight, shrunken and deformed wings and legs, and they may be seen crawling at the hive entrance (DEFRA 2005)
- *Tropilaelaps* can act as vectors for honey bee viruses (OIE 2011b).

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- the presence of *Tropilaelaps* in managed and feral honey bee colonies is not considered to negatively impact on native plant species as pollination of native flora is not dependent on *A. mellifera*
- the susceptibility of Australian native bees to *Tropilaelaps* is unknown.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- *Tropilaelaps* (*T. clareae*) is notifiable in Australia (DAFF 2011)
- if *Tropilaelaps* was identified in Australia, the response policy as outlined in the AUSVETPLAN *Disease Strategy: Bee Pests and Diseases* (Animal Health Australia 2010) would be followed based on how early the incursion was detected, the extent of the incursion and the location of affected hives. Control and eradication of *Tropilaelaps* using stamping out is the default policy (Animal Health Australia 2010)[4] but for the outbreak scenario under consideration it is unlikely that eradication would be feasible
- controls over honey bee movements, products and equipment would be imposed on managed apiaries within designated areas until further decisions were made (Animal Health Australia 2010)
- movement controls could affect commercial interests of the apiarist and health of honey bee colonies (Animal Health Australia 2010)
- if the decision was made not to attempt eradication but to recommend that control practices be initiated, state/territory and/or industry-based control measures would be initiated, which may include encouraging industry to develop its own long-term policies and procedures (Animal Health Australia 2010).

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- if *Tropilaelaps* were detected, movement restrictions would be imposed until tracing and surveillance were completed and the results analysed (Animal Health Australia 2010)
- if eradication was not attempted, ongoing control measures may include interstate movement controls (Animal Health Australia 2010)
- the presence of *Tropilaelaps* in managed and feral honey bee colonies may negatively impact on pollination services to horticultural and agricultural crops. The loss of honey bee pollination from agricultural production has been estimated to result in a flow-on loss of A$2 billion and 11 000 jobs (Gordon 2003)
- prices of products from honey bees may increase, thereby reducing consumer demand.

[4] The emergency management of honey bee diseases and pests are in the process of moving under the Australian Emergency Plant Pest Response Deed (EPPRD) and the Australian Emergency Plant Pest Response Plan (PLANTPLAN)

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- loss of Australia's *Tropilaelaps*–free status may reduce international consumer demand for Australian honey bees and bee products
- if *Tropilaelaps* were to become established, renegotiation of trade conditions with trading partners may become necessary.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- if used, the application of chemicals to control *Tropilaelaps* may have an effect on a range of arthropod species and disrupt the food source of wildlife, leading to environmental contamination (including water sources) and increased resistance to the chemicals.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- the cost of beekeeping would increase due to expenses associated with control of *Tropilaelaps*
- non-commercial and small-scale commercial beekeepers may become non-viable
- use of acaricides to control *Tropilaelaps* in honey bee colonies may leave chemical residues in honey bee products. Products may be declared unfit for human consumption and consumers may lose confidence in the domestic market.

Conclusion for overall direct and indirect effects: based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread for the outbreak scenario was estimated to be **moderate** from Table 3. The effect is likely to be recognised on a national level and significant within affected zones, and be highly significant to directly affected parties.

Derivation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario using Table 4 to obtain an estimation of likely consequences.

Therefore, the likelihood of establishment and/or spread ('high') is combined with the estimate of the overall effect of establishment and/or spread ('moderate') which resulted in **moderate** likely consequences.

Unrestricted risk estimation

Risk estimation is the integration of likelihood of release and exposure, and likely consequences of establishment and/or spread to derive the risk associated with release, exposure, establishment and/or spread of *Tropilaelaps* introduced by the importation of queen honey bees into Australia.

Using Table 5, the likelihood of release and exposure ('low') is combined with the likely consequences of establishment and/or spread ('moderate'), which results in a risk estimation of **low**.

Therefore, the unrestricted risk associated with *Tropilaelaps* species was assessed as **low**. As this estimate exceeds Australia's ALOP of 'very low', risk management was considered necessary for this agent.

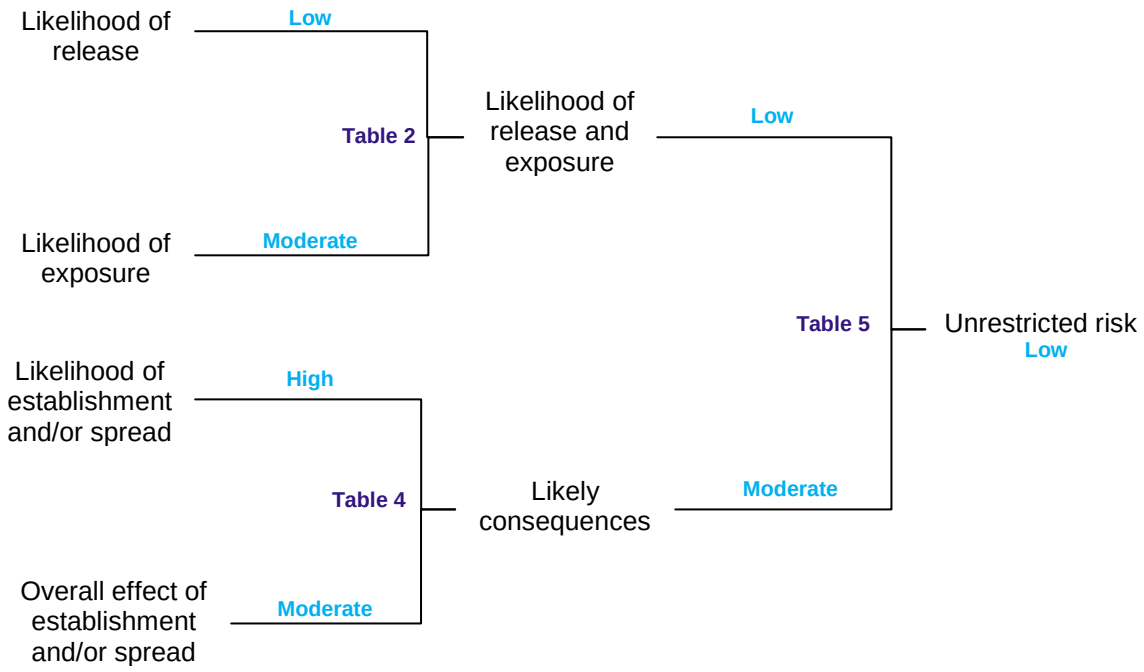

Figure 9. Summary of the risk assessment pathways for *Tropilaelaps*

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4.8 Varroosis

Technical information

Background

Varroosis, also known as *Varroa*, is a disease of the honey bee (*Apis mellifera*) caused by species of mites in the family Varroidae within the order Acari. It is considered to be the most damaging pest for beekeeping worldwide (Solignac 2005). Only Australia, apart from islands in the Torres Strait adjacent to PNG but administered by Australia, remains free of Varroa. (Ellis 2005).

Four species of *Varroa* have been recorded: *V. destructor*, *V. jacobsoni*, *V. rinderi* and *V. underwoodi* (OIE 2008b). Until 2000, *V. destructor* and *V. jacobsoni* were thought to be the same species (Anderson 2000).

The natural host of *Varroa* is the Asian honey bee (*A. cerana*). In the mid 20th century *V. destructor* adapted to successfully parasitise *A. mellifera* and subsequently spread through natural dispersion and the translocation of live honey bees by humans. Of the numerous known haplotypes of *V. destructor*, only a limited number of haplotypes from the Japan 1 (also known as J1) and Korea 1 (also known as K1) haplogroups infest *A. mellifera*; the others are confined to *A. cerana* (Solignac 2005; Navajas 2010). A form of *V. jacobsoni* that is harmful to *A. mellifera* has been found in PNG (Anderson 2008).

Varroosis, caused by the K and J haplotypes of *V. destructor*, is an OIE listed disease (OIE 2011a). Both *V. destructor* and *V. jacobsoni* are notifiable in Australia (DAFF 2011).

OIE requirements

The OIE Code recommendations (OIE 2011b) for the importation of live queen honey bees, worker honey bees and drones with or without associated brood combs are the presentation of an international veterinary certificate attesting that the honey bees come from a country or zone/compartment officially free from varroosis.

Epidemiology and pathogenesis

Varroa is easily spread by direct contact with adult honey bees (The Food and Environment Research Agency 2009; OIE 2011b). Its spread through a number of new geographic ranges has been extensively documented (De Jong 1997; Matheson 1993; Matheson 1996). To date, no eradication program has been successful.

Varroa is a parasite of adult honey bees and their brood (OIE 2011b). Local spread occurs by intercolony drifting of infested adult honey bees, movement of swarms and by honey bees robbing weakened colonies (De Jong 1997). Movement of equipment, transhumance, displacement of colonies for pollination purposes, and the worldwide trade in live honey bees have had a major role in the rapid geographic spread of *Varroa* (Solignac 2005).

The entire lifecycle of *Varroa* occurs in the beehive. The mature mated female *Varroa* enters a brood cell just before the cell is capped. It feeds on the haemolymph of the developing honey bee larva and starts to lay eggs approximately 60 hours after the cell is capped. Eggs

are laid in a strict sequence: firstly, an unfertilised egg that will develop as a male and then, at intervals of approximately 30 hours, up to 5 more fertilised eggs that will develop as females. All stages are obligate parasites and feed on the haemolymph.

The hatched *Varroa* mature rapidly and the male is ready to mate within 190 hours (approximately 8 days) of being laid. The male mates with each female as the females mature. Adult, mated females then emerge from the cell along with the hatching honey bee (Donzé 1994; Oldroyd 1999).

Varroa has a propensity for drone larvae. In *A*. *cerana* colonies, *Varroa* reproduction is almost wholly limited to drone cells but in *A*. *mellifera*, although there is still a strong preference for drone larvae (average eightfold), *Varroa* will also reproduce in worker cells, particularly if drone brood is absent or heavily parasitised (Oldroyd 1999).

Varroa can also feed on the haemolymph of adult honey bees by puncturing and feeding through the intersegmental membrane (OIE 2008b). The lifespan of *Varroa* on larval or adult honey bees varies from a few days to a few months, depending on temperature and humidity (OIE 2011b). *Varroa* can survive no more than 5 days without honey bees or brood but can live in a comb with sealed brood for up to 30 days (Frazier 2005).

When introduced to a new area, infestations of individual honey bee colonies increase slowly over several years. There is little sign of damage initially and the infestation often goes unnoticed while the number of *Varroa* in a colony is low; during this time *Varroa* can spread to other colonies (De Jong 1997). A survey undertaken in New Zealand in 2000, suggested that *Varroa* may have been present for 3–4 years before it was discovered (Benard 2000). Heavy infestations usually develop after 3–4 years (OIE 2008b).

Varroosis is considered to be the most severe threat to beekeeping worldwide (De Jong 1997). There are reports of the loss of thousands of colonies of managed honey bees and the loss of feral populations following the introduction of *Varroa* into a new geographic range (Beetsma 1994; Seeley 2007).

Damage is mostly caused during the development of the honey bee. Due to injuries caused by *Varroa* puncturing the larvae and pupae, and loss of haemolymph, the average body weight of newly emerged worker honey bees is reduced by up to 25 per cent (Beetsma 1994). Honey bees that have been infested during the brood phase show various ill effects and the parasitism is critical if more than one *Varroa* is in the brood cell (Beetsma 1994; OIE 2008b). *Varroa* can also be a vector for honey bee viruses such as acute paralysis virus and deformed wing virus (Ribière 2008).

Clinical signs

Clinical signs of infestation are often first observed in the latter part of the season. While the numbers of *Varroa* usually increase slowly at the start of the season, with maximum numbers being reached late in the season, clinical signs may be seen at any time (OIE 2008b).

Honey bees infested during the brood phase by a single parasitic *Varroa* may show a reduced life span, behavioural changes and increased disease susceptibility (OIE 2008b). Shrunken wings and shortened abdomen, due to increased susceptibility to deformed wing virus and acute paralysis virus, may also be observed (OIE 2008b).

Severely infested colonies can appear restless with neglected brood, and often clinical signs of European foulbrood are also present (De Jong 1997). Generally, colonies that are not treated die within 2–4 years (De Jong 1997).

Diagnosis

Varroa infestation may be diagnosed by examining adult honey bees, brood or hive debris.

Adult, female *Varroa* can be seen with the naked eye but they are difficult to detect as they attach to the adult honey bee between the abdominal segments or between body regions (Shimanuki 2000). Individual *Varroa* are oval, flat, pale to reddish-brown, and 1.1 mm long and 1.5 mm wide.

Examination of adult honey bees for *Varroa* requires the collection of 200 to 300 honey bees. *Varroa* can be dislodged by shaking the honey bees in liquids such as alcohol, hot water or detergent solution; 70 per cent alcohol (ethyl, methyl or isopropyl) is recommended (Shimanuki 2000). Dislodged *Varroa* are collected by passing the honey bees and alcohol through a wire screen and sieving alcohol through cotton cloth.

An alternative method using powdered sugar does not require honey bees to be killed; they are coated with powdered sugar in a jar, shaken and the sugar and detached *Varroa* sieved onto paper for examination. Methods involving treating honey bees with ether or subjecting them to heat to remove *Varroa* have also been described (Shimanuki 2000) but are not commonly used.

Examination of brood is done by uncapping brood (preferably drone) and observing the dark *Varroa* against the white bodies of the brood (Shimanuki 2000).

Hive debris can be examined for *Varroa* by floatation (Shimanuki 2000). Collection and examination of hive debris is facilitated by placing sticky boards above the bottom boards and under a wire frame.

Using these methods, in their assessment of diagnostic methods for low levels of *Varroa* infestation, Fries et al. (1991) concluded that when sealed brood was present, examination of hive debris was more effective than sampling of brood, and brood sampling was more effective than sampling of live honey bees. In colonies without sealed brood, examination of hive debris and of live honey bee samples, were approximately equally efficient. The earliest and most precise diagnosis requires the application of medication that kills *Varroa* directly or forces them to drop off the honey bees (OIE 2008b).

Control

The main aim of *Varroa* control is to keep the population below a level that is harmful for colony health, production and pollination (The Food and Environment Research Agency 2009). Both chemical and non-chemical treatments have been used to control *Varroa*.

Many of the same chemicals used to kill *Tropilaelaps* will control *Varroa* (OIE 2008a). However, use of acaricides in honey bee colonies can leave chemical residues in honey bee products (Wallner 1999). Exposure of honey bees to these chemicals and their accumulation over time in wax have been implicated in colony loss syndromes (Johnson 2010; Le Conte 2010).

Acaricides are applied in various ways: in feed, directly on the adult honey bees, as fumigants, as contact strips or by evaporation (The Food and Environment Research Agency 2009). Synthetic pyrethroids have been used successfully but *Varroa* have developed resistance in some countries (Milani 1999). Non-pyrethroid chemicals such as amitraz, coumaphos, thymol, and acids such as formic, lactic and oxalic, have also been used with varying success (The Food and Environment Research Agency 2009).

Hive management techniques such as drone brood removal, comb trapping, artificial swarm and the use of open mesh floors can be used to reduce *Varroa* numbers. However, these methods are only suitable for restricted periods of the year due to seasonal variations in honey bee colony activities. Generally, if there is a heavy *Varroa* infestation, these methods provide insufficient control and need to be used in conjunction with acaricides (The Food and Environment Research Agency 2009).

Several programs based on breeding *Varroa*-resistant honey bees for long-term control have been developed with some success (Büchler 2010; Rinderer 2010).

Conclusion

Varroa are not present in Australia and are likely to be present in exporting countries. The OIE Code recommendations (OIE 2011b) for the importation of live queen honey bees, worker honey bees and drones with or without associated brood combs are the presentation of an international veterinary certificate attesting that the honey bees come from a country or zone/compartment officially free from varroosis. Australia's previous biosecurity requirements differ from those in the OIE Code. Therefore, DAFF concluded that further risk assessment was required.

Risk assessment

For details of the method used in this risk assessment see Chapter 2. A summary of the risk assessment is shown in Figure 10.

Release assessment

The following factors were considered relevant to the estimate of the likelihood of *Varroa* being present on imported queen honey bees.

- varroosis occurs worldwide, however Australia remains free (Ellis 2005)
- *Varroa* is a parasite of adult honey bees and their brood (OIE 2011b)
- the lifespan of *Varroa* on larval or adult honey bees varies from a few days to a few months, depending on the temperature and humidity (OIE 2011b)
- there is little sign of damage initially and the infestation often goes unnoticed while the number of *Varroa* in a colony is low; during this time it can spread to other colonies (De Jong 1997).

Conclusion: based on this information, the likelihood of release of *Varroa* associated with the importation of queen honey bees was estimated to be **high**.

Exposure assessment

The exposure group considered was managed and feral honey bee colonies and the most likely exposure pathway is direct contact between imported queen honey bees and domestic managed colonies through the introduction of imported honey bees into domestic colonies.

The following factors were considered relevant to the estimate of the likelihood of susceptible honey bees being exposed to *Varroa* via imported queen honey bees:

- *Varroa* is easily spread by direct contact with adult honey bees (The Food and Environment Research Agency 2009; OIE 2011b)
- local spread occurs by intercolony drifting of infested adult honey bees, movement of swarms and by honey bees robbing weakened colonies (De Jong 1997)
- movement of equipment, transhumance, displacement of colonies for pollination purposes, and the worldwide trade in live honey bees have had a major role in the rapid geographic spread of *Varroa* (Solignac 2005).

Conclusion: based on this information, the likelihood of susceptible honey bees being exposed to *Varroa* via infested imported queen honey bees was estimated to be **high**.

Estimation of the likelihood of release and exposure

The likelihood of release and exposure is estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (Table 2). With the likelihood of release estimated to be 'high' combined with the likelihood of exposure estimated to be 'high', the likelihood of release and exposure was estimated to be **high**.

Consequence assessment

The consequence assessment describes the potential consequences associated with hazard entry and exposure, and estimates the likelihood of them occurring. This involves estimating the likelihood of establishment and/or spread of the hazard for the most likely outbreak scenario, and determining the direct or indirect effects should this outbreak scenario occur. Combining the likelihood of establishment and/or spread for this outbreak scenario with the corresponding overall effect gives an estimation of likely consequences.

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred, a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario was determined by describing the likely extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to *Varroa* is considered to be establishment and/or spread to populations of susceptible honey bees within multiple states/territories through direct contact.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of susceptible honey bees to *Varroa*:

- varroosis is extremely communicable
- the lifespan of *Varroa* on larval or adult honey bees varies from a few days to a few months, depending on the temperature and humidity (OIE 2011b)

- heavy infestations usually develop 3–4 years after the primary incursion into a colony (OIE 2008b)
- when introduced to a new area, infestations of individual honey bee colonies increase slowly over several years. There is little sign of damage initially and the infestation often goes unnoticed while the number of *Varroa* in a colony is low; during this time it spreads to other colonies (De Jong 1997)
- *Varroa* may have been present in New Zealand for 3–4 years before it was discovered (Benard 2000)
- movement of equipment, transhumance, displacement of colonies for pollination purposes, and the worldwide trade in live honey bees have had a major role in the rapid geographic spread of *Varroa* (Solignac 2005)
- feral colonies of honey bees are widely distributed, interacting with managed hives and providing ample scope for rapid dissemination of *Varroa*. Honey bees, hive equipment and apiary products are moved over long distances and often interstate (Animal Health Australia 2010).

Conclusion: based on these considerations, it was determined that the likelihood of establishment and/or spread of *Varroa* for the exposure group was **high**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect impacts of *Varroa* were estimated at the national, state or territory, district/region and local levels. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria.

The following factors were considered relevant to a conclusion on the effects of the establishment and/or spread of *Varroa*.

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- varroosis is considered to be the most severe threat to beekeeping worldwide (De Jong 1997)
- there are reports of the loss of thousands of colonies of managed *A. mellifera* and the loss of feral populations following the introduction of *Varroa* into a new geographic range (Beetsma 1994; Seeley 2007)
- *Varroa* feeding on larvae can produce weakened honey bees—lower body weights, reduced life span, behavioural changes and increased disease susceptibility have been reported (Beetsma 1994; OIE 2008b)
- *Varroa* is a vector for honey bee viruses (OIE 2011b)
- generally, colonies that are not treated die within 2–4 years (De Jong 1997).

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- there are reports of the loss of feral populations of *A. mellifera* following the introduction of *Varroa* into a new geographic range (Seeley 2007)

- the presence of *Varroa* in managed and feral honey bee colonies is not considered to negatively impact on pollination of native plant species as native flora is not dependent on *A. mellifera*
- the susceptibility of Australian native bees to *Varroa* is unknown.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- varroosis caused by *V. destructor* and *V. jacobsoni* is nationally notifiable in Australia (DAFF 2011)
- if *Varroa* were identified in Australia, the response policy as outlined in the AUSVETPLAN *Disease Strategy: Bee Pests and Diseases* (Animal Health Australia 2010) would be followed based on how early the incursion was detected, the extent of the incursion and the location of affected hives. Although control and eradication of *Varroa* using stamping out is the default policy (Animal Health Australia 2010)[5], it is unlikely that eradication would be considered feasible for the current outbreak scenario
- controls over honey bee movements, products and equipment would be imposed on managed apiaries within designated areas until further decisions were made (Animal Health Australia 2010)
- movement controls could affect commercial interests of the apiarist and health of honey bee colonies (Animal Health Australia 2010)
- if the decision was made not to attempt eradication but to recommend that control practices be initiated, state/territory and/or industry-based control measures would be initiated, which may include encouraging industry to develop its own long-term policies and procedures (Animal Health Australia 2010).

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- if *Varroa* were detected, movement restrictions would be imposed until tracing and surveillance were completed and the results analysed (Animal Health Australia 2010)
- if eradication was not attempted, ongoing control measures may include interstate movement controls (Animal Health Australia 2010)
- the presence of *Varroa* in managed and feral honey bee colonies will negatively impact on pollination services to horticultural and agricultural crops. The loss of honey bee pollination from agricultural production has been estimated to result in a flow-on loss of A$2 billion and 11 000 jobs (Gordon 2003)
- prices of products from honey bees may increase, thereby reducing consumer demand.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- varroosis occurs worldwide, however Australia remains free of infestation (Ellis 2005)

[5] The emergency management of honey bee diseases and pests are in the process of moving under the Australian Emergency Plant Pest Response Deed (EPPRD) and the Australian Emergency Plant Pest Response Plan (PLANTPLAN)

- loss of Australia's *Varroa*–free status may reduce international consumer demand for Australian honey bees and their products
- if *Varroa* were to become established, renegotiation of trade conditions may be necessary.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- if used, application of chemicals to control *Varroa* may have an effect on a range of arthropod species and disrupt the food source of wildlife, lead to environmental contamination (including water sources) and increased resistance to the chemicals.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- the cost of beekeeping would increase due to expenses associated with control of *Varroa*
- non-commercial and small-scale commercial beekeepers may become non-viable
- use of acaricides to control *Varroa* can leave chemical residues in honey bee products (Wallner 1999). Products may be declared unfit for human consumption and consumers may lose confidence in the domestic market.

Conclusion for overall direct and indirect effects: based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread for the outbreak scenario was estimated to be **high** from Table 3. The effect is likely to be significant at the national level, highly significant within affected zones, and to be of national concern.

Derivation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario using Table 4 to obtain an estimation of likely consequences.

The likelihood of establishment and/or spread ('high') is combined with the estimate of the overall effect of establishment and/or spread ('high') which results in **high** likely consequences.

Unrestricted risk estimation

Risk estimation is the integration of likelihood of release and exposure, and likely consequences of establishment and/or spread to derive the risk associated with release, exposure, establishment and/or spread of *Varroa* introduced by imported queen honey bees into Australia.

Using Table 5, the likelihood of release and exposure ('high') is combined with the likely consequences of establishment and/or spread ('high'), resulting in a risk estimation of **high**.

Therefore, the unrestricted risk associated with *Varroa* was assessed as **high**. As this exceeds Australia's ALOP of 'very low', risk management was considered necessary.

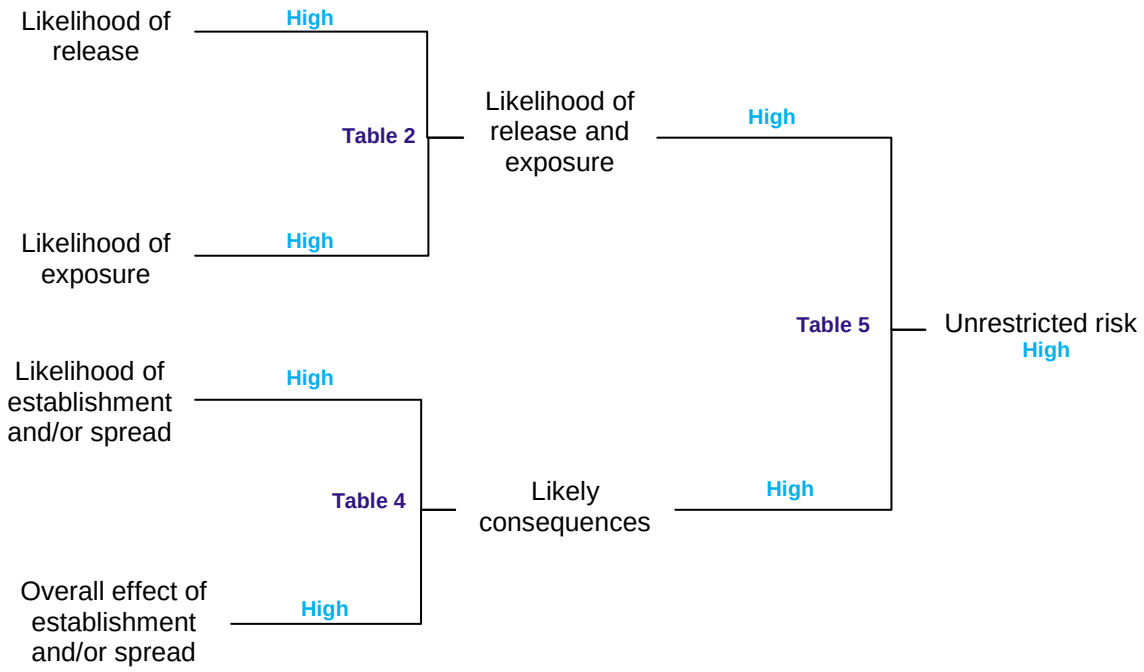

Figure 10. Summary of the risk assessment pathways for *Varroa*

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4.9 Acute paralysis virus

Technical information

Background

Acute paralysis virus (APV) was first identified in 1963 (Bailey 1963). It is a single-stranded RNA virus in the family Dicistroviridae (Christian 2005).

APV has been shown to occur in low concentrations in apparently healthy adult *Apis mellifera* (Bailey 1963; Bailey 1981) and in bumble bees (*Bombus* species) (Bailey 1964) with no obvious signs at the individual or colony level (de Miranda 2010). APV has been detected in honey bees in Africa, North, Central and South America, Asia, Europe, Middle East and New Zealand (Anderson 1991; Anderson 1995; Allen 1996; Ellis 2005), without associated disease or mortality (Allen 1996).

However, in Europe and the USA, high concentrations of APV have been detected in dead adult honey bees and diseased brood in colonies infested with *Varroa destructor*, and pathogen incidence studies have implicated APV as the cause of mortality in *Varroa*-infested colonies (Allen 1996; Ribière 2008).

APV has not been confirmed in Australia. Although APV was initially reported in serological tests as being present in Australia (Reinganum 1969), subsequent studies using specific APV antiserum failed to confirm its presence (Anderson 1983; Anderson 1984; Dall 1985; Hornitzky 1987; Anderson 1988; Anderson 1989). Hence, the initial report (Reinganum 1969) is now regarded as a false-positive, probably caused by non-specific host proteins in the antiserum used. A survey in 1993 revealed the presence of five known honey bee viruses but APV was not among them (Anderson 1993).

APV is not a notifiable disease in Australia (DAFF 2011).

OIE requirements

APV is not an OIE listed disease (OIE 2011) and there are no OIE recommendations.

Epidemiology and pathogenesis

APV has been detected in both brood and adult honey bees (Chen 2007).

It accumulates in the head of acutely paralysed honey bees, especially in the hypopharyngeal glands and brain (Bailey 1969), and infectious APV particles can be detected in faeces (Bailey 1964). In nature, the virus may be spread via salivary gland secretions of adult honey bees and the food to which these secretions are added (Bailey 1976). It may exist as an inapparent infection of the gastrointestinal tract (Anderson 1991) and in tissues that are not immediately essential to the life of the honey bee (Bailey 1981).

APV has been detected in the pollen loads of foraging honey bees but not in the pollen of plants, suggesting that it is not a plant virus and that foodborne transmission may occur (Bailey 1981; Chen 2006). APV has also been detected in drone semen, implicating mating

as a possible route of horizontal and vertical virus transmission (Yue 2006). APV has not been detected in the ovaries of queen honey bees (Chen 2006b).

When injected into pupae or adult honey bees, APV is extremely virulent. Infectivity studies determined the LD_{50}[6] of APV when injected into apparently healthy honey bees was equivalent to 130 intact particles per honey bee, compared with more than 1011 particles per honey bee by feeding, and 108 to 109 particles per honey bee by spraying (Bailey 1963).

APV was initially reported to be transmissible by *V. destructor* (formerly known as *V. jacobsoni*) (Batuev 1979). Since then, it has been implicated in *Varroa*-induced colony losses, particularly in Europe in the 1980s–90s (de Miranda 2010). APV has been found in honey bee samples from apiaries where no APV-positive *Varroa* were detected, suggesting that APV can be transmitted by contact between individual honey bees (Tentcheva 2004).

It is possible that *V. destructor* transfers APV when feeding on pupae and adult honey bees. Following contact with APV-infected pupae, adult female *V. destructor* have been shown to transfer the virus to uninfected pupae in 50–89.5 per cent of cases; transmission rates are correlated with the period of feeding on infected pupae and the number of feeds on the same naïve pupae (Wiegers 1988; Ball, as cited in Genersch 2010).

Varroa appears to act only as a mechanical vector of APV particles and does not allow or support virus replication; there is no latent period between acquiring the virus and viral transmission, and the transfer efficiency drops to zero when the same *Varroa* is successively introduced onto 4–5 different naïve pupae (Wiegers 1988). In addition to acting as a viral vector, *Varroa* is also believed to be an activator of inapparent APV in infected honey bees (Chen 2007).

The prevalence, regional distribution and seasonal incidence of APV vary across apiaries (de Miranda 2010). APV tends to increase in prevalence and titre as the season progresses, peaking in the late northern hemisphere summer which coincides with peak *V. destructor* populations (Tentcheva 2004; de Miranda 2010).

Tentcheva (2004) demonstrated the prevalence of APV in adult honey bees, pupae and *Varroa* in 10 colonies from each of 36 French apiaries. While the sampled colonies were apparently healthy, APV was detected in adult honey bees at least once in 58 per cent of the sampled apiaries, and in pupae at least once in 23 per cent of sampled apiaries. APV was detected in *V. destructor* in 36 per cent of the apiaries. The incidence of APV was higher in summer and autumn, which coincided with the peak in the *V. destructor* population.

A study of Danish apiaries with winter mortality showed an APV prevalence of 14 per cent (Nielsen 2008). Siede (2008) determined the prevalence of APV in 110 colonies in 11 apiaries in Germany to be 73 per cent in 2004 and 80 per cent in 2005 (Siede 2008).

Clinical signs

APV normally exists as an inapparent infection in honey bee colonies. Clinical signs in artificially infected honey bees are more acute than those of natural disease (Bailey 1963). Honey bees injected with APV typically show signs of paralysis (trembling) within 2 to 4 days and then die within the following 24 hours (Bailey 1963).

[6] LD_{50} is the dilution of each preparation that would have killed half of the bees in a group

Although mostly symptomless in individual adult honey bees, pupae and larvae, APV can be lethal at the colony level when in association with *V. destructor* (de Miranda 2008). This can result in the appearance of diseased larvae and pupae due to the lack of adults tending to the brood (de Miranda 2010).

Diagnosis

Diagnostic techniques have moved from serology-based approaches to molecular protocols that detect virus genetic material (de Miranda 2010). Diagnostic methods using antisera that have been investigated include immunodiffusion and enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay is useful for screening large numbers of samples cheaply and easily, and has sufficient sensitivity to detect viruses at subclinical levels (de Miranda 2010).

Molecular techniques such as reverse transcription polymerase chain reaction (RT-PCR) have largely replaced serological techniques. The limiting factor for molecular techniques is the availability of accurate nucleotide sequence data. RT-PCR protocols are available for detection of structural and functional genes of APV (de Miranda 2008; de Miranda 2010).

Reverse transcription quantitative PCR has become an important tool for the investigation of pathogenesis of viral infections in honey bees (Dainat 2011).

Control and management

Viral infections of honey bees can have serious impacts on the profitability of the beekeeping industry. However, to date, limited work has been undertaken on suitable treatments and control measures (Genersch 2010).

An integrated pest management program for management of viruses should include accurate diagnosis of diseases to allow development and implementation of control strategies, good beekeeping management practices (such as controlling *Varroa* populations), enhancing natural immunity to infection and selecting and breeding disease-resistant strains of honey bee (Aubert 2008;de Miranda 2008).

Conclusion

APV has not been confirmed to be present in Australia and is likely to be present in exporting countries. There are no recommendations in the OIE Code. Therefore, DAFF concluded that further risk assessment was required.

Risk assessment

For details of the method used in this risk assessment see Chapter 2. A summary of the risk assessment is shown in Figure 11.

Release assessment

The following factors were considered relevant to the estimate of the likelihood of APV being present in imported queen honey bees.

- APV has been detected in both brood and adult honey bees (Chen 2007)

- APV has been detected in honey bees in Africa, North, Central and South America, Asia, Europe, Middle East and New Zealand (Anderson 1991; Anderson 1995; Allen 1996; Ellis 2005), often without associated disease or mortality (Allen 1996).
- the prevalence of APV varies across apiaries (de Miranda 2010)
- in apparently healthy sampled colonies in France, APV was detected in adult honey bees at least once in 58 per cent, and in pupae at least once in 23 per cent, of apiaries (Tentcheva 2004)
- a study of Danish apiaries with winter mortality gave an APV infection rate of 14 per cent (Nielsen 2008)
- the prevalence of APV in 110 colonies in 11 apiaries in Germany was determined to be 73 per cent in 2004 and 80 per cent in 2005 (Siede 2008).

Conclusion: based on this information, the likelihood of release of APV associated with the importation of queen honey bees was estimated to be **moderate**.

Exposure assessment

The exposure group considered was managed and feral honey bee colonies and the most likely exposure pathway is the introduction of imported honey bees into domestic colonies.

The following factors were considered relevant to the estimate of the likelihood of susceptible honey bees being exposed to APV via infected imported queen honey bees:

- although *Varroa* has a role in spreading the virus, APV has been found in honey bee samples from apiaries where no APV-positive *Varroa* were detected, suggesting that APV can be transmitted by contact between individual honey bees (Tentcheva 2004)
- the virus may be spread via salivary gland secretions of adult honey bees and the food to which these secretions are added (Bailey 1976)
- APV has been detected in pollen loads of foraging honey bees but not in the pollen of plants, suggesting foodborne transmission may occur (Chen 2006)
- APV has also been detected in drone semen, implicating mating as a possible route of horizontal and vertical virus transmission (Yue 2006).

Conclusion: based on this information, the likelihood of susceptible honey bees being exposed to APV via infected imported queen honey bees was estimated to be **moderate**.

Estimation of the likelihood of release and exposure

The likelihood of release and exposure is estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (Table 2). With the likelihood of release estimated to be **moderate** combined with the likelihood of exposure estimated to be **moderate**, the likelihood of release and exposure was estimated to be **low**.

Consequence assessment

The consequence assessment describes the potential consequences associated with hazard entry and exposure, and estimates the likelihood of them occurring. This involves estimating the likelihood of establishment and/or spread of the hazard for the most likely outbreak scenario, and determining the direct or indirect effects should this outbreak scenario occur. Combining the likelihood of establishment and/or spread for this outbreak scenario with the corresponding overall effect gives an estimation of likely consequences.

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred, a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario was determined by describing the likely extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to APV is considered to be establishment and/or spread through direct contact to local populations of susceptible honey bees.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of susceptible honey bees to APV:

- APV can, and normally does, produce inapparent infection (Bailey 1963)
- *Varroa* appears to act as a mechanical vector of APV (Wiegers 1988). In addition to acting as a viral vector, *Varroa* is also believed to be an activator of APV in infected honey bees (Chen 2007)
- *Varroa* is not present in Australia
- although *Varroa* has a role in spreading the virus, APV has been found in honey bee samples from apiaries where no APV-positive *Varroa* were detected, suggesting that APV can be transmitted by contact between individual honey bees (Tentcheva 2004)
- APV may be spread via salivary gland secretions of adult honey bees and the food to which these secretions are added (Bailey 1976)
- APV has been detected in pollen loads of foraging honey bees but not in the pollen of plants, suggesting foodborne transmission may occur (Chen 2006)
- APV has also been detected in drone semen, implicating mating as a possible route of horizontal and vertical virus transmission (Yue 2006).

Conclusion: based on these considerations, it was determined that the likelihood of establishment and/or spread of APV for the exposure group was **moderate**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect impacts of APV were estimated at the national, state or territory, district/region and local levels. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria.

The following factors were considered relevant to a conclusion on the effects of the establishment and/or spread of APV.

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- APV can and normally does produce inapparent infection. Clinical signs in artificially infected honey bees are more acute than those of natural disease (Bailey 1963). Honey bees injected with APV usually first show signs of paralysis (trembling) in 2–4 days and then died within a day (Bailey 1963)
- although mostly symptomless in individual adult honey bees, pupae and larvae, APV can be lethal at the colony level in association with *Varroa* (de Miranda 2008). This

can result in the appearance of diseased larvae and pupae due to the lack of adults tending to the brood (de Miranda 2010).

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- the presence of APV in managed and feral honey bee colonies is not considered to negatively impact on native plant species as pollination of native flora is not dependent on *A. mellifera*
- APV has not been detected in Australian native bee species (Anderson 1982)
- the susceptibility of Australian native bees to APV is unknown.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- APV is not notifiable in Australia (DAFF 2011), it is not included in the AUSVETPLAN *Disease Strategy: Bee Pests and Diseases*
- if APV was detected in Australia it is unlikely that eradication and control would be implemented.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- if APV was detected in Australia, it is unlikely that movement restrictions would be imposed
- if APV was detected in Australia, it is unlikely that eradication would be attempted.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- given its worldwide distribution, if APV was to become established, renegotiation of trade conditions with trading partners would probably not be necessary.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- if APV was detected in Australia, it is unlikely to lead to any indirect effects on the environment.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- if APV was detected in Australia, it is unlikely to lead to any effects on communities.

Conclusion for overall direct and indirect effects: based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread for the outbreak scenario was estimated to be **very low** from Table 3. The effect is likely to be **minor** to directly affected parties. The effect is unlikely to be discernable at any other level.

Derivation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario using Table 4 to obtain an estimation of likely consequences.

The likelihood of establishment and/or spread ('moderate') is combined with the estimate of the overall effect of establishment and/or spread ('very low') which results in **very low** likely consequences.

Unrestricted risk estimation

Risk estimation is the integration of likelihood of release and exposure, and likely consequences of establishment and/or spread to derive the risk associated with release, exposure, establishment and/or spread of APV introduced by imported queen honey bees into Australia.

Using Table 5 the likelihood of release and exposure ('low') is combined with the likely consequences of establishment and/or spread ('very low'), resulting in a risk estimation of **negligible**.

Therefore, as the unrestricted risk achieves Australia's ALOP of 'very low', no specific risk management was considered necessary.

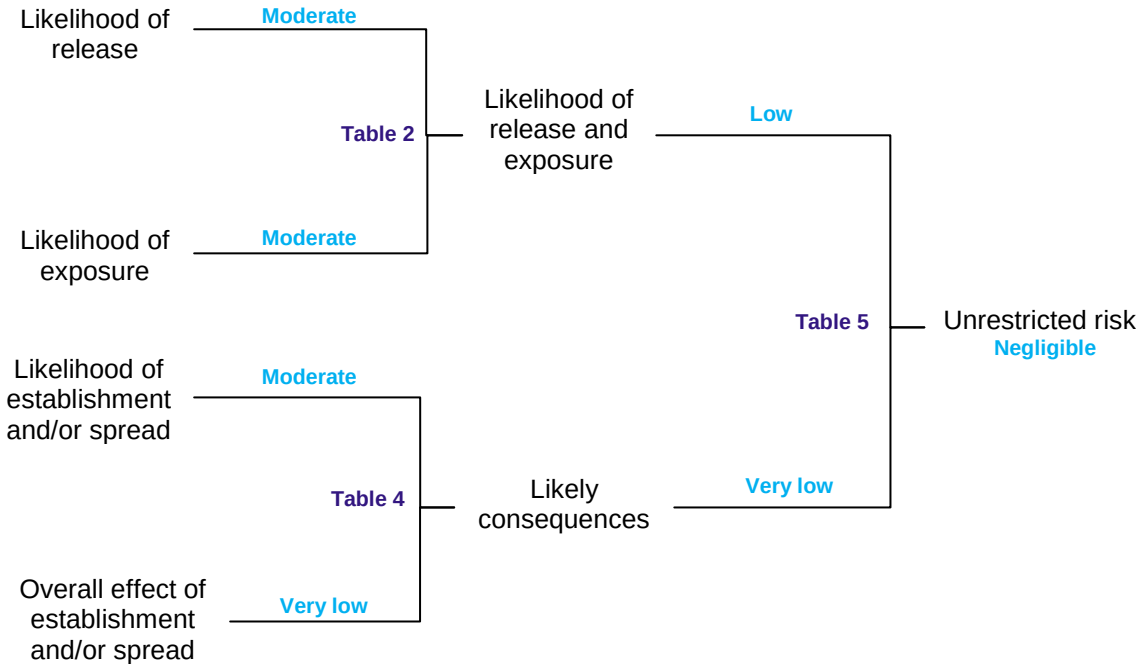

Figure 11. Summary of the risk assessment pathways for acute paralysis virus

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4.10 Deformed wing virus

Technical information

Background

Deformed wing virus (DWV) was initially isolated from adult honey bees (*Apis mellifera*) from Japan in 1982 (de Miranda and Genersch 2010) and subsequently identified as a cause of brood and adult honey bee mortality in colonies infested with *Varroa destructor* in many countries (Allen and Ball 1996; Ribière et al. 2008). It is possible that the virus was present in some countries but was not detected, due to the unstable nature of purified DWV extracts obtained from honey bees (Bailey 1994).

DWV has been detected in *A*. *mellifera* in Africa, North, Central and South America, Asia, Europe, the Middle East and New Zealand, and in *A*. *cerana* in China (Allen and Ball 1996; Antúnez 2005; Ellis and Munn 2005). DWV has also been detected in bumble bees (*Bombus terrestris* and *B. pascuorum*) exhibiting wing deformities (Genersch et al. 2006).

DWV is not a notifiable disease in Australia (DAFF 2011).

OIE requirements

DWV is not an OIE listed disease (OIE 2011). There are no OIE recommendations for DWV.

Epidemiology and pathogenesis

DWV is a single-stranded RNA virus in Group D of the floating genus Iflavirus (Carter 2008), in the picorna-like family Iflaviridae (de Miranda and Genersch 2010). DWV shows a weak reaction with sera raised against Egypt bee virus (Bailey 1979; Mayo et al. 2005).

DWV appears to be the most prevalent viral infection in *A*. *mellifera* in recent years, with surveys finding DWV infection in up to 100 per cent of apiaries (Chen and Siede 2007). There is some seasonal variation in DWV incidence, increasing from spring to autumn (Tentcheva et al. 2004)—this seasonal distribution closely follows that of *Varroa* (de Miranda and Genersch 2010).

Varroa has been shown to be able to transmit DWV from severely infected to healthy brood during feeding activities (Bowen-Walker et al. 1999; Nordström 2003; Shen et al. 2005). DWV has been detected in individual female *Varroa*.

In colonies without *Varroa*, DWV infection generally does not result in visible clinical signs of disease or any apparent negative impact on host fitness (de Miranda and Genersch 2010). DWV infections with clear clinical signs of disease are associated with transmission of DWV by *V*. *destructor* (de Miranda and Genersch 2010). Although the exact mechanism is unclear, the consensus is that transmission of DWV to pupae through parasitising *Varroa* is the prerequisite for the development of deformed wings (de Miranda and Genersch 2010). However, even in *Varroa* -infested colonies, most DWV-infected honey bees do not show any visible signs of infection, indicating that DWV causes asymptomatic infections (de Miranda and Genersch 2010).

DWV has been associated with honey bee colony collapse induced by *Varroa* and loss of infested colonies (Carreck et al. 2010; de Miranda and Genersch 2010). The contribution of DWV infection in association with *Varroa* to colony decline is not fully understood (Ribière et al. 2008). DWV is more prevalent in colonies infested with *Varroa* compared to previous long-term observations of uninfested colonies in Britain before arrival of *Varroa* (Carreck et al. 2010).

The majority of DWV-positive *V. destructor* only passively acquire and mechanically transmit the virus (de Miranda and Genersch 2010). However, DWV replication occurs in some *V. destructor*, indicating active infection; virus replication in *Varroa* has been correlated with morphologically deformed honey bees (Yue and Genersch 2005; Gisder 2009). It has been suggested that infecting *Varroa* require a threshold DWV titre (achieved through viral replication in *Varroa*) for infected honey bees to emerge with deformed wings (Gisder 2009).

DWV has also been detected in *Tropilaelaps mercedesae* (Dainat 2009; Forsgren et al. 2009) and in the small hive beetle (*Aethina tuminda*) (Eyer 2009).

Queen honey bee and colony mortality, attributed to DWV infections, were reported in Britain and South Africa before *Varroa* became established in these regions, although none of the characteristic pathology associated with DWV was observed (Ball, as cited in de Miranda 2010).

DWV has been detected in honey and pollen loads collected by honey bees (Chen et al. 2006) and in honey bee larval food (Yue and Genersch 2005), suggesting that foodborne transmission of the virus may occur. DWV has also been found in honey bee faeces (Chen 2006), suggesting faecal-oral transmission.

Vertical transmission of DWV may occur. DWV has been detected in the ovaries of queen honey bees (Chen 2006) and in all honey bee developmental stages, including eggs, pupae and larvae (Chen 2005). DWV has also been detected in drone semen, implicating mating as a possible route of horizontal and vertical virus transmission (Yue et al. 2006). De Miranda and Fries (2008) demonstrated venereal transmission from DWV-infected semen to the ovaries of DWV-negative and subsequent vertical transmission of virus from queen honey bees to progeny. Yue et al. (2007) demonstrated virus transmission to eggs from DWV-negative queen honey bees impregnated with DWV-positive semen. None of the DWV-positive drones or workers produced in the study showed any visible clinical signs of disease, suggesting that in the absence of *Varroa*, even the combination of horizontal and vertical transmission routes within a colony does not have a high negative impact on the fitness and fecundity of infected honey bees (Yue et al. 2007).

Clinical signs

When present, the typical clinical signs of DWV are shrunken, crumpled wings, decreased body size and discolouration of adult honey bees (Chen and Siede 2007). However, adult honey bees with high titres of the virus may not show clinical signs (Ribière et al. 2008). DWV infection is detected in adult honey bees and in all stages of development (Chen and Siede 2007). A small proportion of pupae infected with DWV may die; some will develop into adult honey bees with a shortened lifespan and the characteristic morphological deformities although most emerge as apparently normal, but covertly infected, individuals (Ribière et al. 2008). When honey bees are infected post-emergence, no physical signs of infection are

apparent (Ribière et al. 2008). Asymptomatic infections can occur (de Miranda and Genersch 2010).

Diagnosis

Historically, studies on the incidence and prevalence of DWV were hampered because traditional diagnostic techniques were low in sensitivity and specificity (Yue and Genersch 2005). Development of molecular technologies has provided a powerful method for specific, sensitive and rapid identification of honey bee viruses (Chen 2006).

Publication of the complete nucleotide sequences of the DWV genome led to the development of several RT-PCR protocols for detection of DWV (for example, Chen 2005; Tentcheva 2006).

RT-PCR protocols published for the detection of DWV are summarised by de Miranda (2008). Guidelines for designing RNA-virus diagnostic primers and avoiding misdiagnosis are published in de Miranda (2008).

Based on a quantitative description of DWV infection in honey bee colonies in the USA, real-time quantitative RT-PCR was described as a specific, sensitive, robust and reproducible assay with practical applications in the diagnosis of honey bee viral diseases (Chen 2005) and has become an important tool for investigation of pathogenesis of viral infections in honey bees (Dainat et al. 2011).

Control and management

Viral infections of honey bees can have serious impacts on the profitability of the beekeeping industry. However, to date, limited work has been undertaken on suitable treatments and control measures (Genersch and Aubert 2010).

An integrated pest management program for management of viruses should include accurate diagnosis of diseases to allow development and implementation of control strategies, good beekeeping management practices (such as controlling *Varroa* populations), enhancing natural immunity to infection and selecting and breeding disease-resistant strains of honey bees (Aubert 2008; de Miranda 2008).

Conclusion

DWV has not been confirmed to be present in Australia and is likely to be present in exporting countries. There are no recommendations in the OIE Code. Therefore, DAFF concluded that further risk assessment was required.

Risk assessment

For details of the method used in this risk assessment see Chapter 2. A summary of the risk assessment is shown in Figure 12.

Release assessment

The following factors were considered relevant to the estimate of the likelihood of DWV being present in imported queen honey bees.

- DWV is widespread and has been detected in *A*. *mellifera* in Africa, North, Central and South America, Asia, Europe, the Middle East and New Zealand, and in *A*. *cerana* in China (Allen and Ball 1996; Antúnez 2005; Ellis and Munn 2005).
- DWV appears to be the most prevalent viral infection in *A*. *mellifera* in recent years, with surveys finding DWV infection in up to 100 per cent of apiaries (Chen and Siede 2007).

Conclusion: based on this information, the likelihood of release of DWV associated with the importation of queen honey bees was estimated to be **high**.

Exposure assessment

The most likely exposure group considered was managed and feral honey bee colonies and the most likely pathway is the introduction of imported honey bees into domestic colonies.

The following factors were considered relevant to the estimate of the likelihood of susceptible honey bees being exposed to DWV via imported queen honey bees:

- *Varroa* have been shown to be able to transmit the virus from severely infected to healthy brood during feeding activities (Bowen-Walker et al. 1999; Nordström 2003; Shen et al. 2005)
- *Varroa* is not present in Australia
- DWV has been detected in honey and pollen loads (Chen et al. 2006), and in honey bee larval food (Yue and Genersch 2005), suggesting foodborne transmission of the virus may occur. DWV has also been found in honey bee faeces (Chen 2006), suggesting faecal-oral transmission
- vertical transmission of DWV may occur. DWV has been detected in the ovaries of queen honey bees (Chen 2006) and in all honey bee developmental stages, including eggs, pupae and larvae (Chen 2005). DWV has also been detected in drone semen, implicating mating as a possible route of horizontal and vertical virus transmission (Yue et al. 2006).

Conclusion: based on this information, the likelihood of susceptible honey bees being exposed to DWV via infested imported queen honey bees was estimated to be **moderate**.

Estimation of the likelihood of release and exposure

The likelihood of release and exposure is estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (Table 2). With the likelihood of release estimated to be ‘high’ combined with the likelihood of exposure estimated to be ‘moderate’, the likelihood of release and exposure was estimated to be **moderate**.

Consequence assessment

The consequence assessment describes the potential consequences associated with hazard entry and exposure, and estimates the likelihood of them occurring. This involves estimating the likelihood of establishment and/or spread of the hazard for the most likely outbreak scenario, and determining the direct or indirect effects should this outbreak scenario occur. Combining the likelihood of establishment and/or spread for this outbreak scenario with the corresponding overall effect gives an estimation of likely consequences.

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred, a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario was determined by describing the likely extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to DWV is considered to be establishment and/or spread to populations of susceptible honey bees locally (restricted to a single locality or town), through direct contact.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of susceptible honey bees to DWV.

- *Varroa* has been shown to be able to transmit the virus from severely infected to healthy brood during feeding activities (Bowen-Walker et al. 1999; Nordström 2003; Shen et al. 2005)
- *Varroa* is not present in Australia
- DWV has been detected in honey and pollen loads (Chen et al. 2006), and in larval food (Yue and Genersch 2005), suggesting foodborne transmission of virus may occur. DWV has also been found in honey bee faeces (Chen 2006), suggesting faecal-oral transmission
- vertical transmission of DWV may occur. DWV has been detected in the ovaries of queen honey bees (Chen 2005) and in all honey bee developmental stages, including eggs, pupae and larvae (Chen 2005). DWV has also been detected in drone semen, implicating mating as a possible route of horizontal and vertical virus transmission (Yue et al. 2006)
- even in *Varroa*-infested colonies, most DWV-infected honey bees do not show any visible signs of infection (de Miranda and Genersch 2010).

Conclusion: based on these consequences, it was determined that the likelihood of establishment and/or spread of DWV for the exposure group was **moderate**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect impacts of DWV were estimated at the national, state or territory, district/region and local levels. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria.

The following factors were considered relevant to a conclusion on the effects of the establishment and/or spread of DWV.

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- in colonies without *Varroa*, DWV infection generally does not result in visible clinical signs of disease or any apparent negative impact on host fitness (de Miranda and Genersch 2010)
- DWV infections with clear clinical signs of disease are associated with transmission of DWV by *Varroa* (de Miranda and Genersch 2010)

- transmission of DWV to pupae through *Varroa* is believed to be the prerequisite for the development of deformed wings (de Miranda and Genersch 2010). However, even in *Varroa* -infested colonies, most DWV-infected honey bees do not show any visible signs of infection (de Miranda and Genersch 2010)
- a small proportion of pupae infected with DWV may die; some will develop into adult honey bees with a shortened lifespan and the characteristic morphological deformities although most emerge as apparently normal but covertly infected individuals (Ribière et al. 2008)
- DWV has been associated with honey bee colony collapse induced by *V. destructor* and loss of infested colonies (Carreck et al. 2010; de Miranda and Genersch 2010)
- *Varroa* species are not present in Australia.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- the presence of DWV in managed and feral honey bee colonies is not considered to negatively impact on native plant species as pollination of native flora is not dependent on *A. mellifera*
- the susceptibility of Australian native bees to DWV is unknown.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- DWV is not notifiable in Australia (DAFF 2011), it is not included in the AUSVETPLAN *Disease Strategy: Bee Pests and Diseases* response policy
- if DWV was detected in Australia it is unlikely that eradication and control would be implemented.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- if DWV was detected in Australia, it is unlikely that movement restrictions would be imposed
- if DWV was detected in Australia, it is unlikely that eradication would be attempted.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- if DWV was to become established, renegotiation of trade conditions with trading partners would probably not be necessary.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- if DWV was detected in Australia, it is unlikely to lead to any indirect effects on the environment.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- if DWV was detected in Australia, it is unlikely to lead to any effects on communities.

Conclusion for overall direct and indirect effects: based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread for the outbreak scenario was estimated to be **very low** from Table 3. The effect is likely to be **minor** to directly affected parties. The effect is unlikely to be discernable at any other level.

Derivation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario using Table 4 to obtain an estimation of likely consequences.

The likelihood of establishment and/or spread ('moderate') is combined with the estimate of the overall effect of establishment and/or spread ('very low') which results in **very low** likely consequences.

Unrestricted risk estimation

Risk estimation is the integration of likelihood of release and exposure, and likely consequences of establishment and/or spread to derive the risk associated with release, exposure, establishment and/or spread of DWV introduced by imported queen honey bees into Australia.

Using Table 5, the likelihood of release and exposure ('moderate') is combined with the likely consequences of establishment and/or spread ('very low'), resulting in a risk estimation of **very low**.

Therefore, as the unrestricted risk achieves Australia's ALOP of 'very low', no specific risk management was considered necessary.

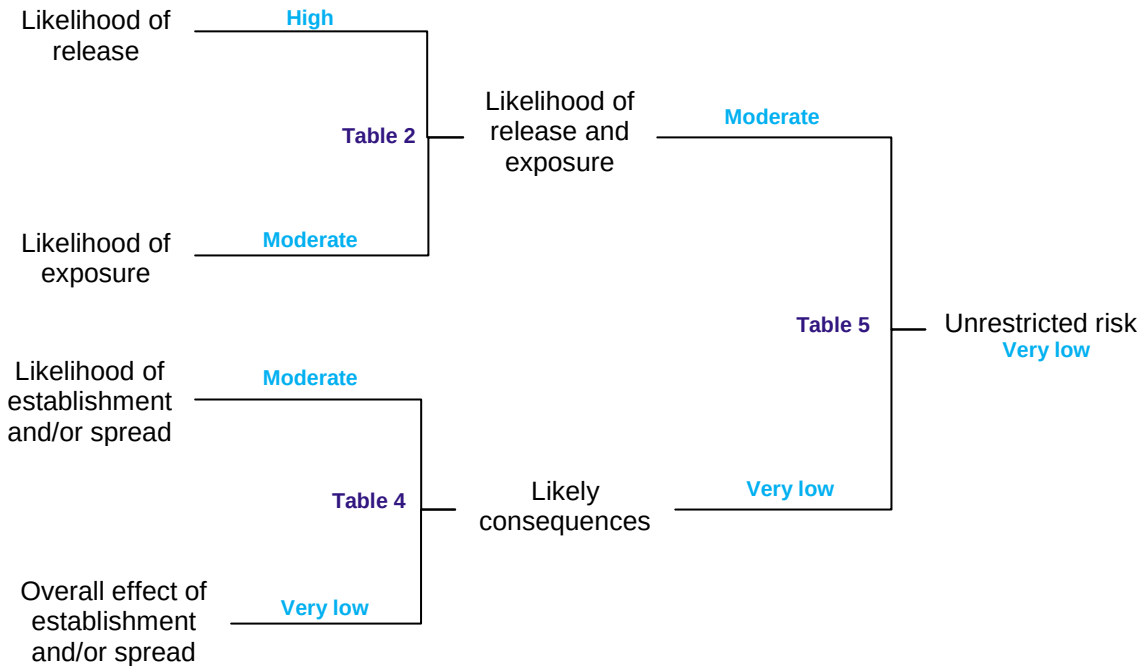

Figure 12. Summary of the risk assessment pathways for deformed wing virus

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4.11 Slow paralysis virus

Technical information

Background

Slow paralysis virus (SPV) is a rare virus of honey bees (*Apis mellifera*) and has only recently been characterised (de Miranda et al. 2010). It is an RNA virus and it has not yet been assigned to a genus or family (Mayo et al. 2005).

SPV was originally detected during field surveys for bee virus X (Bailey and Woods 1974) and it has been associated with colony collapse disorder (Martin et al. 1998).

SPV has been reported in Fiji, Western Samoa, Switzerland and the United Kingdom (Bailey 1981; Anderson 1990; Allen and Ball 1996; de Miranda et al. 2010). Its status in Australia is uncertain. Although a review by Hornitzky (1985) reported SPV as one of nine viruses detected in Australia since 1968, none of the literature cited in this review actually reports the finding of SPV. In a later paper, Hornitzky (1987) describes a study examining specimens of bees and brood of primarily eastern Australian origin; SPV was not detected despite SPV antiserum being included in the survey.

SPV is not a notifiable disease in Australia (DAFF 2011).

OIE requirements

SPV is not an OIE listed disease (OIE 2011) and there are no OIE recommendations.

Epidemiology and pathogenesis

SPV seems to persist in honey bees primarily as an inapparent infection (Ribière et al. 2008). It has not been found in species other than *Apis*.

The virus can be transmitted to adult honey bees by *Varroa destructor* (Santillán-Galicia et al. 2010). Experimentally, *Varroa* has an important role in virus transmission within adult honey bee populations while bee-to-bee transmission plays only a minor part (Santillán-Galicia et al. 2010). Transmission of SPV is thought to occur during the feeding activities of *Varroa* (Santillán-Galicia et al. 2010).

SPV has been associated with the loss of infested colonies and *Varroa* is probably critical for SPV-induced colony mortality (Carreck et al. 2010; de Miranda et al. 2010; Santillán-Galicia et al. 2010).

SPV has been shown to kill pupae in approximately 5 days and adults in 10–12 days, which may affect the chances of SPV being transmitted and surviving in the honey bee population (Bailey 1981; Santillán-Galicia et al. 2010).

Studies in the United Kingdom have shown that SPV first appeared in colonies late in the season, when the largest *Varroa* populations occurred and when multiple *Varroa* infestations of brood cells most frequently occurred. This was often followed by colony death. When SPV infection declined, colonies could recover and survive over winter (Carreck 2005).

SPV has a low prevalence in Europe. The natural prevalence of SPV in 847 colonies in 162 apiaries across 5 European countries was found to be less that 2 per cent. Positive samples were found only in England and Switzerland, in colonies with variable degrees of *Varroa* infestation (de Miranda et al. 2010). SPV is more prevalent in colonies infested with *Varroa*, compared to previous long-term observations of non-infested colonies in Britain before the arrival of *Varroa* (Carreck et al. 2010; de Miranda et al. 2010).

Clinical signs

An experimental study showed that adult honey bees died approximately 12 days after being injected with SPV preparations, and typically showed signs of paralysis of the two front pairs of legs for a day or two before death (Bailey and Woods 1974).

While SPV can be detected in larvae and pupae, they show no clinical signs of disease (de Miranda 2008).

Diagnosis

Development of molecular technologies has provided a powerful method for specific, sensitive and rapid identification of bee viruses (Chen et al. 2005). The complete genome sequence of SPV has been determined (de Miranda et al. 2010).

Several RT-PCR assays have been developed for SPV detection and quantification (de Miranda 2008; de Miranda et al. 2010) and it has become an important tool for investigation of the pathogenesis of viral infections in honey bees (Dainat et al. 2011).

Control and management

Viral infections of honey bees can have serious impacts on the profitability of the beekeeping industry. However, possible treatments against viral infections in honey bees have never been seriously considered (Genersch and Aubert 2010).

An integrated pest management program for management of viruses should include accurate diagnosis of diseases to allow development and implementation of control strategies, good beekeeping management practices (such as controlling *Varroa* populations), enhancing natural immunity to infection and selecting and breeding disease-resistant strains of honey bees (Aubert 2008; de Miranda 2008).

Conclusion

SPV has not been confirmed to be present in Australia and is likely to be present in some exporting countries. There are no recommendations in the OIE Code. Therefore, DAFF concluded that further risk assessment was required.

Risk assessment

For details of the method used in this risk assessment see Chapter 2. A summary of the risk assessment is shown in Figure 13.

Release assessment

The following factors were considered relevant to the estimate of the likelihood of SPV being present in imported queen honey bees.

- SPV has been reported in Fiji, Western Samoa, Switzerland and the United Kingdom (Bailey 1981; Anderson 1990; Allen and Ball 1996; de Miranda et al. 2010)
- SPV has a low prevalence in Europe. The natural prevalence of SPV across five European countries was found to be less than two per cent (de Miranda et al. 2010).

Conclusion: based on this information, the likelihood of release of SPV associated with the importation of queen honey bees was estimated to be **very low**.

Exposure assessment

The exposure group considered was managed and feral honey bee colonies and the most likely exposure pathway is the introduction of imported honey bees into domestic colonies.

The following factors were considered relevant to the estimate of the likelihood of susceptible honey bees being exposed to SPV via imported queen honey bees:

- SPV can be transmitted to adult honey bees by *Varroa* (Santillán-Galicia et al. 2010). Experimentally, *Varroa* has an important role in virus transmission within adult honey bee populations while bee-to-bee transmission plays only a minor part (Santillán-Galicia et al. 2010)
- *Varroa* is not present in Australia .

Conclusion: based on this information, the likelihood of susceptible honey bees being exposed to SPV via infested imported queen honey bees was estimated to be **very low**.

Estimation of the likelihood of release and exposure

The likelihood of release and exposure is estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (Table 2). With the likelihood of release estimated to be ‘very low’ combined with the likelihood of exposure estimated to be ‘very low’, the likelihood of release and exposure was estimated to be **extremely low**.

Consequence assessment

The consequence assessment describes the potential consequences associated with hazard entry and exposure, and estimates the likelihood of them occurring. This involves estimating the likelihood of establishment and/or spread of the hazard for the most likely outbreak scenario, and determining the direct or indirect effects should this outbreak scenario occur. Combining the likelihood of establishment and/or spread for this outbreak scenario with the corresponding overall effect gives an estimation of likely consequences.

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred, a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario was determined by describing the likely extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to SPV is considered to be establishment and/or spread to populations of susceptible honey bees locally (restricted to a single locality or town), through direct contact.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of susceptible honey bees to SPV:

- SPV mainly persists in honey bees as an inapparent infection (Ribière et al. 2008)
- SPV can be transmitted to adult honey bees by *Varroa* (Santillán-Galicia et al. 2010). Experimentally, *Varroa* has an important role in virus transmission within adult honey bee populations while bee-to-bee transmission plays only a minor part (Santillán-Galicia et al. 2010)
- *Varroa* is not present in Australia .

Conclusion: based on these considerations for the identified outbreak scenario, the likelihood of establishment and/or spread of SPV for the exposure group was **moderate**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect impacts of SPV were estimated at the national, state or territory, district/region and local levels. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria.

The following factors were considered relevant to a conclusion on the effects of the establishment and/or spread of SPV:

Direct effects

The effect on the life or health (including production effects) of susceptible animals

- SPV seems to persist in honey bees primarily as an inapparent infection (Ribière et al. 2008)
- SPV has been associated with the loss of infested colonies and *Varroa* is probably critical for SPV-induced colony mortality (Carreck et al. 2010; de Miranda et al. 2010; Santillán-Galicia et al. 2010)
- when injected with preparations of SPV, adult honey bees died after approximately 12 days, typically showing paralysis of the two front pairs of legs for a day or two before death (Bailey and Woods 1974)
- *Varroa* is not present in Australia .

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- the presence of SPV in managed and feral honey bee colonies is not considered to negatively impact on native plant species as pollination of native flora is not dependent on *A. mellifera*
- the susceptibility of Australian native bees to SPV is unknown.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- SPV is not notifiable in Australia (DAFF 2011), it is not included in the AUSVETPLAN *Disease Strategy: Bee Pests and Diseases*
- if SPV was detected in Australia it is unlikely that eradication and control would be implemented.

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- if SPV was detected in Australia, it is unlikely that movement restrictions would be imposed
- if SPV was detected in Australia, it is unlikely that eradication would be attempted.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- if SPV was to become established, renegotiation of trade conditions with some trading partners may be necessary.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- if SPV was detected in Australia, it is unlikely to lead to any indirect effects on the environment.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- if SPV was detected in Australia, it is unlikely to lead to any effects on communities.

Conclusion for overall direct and indirect effects: based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread for the outbreak scenario was estimated to be **very low** from Table 3. The effect is likely to be **minor** to directly affected parties. The effect is unlikely to be discernable at any other level.

Derivation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario using Table 4 to obtain an estimation of likely consequences.

The likelihood of establishment and/or spread ('moderate') is combined with the estimate of the overall effect of establishment and/or spread ('very low') which results in **very low** likely consequences.

Unrestricted risk estimation

Risk estimation is the integration of likelihood of release and exposure, and likely consequences of establishment and/or spread to derive the risk associated with release, exposure, establishment and/or spread of SPV introduced by imported queen honey bees into Australia.

Using Table 5, the likelihood of release and exposure ('extremely low') is combined with the likely consequences of establishment and/or spread ('very low'), resulting in a risk estimation of **negligible.**

Therefore, as the unrestricted risk associated with SPV meets Australia's ALOP of 'very low', no specific risk management was considered necessary.

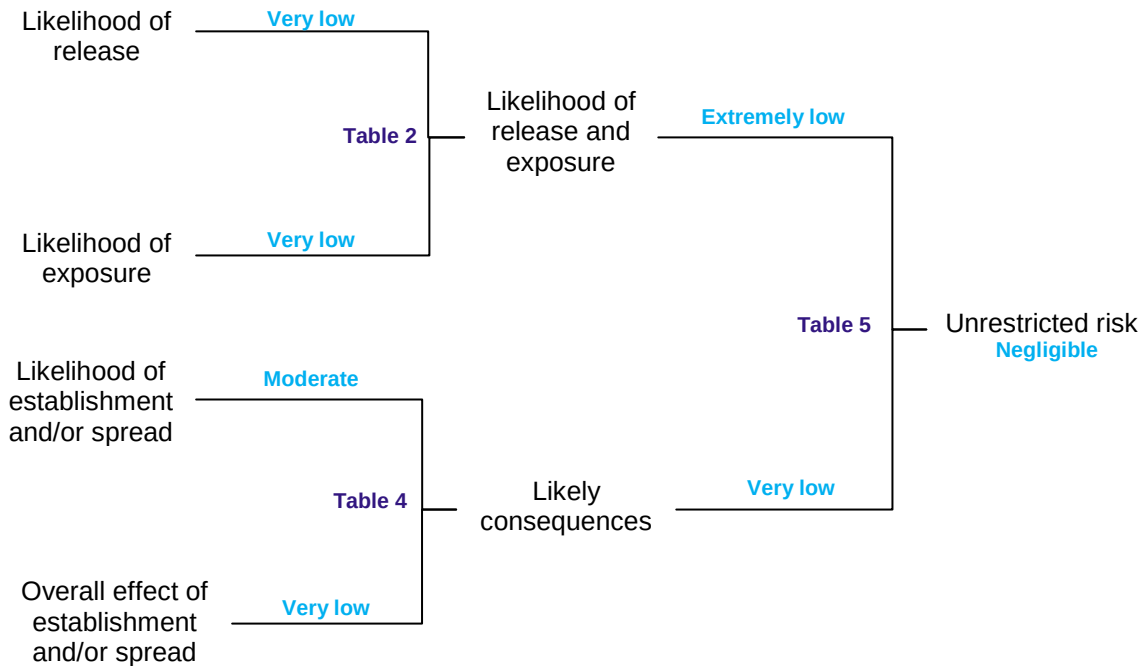

Figure 13. Summary of the risk assessment pathways for slow paralysis virus

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4.12 Africanised honey bee (*Apis mellifera scutellata* and its hybrids)

Technical information

Background

The natural range of *Apis mellifera scutellata* (hereafter called 'Scutellata') is the savannah region of sub-Saharan Africa. Scutellata is characterised by behavioural traits that are adaptations to life in the savannah: high reproductive rates and extreme defensiveness to counter frequent predation, and regular migration to exploit highly ephemeral nectar sources (Rinderer 1988).

From 1954–56 there were various imports of Scutellata queen honey bees into Brazil. The goal of these importations was to develop a strain of honey bee that was more suitable for tropical beekeeping than the existing M and C lineage honey bees that were available in Brazil at the time (Schneider 2004). Descendents of these imports formed the basis of a massive feral population of A-lineage Scutellata honey bees that has replaced honey bees of European lineage (hereafter 'European') throughout the neo-tropics and the southern USA (Winston 1992; Clarke 2002; Pinto 2005; Schneider 2004; Whitfield 2006).

From the initial importation, Scutellata expanded its range by 200–500 km per year (Taylor 1977; Winston 1992). By 1986 it had reached Mexico and it first invaded the USA in 1992 (Guzmán-Novoa 1994). This is one of the most successful biological invasions of all time (Winston 1992).

Scutellata is now present throughout sub-Saharan Africa and Central America. In South America, Scutellata extends as far south as 34º of latitude south, where it forms a stable hybrid zone with honey bees of the C and M lineages (Sheppard 1991). In the USA, Scutellata has been identified in the states of Alabama, Arizona, Arkansas, California, Florida, Georgia, Louisiana, Nevada, New Mexico, New York, Oklahoma, South Carolina, Texas, Utah and Virginia (Louisiana Department of Agriculture and Forestry 2009; Georgia Department of Agriculture 2010; Utah Department of Agriculture and Food 2010; Cooperative Agricultural Pest Survey 2011).

Scutellata was expected to increase its range into states to the north, especially along the eastern seaboard of the USA (Taylor 1977; Winston 1992). However as time goes on this range expansion seems less likely.

'Africanised bees' are notifiable as a disease in NSW, SA, Tasmania, Victoria and WA.

OIE requirements

Scutellata is not an OIE listed disease (OIE 2011) and there are no OIE recommendations.

Epidemiology and pathogenesis

Scutellata spread and replace European honey bees primarily by producing larger numbers of reproductive swarms and drones. Otis (1991) observed that a Scutellata colony can produce 6–12 reproductive colonies per year and, via the offspring of the offspring colonies,

up to 60 descendents in a year. There are also other more subtle processes that contribute to ‘Africanisation’. First, Scutellata drones enter European colonies, and by their presence, suppress drone rearing by the host colony (Rinderer 1985). This process increases the proportion of Scutellata drones present in the environment and thus the likelihood that queen honey bees will mate with Scutellata drones rather than European drones. Second, migrating Scutellata swarms often enter European colonies.

By processes not understood, the Scutellata queen honey bee replaces the European queen honey bee, converting the previously European colony into a Scutellata one (Vergara 1989; Danka 1992). Finally, because Scutellata queen honey bees have a shorter development time than European queen honey bees, a queen honey bee that is mated to both Scutellata and European drones is more likely to produce offspring queen honey bees that are of Scutellata paternity. This is because the first offspring queen honey bee to hatch seeks out and kills her sisters in their pupal queen cells. Thus the first queen honey bee to hatch is most likely to have Scutellata paternity and the most likely to inherit the colony (Schneider 2003). Scutellata is resistant to some of the major pests of European honey bees, particularly SHB and *Varroa*.

Scutellata’s adaptations to the African savannah make it undesirable for modern beekeeping as practised in Australia as:

- Scutellata colonies have high absconding rates—forming a migratory swarm that seeks better environmental conditions when the colony faces starvation
- Scutellata will continue to breed even when there is a lack of food available in the field, utilising resources for reproduction rather than honey storage
- Scutellata colonies are difficult to work with, requiring full honey bee suits and heavy gloves at all times. When beekeepers work in Scutellata apiaries the colonies become extremely defensive and invariably attack any mammal or bird within a few hundred metres
- Scutellata apiaries are recommended to be situated a minimum of 200–500 metres away from places occupied by humans, livestock or companion animals (Caron 2001)
- If introduced into Australia they would be likely to greatly increase the size of the feral population. An increased density of feral honey bees would likely have effects on those native fauna that compete with feral honey bees for nest sites
- They pose a significant risk to the public via incidental stinging attacks. The attacks involve hundreds of stinging honey bees, and death by stinging or the consequences of panic is possible. In Mexico between 1988 and 1993 about 15 per cent of such attacks were fatal and 190 deaths from Scutellata attacks were recorded (Guzmán-Novoa 1994). Nonetheless, the individual stings are no more venomous than honey bees of other subspecies (Schumacher 1995). Both private and public landowners are likely to become more reticent to allow beekeeping activities on their properties because of the possibility of stinging incidents, stock losses, and fear of litigation. This would have a significant negative impact on beekeepers and landowners who hire honey bees for pollination
- Scutellata are unsuitable for paid pollination services. It is dangerous to have large numbers of ‘killer bees’ in orchards where untrained and unprotected people are likely to come in contact with the colonies. However Ratnieks and Visscher (Ratnieks

1996) report that in Mexico regular requeening of colonies with European queen honey bees and changes in management practices has allowed managed pollination to be maintained.

Clinical signs

Extreme defensive behaviour and stinging incidents are often the first indication of the presence of Scutellata colonies. Assessments of defensive behaviour, the amount and pattern of brood (more with Africanised colonies) and the difficulty in locating the queen honey bee have been used in the field as indications of the presence of Scutellata (Spivak 1991).

Diagnosis

Identification

Scutellata are small yellow bees and are not sufficiently different from honey bees of other subspecies to be reliably distinguishable by eye. Large colonies are extremely aggressive but this too is not a reliable diagnosis—so are some European colonies. Small colonies of Scutellata are often not aggressive and this can lead to misdiagnosis.

Testing

Comb measurement

If comb has been constructed naturally (i.e. has not been built with the aid of foundation comb), a rough diagnosis can be obtained by measuring the width of 10 consecutive worker cells. For this procedure, each cell is defined as having one cell wall. The outside of the wall of the first cell is included in the measure. The last cell is measured up to the edge of the eleventh wall. Only fully formed worker cells in the central portion of a comb should be measured (Rinderer 1986). Scutellata cells average 4.8–4.9 cm (range 4.5–5.0 cm) whereas European cells average 5.2–5.3 cm (range 5.0–5.5 cm) (Rinderer 1982). Colonies producing worker comb having an average of three measurements of 4.9 cm or below are almost certain to be Africanised. Colonies producing worker comb having an average of three measurements of 5.2 cm or above are almost certain to be European. Colonies with intermediate values should be considered unidentifiable by this procedure, but suspected to be Africanised (Rinderer 1986).

Morphometrics

Morphometrics, the study of size and shape of organisms, can provide a diagnosis of Africanisation (Daly 1978). It can detect hybrids as well as purebred individuals but the sensitivity and accuracy of the method declines as the level of Africanisation decreases (Guzmán-Novoa 1994).

This is a laboratory procedure involving precise measurement of forewing length using a dissecting microscope fitted with a calibrated ocular micrometer. Ten forewing lengths of adult worker honey bees are measured as shown in Figure 14 and the average calculated. The probability that the sample is Scutellata and the probability that the sample is European can then be estimated (Rinderer 1986).

The average forewing length of Scutellata honey bees is 8.87 mm, and the average forewing length of European honey bees is 9.20 mm (Rinderer 1986).

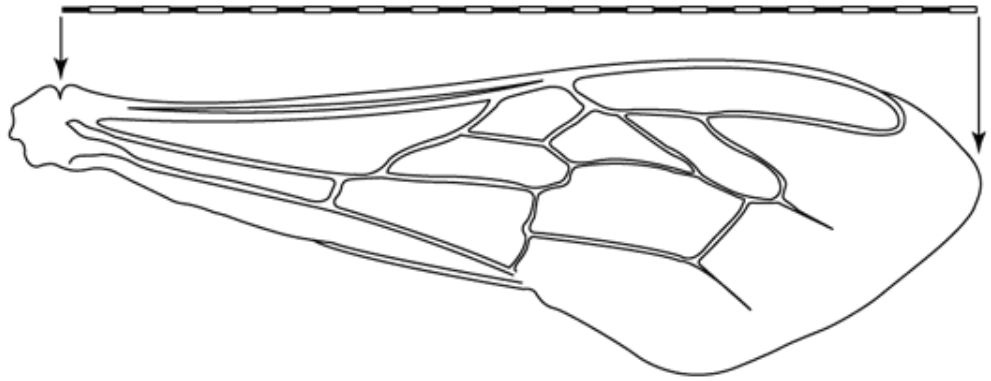

Figure 14. Correct measurement of forewing length (from Rinderer 1986)

Mitochondrial genome

Testing of honey bee maternally inherited mitochondrial genome is available. Deoxyribonucleic acid (DNA) is extracted by a standard protocol (for example, Holmes 2010).

Pinto et al. (Pinto 2003) validated a diagnostic restriction site for Scutellata identified by the work of Crozier et al. (1991) using a PCR amplified DNA assay. The restriction site was scored as present (not Scutellata) or absent. Pinto et al. (2003) performed the test on a sample of 451 colonies from the southern USA with most being able to be classed as honey bees of A lineage or bees of C and M lineage. The only exception was that some honey bees of the *A*. *m*. *iberica* type from southern Spain and France were diagnosed as being of A lineage and therefore, Scutellata (Franck 1998). There were some imports of *A*. *m*. *iberica* into WA early last century, but these appear to have been of M lineage (Chapman 2008).

All subspecies of *A*. *mellifera* can mate and produce fertile offspring. This means that a diagnosis cannot be based solely on the mitochondrial genome associated with the A lineage. For example, if a queen honey bee of C lineage mates with Scutellata males, the offspring queen honey bee will show C lineage mitochondria (inherited maternally), but will be 50 per cent Scutellata in the nuclear genome. If there are repeated backcrosses to Scutellata males, it is possible to have a colony that is Scutellata in phenotype and nuclear genotype, but still showing C lineage in the mitochondria. Therefore it is important to test both the nuclear and mitochondrial genomes for evidence of Scutellata genetic background. Evidence of A lineage in either the nuclear or mitochondrial genome is sufficient for a positive diagnosis.

Identification of evidence of Africanisation in the nuclear genome

Whitfield et al. (2006) identified seven single nucleotide polymorphisms that are at high frequency in *A*. *m*. *scutellata* and low frequency in honey bees of the C lineage. However, all these single nucleotide polymorphisms also have high frequency in honey bees of the M linage and would give false positives for feral honey bees from both Australia and the USA at high frequency. Therefore the use of single nucleotide polymorphisms is unsuitable for the diagnosis of honey bees of African descent.

There are no known DNA microsatellites that show fixed difference between honey bees of African origin and honey bees of C and M lineage (Clarke 2002). Therefore this method cannot be used for definitive diagnosis.

Control

The experiences of Scutellata management in the Americas show that once established there is no possibility of eradication. Restriction of movement (other than into WA and Tasmania) would be futile, and would cause further undesirable disruption to the beekeeping industry.

Possible management measures and restrictions include*:*

- requeening with non-Scutellata stock
- methods of transport including mandatory netting of loads
- restriction of beekeeping activities in urban areas
- location of apiaries, including distances from roads, dwellings, and walking tracks
- appropriate warning signage around apiaries
- development of reliable sources of non-Scutellata stock. This could include establishment of queen honey bee breeding businesses south of 34º South. Unfortunately, the season during which queen honey bees can be produced grows progressively shorter as one travels south.

Conclusion

A. m. scutellata and its Africanised hybrids are not present in Australia and are likely to be present in some exporting countries. There are no recommendations in the OIE Code. Therefore, DAFF concluded that a risk assessment was required.

Risk assessment

For details of the method used in this risk assessment see Chapter 2. A summary of the risk assessment is shown in Figure 15.

Release assessment

The following factors were considered relevant to the estimate of the likelihood of Scutellata being present as imported queen honey bees.

- Scutellata is now present throughout sub-Saharan Africa and Central America. In South America, Scutellata extends as far south as 34º South, where it forms a stable hybrid zone with honey bees of the C and M lineages (Sheppard 1991)
- In the USA, Scutellata has been identified in the states of Alabama, Arizona, Arkansas, California, Florida, Georgia, Louisiana, Nevada, New Mexico, New York, Oklahoma, South Carolina, Texas, Utah and Virginia (Louisiana Department of Agriculture and Forestry 2009; Georgia Department of Agriculture 2010; Utah Department of Agriculture and Food 2010; CAPS 2011)
- Scutellata are small yellow bees and are not sufficiently different from honey bees of a number of other *Apis mellifera* sub-species to be reliably distinguishable by eye. Large colonies are extremely aggressive but this too is not a reliable diagnosis—so are some European colonies.

Conclusion: based on this information, the likelihood of release of Scutellata associated with the importation of queen honey bees was estimated to be **moderate**.

Exposure assessment

The exposure group considered is managed and feral honey bee colonies and the most likely exposure pathway is the introduction of imported honey bees with Africanised genetics to a managed honey bee colony.

The following factors were considered relevant to the estimate of the likelihood of honey bees being exposed to Africanised honey bee genetics via imported queen honey bees:

- Scutellata spread and replace European honey bees primarily by producing larger numbers of reproductive swarms and drones
- Scutellata is characterised by behavioural traits that lead them to replace European honey bees when conditions are favourable: high reproductive rates and extreme defensiveness to counter frequent predation, and regular migration to exploit highly ephemeral nectar sources (Rinderer 1988).

Conclusion: based on this information, the likelihood of susceptible honey bees being exposed to Africanised honey bee genetics via imported queen honey bees with Africanised genetics was estimated to be **moderate**.

Estimation of the likelihood of release and exposure

The likelihood of release and exposure is estimated by combining the likelihood of release and the corresponding likelihood of exposure using the matrix of rules for combining descriptive likelihoods (Table 2). With the likelihood of release estimated to be 'moderate' combined with the likelihood of exposure estimated to be 'moderate', the likelihood of release and exposure was estimated to be **low**.

Consequence assessment

The consequence assessment describes the potential consequences associated with hazard entry and exposure, and estimates the likelihood of them occurring. This involves estimating the likelihood of establishment and/or spread of the hazard for the most likely outbreak scenario, and determining the direct or indirect effects should this outbreak scenario occur. Combining the likelihood of establishment and/or spread for this outbreak scenario with the corresponding overall effect gives an estimation of likely consequences.

Likelihood of establishment and/or spread associated with the outbreak scenario

Once exposure of susceptible honey bees has occurred, a number of possible outbreak scenarios could follow, ranging from no spread to widespread establishment.

The most likely outbreak scenario was determined by describing the likely extent of establishment and/or spread at detection. The most likely outbreak scenario following exposure to Africanised honey bee genetics is considered to be establishment and/or spread to populations of honey bees within multiple states/territories.

The following factors were considered relevant to the estimate of the likelihood of establishment and/or spread associated with exposure of honey bees to Africanised honey bee genetics:

- following importation, descendents of Scutellata imports formed the basis of a massive feral population of Scutellata that has replaced European honey bees throughout the neo-tropics and southern USA (Winston 1992; Clarke 2002; Pinto 2005; Schneider 2004; Whitfield 2006). From the initial importation, Scutellata expanded its range by 200–500 km per year (Taylor 1977; Winston 1992). By 1986 it had reached Mexico, and first invaded the USA in 1992 (Guzmán-Novoa 1994) and is now present in a number of southern and western states. This is one of the most successful biological invasions of all time (Winston 1992)
- Scutellata is characterised by behavioural traits that are adaptations to life in the African savannah: high reproductive rates and extreme defensiveness to counter frequent predation, and regular migration to exploit highly ephemeral nectar sources (Rinderer 1988)
- Scutellata are small, yellow honey bees and are not sufficiently different from honey bees of a number of other *A*. *mellifera* sub-species to be reliably distinguishable by eye. Large colonies are extremely aggressive but this too is not a reliable diagnosis—so are some European colonies. If an imported Scutellata queen honey bee were kept in a small nucleus colony, the colony would not be unusually aggressive
- the pattern of distribution of Scutellata in South Africa, southern USA and Argentina strongly suggests that Scutellata could establish large feral populations in all dry savannah regions of Australia north of 34º South. Thus the majority of Australia is vulnerable to the establishment of a feral Scutellata population. This region includes the majority of the preferred beekeeping country in Australia
- the experiences of Scutellata management in the Americas show that, once established, there is no possibility of eradication.

Conclusion: based on these considerations, it was determined that the likelihood of establishment and/or spread of Africanised honey bee genetics for the exposure group was **high**.

Determination of the effects resulting from the outbreak scenario

Following estimation of establishment and/or spread of a hazard is the determination of the effects (health, environmental and socioeconomic) resulting from that outbreak scenario. For the most likely outbreak scenario, the direct and indirect impacts of Africanised honey bee genetics were estimated at the national, state or territory, district/region and local levels. Adverse effects are evaluated in terms of seven (two direct and five indirect) criteria.

The following factors were considered relevant to a conclusion on the effects of the establishment and/or spread of Africanised honey bee genetics.

Direct effects

The effect on the life or health (including production effects) of susceptible animals including public health consequences

- Scutellata spreads and replaces European honey bees primarily by producing larger numbers of reproductive swarms and drones. Otis (1991) observed that a Scutellata colony can produce 6–12 reproductive colonies per year, and, via the offspring of the offspring colonies, up to 60 descendents in a year. There are also other more subtle processes that contribute to Africanisation

- Scutellata is completely resistant to some of the major pests of C lineage honey bees, particularly the small hive beetle (*Athena tumida*)
- a complete replacement of European (C and M) lineage feral honey bees with Scutellata throughout all of Australia north of 34º South would likely be seen
- Scutellata pose a significant risk to the public via incidental stinging attacks. When beekeepers work in Scutellata apiaries the colonies become extremely defensive and invariably attack any mammal or bird within a few hundred metres. The attacks involve hundreds of stinging honey bees, and death by stinging or the consequences of panic is possible
- a recent analysis of the public health costs if *A. cerana* became established in Australia suggested that the annual direct public health costs would be A$7.4 million (Ryan 2010). The costs associated with an endemic Scutellata population would likely be higher.

The effect on the living environment, including life and health of wildlife, and any effects on the non-living environment

- if introduced into Australia, Scutellata may increase the size of the feral honey bee population. An increased density of feral honey bees may have negative effects on those native fauna that compete with feral honey bees for nest sites and for pollen and nectar.

Indirect effects

The effect on new or modified eradication, control, monitoring or surveillance and compensation strategies or programs

- Scutellata is not nationally notifiable in Australia. Scutellata is included in the AUSVETPLAN *Disease Strategy: Bee Pests and Diseases* (Animal Health Australia 2010)[7]
- controls over honey bee movements would be imposed on managed apiaries within designated areas until further decisions were made (Animal Health Australia 2010)
- movement controls and/or inspections may be imposed on vehicles (e.g. trucks, trains) leaving the Scutellata control zone
- if the decision was made not to attempt eradication but to recommend that control practices be initiated, state/territory and/or industry-based control measures may be initiated, which may include encouraging industry to develop its own long-term policies and procedures (Animal Health Australia 2010). This may include:
    - a recommended frequency of mandatory requeening with non-Scutellata stock
    - methods of transport including mandatory netting of loads
    - restriction of beekeeping activities in urban areas
    - location of apiaries, including distances from roads, dwellings, and walking tracks
    - appropriate warning signage around apiaries.

[7] The emergency management of honey bee diseases and pests are in the process of moving under the Australian Emergency Plant Pest Response Deed (EPPRD) and the Australian Emergency Plant Pest Response Plan (PLANTPLAN)

The effect on domestic trade or industry, including changes in consumer demand and effects on other industries supplying inputs to, or using outputs from, directly affected industries

- as a result of the detection of Scutellata, movement restrictions would be imposed until tracing and surveillance were completed and results analysed (Animal Health Australia 2010)
- if eradication was not attempted, control measures may include interstate movement controls (Animal Health Australia 2010)
- Scutellata apiaries are recommended to be situated a minimum of 200-500 metres away from places occupied by humans, livestock or companion animals (Caron 2001)
- Scutellata's behavioural and productive characteristics make it undesirable for modern beekeeping as practised in Australia
- prices of products from honey bees may increase, thereby reducing consumer demand
- both private and public landowners are likely to become more reticent to allow beekeeping activities on their properties because of the possibility of stinging incidents, stock losses, and fear of litigation. This would have a significant negative impact on beekeepers and landowners who must hire bees for pollination
- Scutellata are unsuitable for paid pollination services.

The effect on international trade, including loss of and restriction of markets, meeting new technical requirements to enter or maintain markets, and changes in international consumer demand

- the presence of Scutellata in Australia may reduce international consumer demand for Australian honey bees
- if Scutellata were to become established, renegotiations of trade conditions would be necessary.

The effect on the environment, including biodiversity, endangered species and the integrity of ecosystems

- a reduction in native pollinators due to competition with feral Scutellata could have negative effects on native flora which are dependent on native pollinators.

The effect on communities, including reduced rural and regional economic viability and loss of social amenity, and any 'side effects' of control measures

- the financial cost of beekeeping may increase due to expenses associated with control of Scutellata
- introduction of Scutellata into Australia would likely see many beekeepers, both hobbyist and commercial, exiting the industry, as has occurred in South and Central America (Winston 1992). Working with Scutellata honey bees can be extremely unpleasant, potentially dangerous, and requires trained personnel.

Conclusion for overall direct and indirect effects: based on the geographic level and magnitude of effects, the overall effect of establishment and/or spread for the outbreak scenario was estimated to be **high** from Table 3. The effect is likely to be significant at the national level, highly significant within affected zones, and be of national concern.

Derivation of likely consequences

The estimate of the overall effect associated with the outbreak scenario was combined with the likelihood of establishment and/or spread for the scenario using Table 4 to obtain an estimation of likely consequences.

The likelihood of establishment and/or spread ('high') is combined with the estimate of the overall effect of establishment and/or spread ('high') which results in **high** likely consequences.

Unrestricted risk estimation

Risk estimation is the integration of likelihood of release and exposure, and likely consequences of establishment and/or spread to derive the risk associated with release, exposure, establishment and/or spread of Scutellata introduced by imported queen honey bees into Australia.

Using Table 5, the likelihood of release and exposure ('low') is combined with the likely consequences of establishment and/or spread ('high'), resulting in a risk estimation of **moderate**.

Therefore, as the unrestricted risk associated with Africanised honey bees exceeds Australia's ALOP of 'very low', risk management was considered necessary.

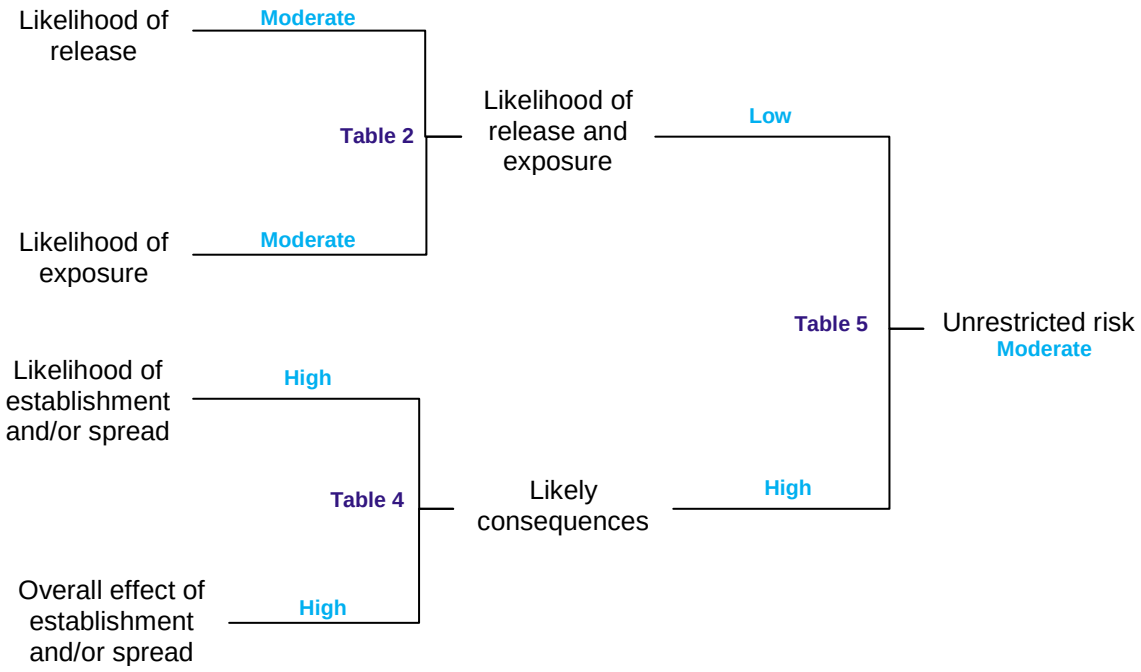

Figure 15. Summary of the risk assessment pathways for Scutellata

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4.13 Cape honey bee (*Apis mellifera capensis*)

Technical information

Background

Apis mellifera capensis (hereafter called 'Capensis') is a native of southern Africa and is unusual among honey bee species and subspecies in that unmated workers produce diploid female offspring as the norm (Anderson 1963; Verma 1983; Baudry 2004). In all other honey bees, worker-laid eggs, if reared, almost always result in haploid males.

During reproductive swarming events and when colonies become queenless, Capensis workers have the potential to become the mother of the replacement queen honey bee (Jordan 2008; Allsopp 2010; Holmes 2010). In groups of queenless workers, one or a few individuals rapidly activate their ovaries and produce queen honey bee-like pheromones in large amounts (Moritz 2000). These individuals suppress ovary activation in their peers, become reproductively dominant (Moritz 2000) and are most likely to become a pseudo-queen (i.e. a reproductively dominant worker that behaves as a queen honey bee) or the mother of the new queen honey bee (Moritz 1996).

Selection for traits that enhance reproductive success is thought to have pre-adapted Capensis workers for lives as reproductive parasites (Moritz 2002; Neumann 2002; Oldroyd 2002; Calis 2003; Dietemann 2006; Beekman 2008; Boot 2008). Capensis is restricted to the Fynbos ecotone of the southern quarter of South Africa. It is when Capensis workers are transferred into *A. m. scutellata* (hereafter 'Scutellata') colonies, whose range includes the savannah regions of southern and central Africa north of the Karroo ectone, that their pre-adaptations to a parasitic life history become most apparent. A hybrid zone exists between the two subspecies, centred in the Karoo ecotone of southern Africa, which has been stable for many decades (Beekman 2008). This suggests that neither subspecies can permanently invade the range of the other, despite Scutellata being highly invasive and providing the source population of 'Africanised' honey bees that have colonised vast regions of the Americas (Spivak 1991; Schneider 2004). Capensis does not move north of the hybrid zone without anthropogenic assistance.

There have been at least three occasions (two historical and one current) when a population of Capensis workers became established within the Scutellata population as social parasites (Lundie 1954; Allsopp 1993; Johannsmeier 1983). In each case a beekeeper or bee researcher moved Capensis colonies beyond the hybrid zone into the Transvaal region to exploit local nectar-producing plants. Genetic analyses of parasitising workers of the current outbreak have revealed an extraordinary lack of genetic diversity. These analyses are consistent with the hypothesis that the parasitic workers are a lineage derived from a single worker that lived in 1991 (Kryger 2001; Baudry 2004; Härtel 2006; Oldroyd 2011).

The 'Capensis clone', defined here as the current lineage of social parasites infesting commercial Scutellata colonies in South Africa, is confined to the commercial beekeeping industry of South Africa, particularly in the provinces of Kwazulu-Natal, Mpumalanga, Gauteng, North West Cape and Northern Cape (Oldroyd 2011). A second clonal lineage is

maintained by honey bee researchers in Germany (Lattorff 2007). This German lineage is apparently not self sustaining, at least in Western Europe. *A*. *m*. *capensis* is not a notifiable disease in Australia (DAFF 2011).

OIE requirements

A. *m*. *capensis* is not an OIE listed disease (OIE 2011) and there are no OIE recommendations.

Epidemiology and pathogenesis

Capensis workers enter a host colony, most often via an anthropogenic vector—beekeepers transporting multiple colonies on trucks, exchanging brood combs between colonies or just on a beekeeper's clothing (Allsopp 1993; Allsopp 1998; Dietemann 2006). Despite the presence of a Scutellata queen honey bee, the Capensis worker activates her ovaries and lays eggs (Dietemann 2006). The resulting larvae are fed lavishly by host workers; so much so that they develop queen honey bee-like reproductive traits such as increased emergence weight and a spermatheca (Beekman 2000; Calis 2002). When these over-fed larvae emerge as adults they too activate their ovaries, lay eggs and repeat the cycle until there are large numbers of parasite workers relative to host workers. Because the parasitic workers do not forage nor care for brood to normal levels, the infested colony soon dies. The process can be regarded as an example of a transmissible social cancer (Martin 2002; Oldroyd 2002).

During the early years of the current infestation of the Scutellata population the Capensis clone was highly virulent and killed hundreds of thousands of colonies (Allsopp 1992). Commercial beekeepers lost 50–75 per cent of their colonies (Oldroyd 2008).

Clinical signs

An infestation can be distinguished from a normal (arrhenotokous) laying worker colony by the absence of drone pupae in worker cells. The typical syndrome includes:

- general dwindling of the colony
- queen-less colony despite the presence of female brood in all stages
- spotty brood pattern
- multiple eggs per worker cell, particularly on comb margins where queen cells might be constructed
- slow moving black workers
- host workers may form a 'court' around a reproductively active worker. Courts form because the host workers are attracted to the queen honey bee-like pheromones produced by the reproductively active worker
- in early stages of infestation fighting between host workers and parasites may be observed.

These signs are unlikely to be recognised even by experienced beekeepers unless they have seen Capensis infestations previously. In the field it is difficult to distinguish worker honey bee-laid female brood from queen bee-laid female brood. In South Africa, host colony Scutellata workers are always yellow, and the black Capensis parasites are often obvious. This situation would not hold in regions in which many strains of feral and domestic bees have black workers.

Diagnosis

The Capensis clone currently infesting commercial colonies in South Africa can be positively identified by DNA microsatellites (Shaibi 2008; Oldroyd 2011).

If an individual carries at least one of the specific alleles there is almost 100 per cent certainty that the individual is derived from the Capensis clone lineage.

Identification of other Capensis

The vast majority of Capensis in South Africa derive from the sexually-reproducing population in the Western and Eastern Cape provinces. Although there are sharp allele frequency differences between the Capensis and Scutellata populations, there are no known fixed genetic differences (Clarke 2001; Franck 2001; Clarke 2002; Whitfield 2006). The only diagnostic feature of Capensis is its ability to reproduce thelytokously. Secondary characteristics include its large number of ovarioles and that about 10 per cent of Capensis workers have a spermatheca. In one study, Capensis workers had a mean of 18 ovarioles per ovary, whereas Scutellata had a mean of 6 ovarioles (Jordan 2008). Allsopp (1992) gives the range of ovarioles in Capensis as 12–15. Typical honey bee sub-species present in Australian have 2–6 ovarioles per ovary (Oldroyd 2008) and never show a spermatheca.

Scutellata almost always have yellow colouration whereas Capensis are mostly black. In the Australian context, colouration of the abdomen is not diagnostic.

Mitochondrial genome

There are four major lineages of honey bee diversity: the Western European lineage (M), the Eastern European lineage (C), the Middle Eastern lineage (O) and the African lineage (A) (Franck 1998; Franck 2001; Whitfield 2006). Capensis is of lineage A, as is Scutellata. Modern beekeeping is mainly based on bees of the C lineage, particularly *A*. *m*. *ligustica* and *A*. *m*. *carnica*, and the original honey bees introduced into Australia from Spain and England were of the M lineage.

Testing, using protocols for the identification of the maternally inherited mitochondrial genome for Scutellata bees by separating samples into either A lineage or C and M lineage (Pinto 2003), will therefore also detect Capensis mitochondrial DNA. This will provide a degree of certainty in diagnosis that is equivalent to the diagnosis of a Scutellata colony. This means that, as for Scutellata, diagnosis cannot be based solely on the mitochondrial genome associated with the A lineage but that evidence of A lineage in the mitochondrial genome (or the nuclear genome) is sufficient for a positive diagnosis.

Control

During the early years of the current infestation in South Africa, the epidemic became known as ‘The Capensis Calamity’ (Allsopp 1992) and draconian measures were put in place by the government. These included restrictions on colony movements, destruction of infested colonies, and paid compensation when colonies were destroyed (Cobey 1999) but were ineffective and the industry was soon left to manage the problem on its own.

Over time beekeepers have adapted their operations to accommodate Capensis. Colonies suspected of being infested are isolated and if black honey bees or other signs of Capensis infestation are observed the colonies are destroyed by burning. Management seeks to minimise the chance of cross infection. Beekeepers emphasise good record keeping

allowing traceback, frequent inspection of colony broodnests and immediate isolation and destruction of infested colonies. Particular care is taken when making splits for increase. To minimise cross infestation, measures should be taken to reduce drifting of returning foragers between colonies. These control measures have been effective and the number of colonies currently infested per year is probably less than 1000.

Historically, South African beekeepers have successfully eradicated Capensis on at least four occasions (Allsopp 1992). The current Capensis clone seems intractable, and has retained virulence for over 20 years.

Conclusion

Capensis is not present in Australia. Its range is restricted to southern Africa and it has not been reported in any other countries. There are no recommendations in the OIE Code. Therefore, DAFF concluded that based on all the available information, further assessment of Capensis was not required.

However, certification of country freedom will be included in Australia's biosecurity measures.

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4.14 Colony collapse disorder

Technical information

Background

Over-wintering losses of honey bee colonies throughout the USA vary from year to year and large scale losses are not unprecedented—episodes of 'disappearing disease of honey bees' were investigated in the 1970s (Wilson and Menapace 1979). A report to the United States Congress (Johnson 2007) in March 2007 mentions particularly heavy losses in the winter of 2003–04 and discusses large die-offs in honey bee colonies due to *Varroa destructor* and tracheal mites in the 1980s and 1990s. Large scale honey bee colony losses have been recorded worldwide (Oldroyd 2007; vanEngelsdorp and Meixner 2010), including in Australia (Anderson 2004).

An increased number of honey bee colony losses amongst large migratory beekeepers based in eastern USA were observed in the northern winter of 2006–07. The losses were in excess of the normal colony losses associated with over-wintering: 30–40 per cent compared to 10–15 per cent which is accepted as the normal loss over a winter (Johnson 2007).

The losses seen in the winter of 2006–07 appeared to follow a distinct pattern (Oldroyd 2007; Frazier et al. 2009):

- a sudden drop in the number of adult honey bees in the hive but with considerable brood and food supplies still present
- an absence of dead honey bees in or around the hive suggesting that the adult honey bees had been lost in the field
- dead colonies tended to be left alone by small hive beetles and wax moths, two pests that normally would rapidly infest dead honey bee colonies, and a lack of cleptoparasitism from neighbouring honey bee colonies.

Originally dubbed 'Fall-dwindle disease' (vanEngelsdorp et al. 2006), the syndrome has become known as colony collapse disorder (CCD). No single aetiological agent for CCD has been identified to date.

CCD is not a notifiable disease in Australia (DAFF 2011)OIE requirements

CCD is not an OIE listed disease (OIE 2011) and there are no OIE recommendations.

Epidemiology

In an attempt to establish the prevalence of the syndrome, USA beekeepers were surveyed between 2006 and 2009. The results estimated over-winter losses of honey bee colonies to range from 25–35.8 per cent (vanEngelsdorp et al. 2008; vanEngelsdorp et al. 2009b; vanEngelsdorp et al. 2011). However, the imprecise nature of the case definition of CCD and the quality of the early survey work hindered efforts to categorise colony losses and establish a prevalence of the syndrome.

In each of these surveys, the identification of a possible CCD episode relied on a single question asking beekeepers what percentage of hives that had died over the previous winter had no dead bees inside the hive or in the apiary. Therefore, estimates of the prevalence of CCD are based on individual beekeepers' observations and their interpretation of this non-specific sign.

A number of European countries also observed increased colony losses at the same time the CCD phenomenon was being reported in the USA. In response, an investigation group known as COLOSS (COLony LOSS) was formed and began to survey beekeepers across Europe to define the scale and cause of colony losses.

A summary of international honey bee colony losses was undertaken in 2009. It reported significant colony losses in Europe, Japan, the Middle East and the USA, but not from Africa, South America or Australia (Neumann and Carreck 2010).

It is now recognised that colony loss and CCD are not synonymous and that only a proportion of colony losses could fit within the set of symptoms that describe CCD (Pettis and Delaplane 2010; Williams et al. 2010). The USA remains the only country where CCD has been documented *sensu stricto* (Williams et al. 2010).

An initial study in response to the reported losses in the USA used a meta-genomic method to look at micro-organisms in American hives (Cox-Foster et al. 2007) and compare colonies considered to be CCD-affected with those assessed to be normal. The study demonstrated an apparent association between what appeared to be an exotic dicastrovirus, Israeli acute paralysis virus (IAPV) and CCD. IAPV was found to be confined, with a single exception, to CCD-affected colonies. While this paper also linked IAPV to the importation of Australian honey bees into the USA, a subsequent study showed that IAPV had been present in the USA for some time before the CCD syndrome was recognised (Chen and Evans 2007).

A number of other honey bee viruses including acute paralysis virus, cloudy wing virus, deformed wing virus and slow paralysis virus, have been implicated in colony losses particularly when associated with *Varroa* (Chen et al. 2004; Chen and Siede 2007; Carreck et al. 2010; Le Conte et al. 2010).

Varroa is a well documented contributor to colony loss, particularly to over-wintering losses (Beetsma 1994; De Jong 1997; Seeley 2007). Untreated colonies usually die with 6–24 months of *Varroa* infestation (Le Conte et al. 2010). *Varroa* has also been implicated and investigated in relation to CCD episodes (Cox-Foster et al. 2007; vanEngelsdorp et al. 2009a; vanEngelsdorp et al. 2010). Although none of these studies found a direct relationship between *Varroa* levels and colonies with CCD symptomatology, Van Englesdorp et al. (2010) concluded that colony health was related to increased acaricide levels with the implication that control of *Varroa* may be important in preventing the appearance of CCD symptoms.

Varroa has been present for some time in all the countries that are reporting increases in colony losses and it may be the crucial factor in the colony losses (Le Conte et al. 2010; Neumann and Carreck 2010). Dahle (2010) and Guzmán-Novoa et al. (2010) concluded that over-wintering losses in at least two countries (Canada and Norway) were almost entirely explained by *Varroa* infestation.

While *Varroa* almost certainly plays a role in CCD, another factor that has been considered is the use of insecticides in the hives. The role of insecticides in CCD was recognised early in the investigations of CCD, with speculation that the reported reluctance of wax moths and small hive beetles to invade dead colonies might be due to toxins within the hives (Oldroyd 2007). The increasing use of acaricides following the spread of *Varroa* (Johnson et al. 2009) and the exposure of foraging bees to pesticides used in cropping systems (Barnett et al. 2007) are the two most likely routes of intoxication.

Honey bee colonies are being increasingly used for paid pollination work in and around crops that are regularly sprayed with insecticides. Large honey bee colony die-offs have been recorded in France and Germany, with a definite link to the use of a relatively new class of insecticide, the neonicotinoids (Promed Mail 2008). Sub-lethal effects on honey bee behaviour have been seen with very low doses of these compounds and with other classes of insecticides used in agriculture (Aliouane et al. 2009; Maini et al. 2010; Rabea et al. 2010). The doses of neonicotinoids that cause changes in honey bee behaviour or mortality may be at the extreme limits of detection (McCarthy 2011).

The two acaricides widely used to control *Varroa*, the pyrethroid tau-fluvalinate and the organophosphate coumaphos, have been shown to be stable with a synergistic effect, absorbed by beeswax within the hive, and to have the potential to build up with repeated treatments (Johnson et al. 2009). Sub-lethal doses of these acaricides may cause mortality amongst honey bees when they are both present in the hive (Johnson et al. 2009). Conversely, vanEngesdorp et al. (2009a; 2010) found higher levels of coumaphos in healthy colonies as opposed to those classified as suffering from CCD. One possible reason was that the healthy colonies had better and more persistent *Varroa* control.

Another contributor to colony losses and perhaps CCD is the microsporidian parasite *Nosema ceranae*. This pathogen was postulated as a major contributing factor to the colony losses seen in Europe (Higes et al. 2006). Natural infection can lead to a long, asymptomatic incubation period that can lead to a sudden collapse in adult honey bee numbers as the queen honey bee is unable to replace the loss of infected workers (Higes et al. 2008). The colony therefore appears to have adequate brood and food reserves and an active queen bee, but very few adult honey bees present, thus conforming to the initial descriptions of CCD.

However, subsequent work has shown that this organism is much more widely spread in *A*. *mellifera* than initially thought and its presence in both Europe and the USA pre-dates the appearance of CCD and colony loss syndromes (Paxton et al. 2007; Chen et al. 2008). Furthermore, data from a five-year cohort study of the prevalence of *Nosema* species in Germany, revealed no relationship between colony mortality and detectable levels of infection with *N*. *ceranae* or the related species, *N*. *apis* (Gisder et al. 2010).

Pettis et al (2012) looked at the interaction of sub-lethal doses of a pesticide (imidacloprid) and *Nosema ceranae*, demonstrating synergism between the two agents. They suggest that subtle interactions between pesticides and pathogens could be a major contributor to increased mortality of honey bee colonies worldwide.

A virus not previously recognised as a pathogen in *A. mellifera*, insect iridovirus, has also been associated with *N*. *ceranae* in colonies showing signs linked with CCD (Bromenshenk

et al. 2010). The authors of this study were unable to clearly define whether the presence of these two agents was a marker, the cause or a consequence of CCD symptoms. At the time of the study's publication, insect iridovirus had not been isolated.

A number of other factors that may play a role in colony loss have been advanced and examined. These include:

- nutrition and the effect malnutrition has on the honey bees' immune systems and on the colonies ability to survive over winter
- the use of genetically modified crops
- management practices such as migratory beekeeping and paid pollination
- the quality of queen honey bees and the genetics of managed honey bees
- the effect climate can play in the availability of resources and the health of honey bee colonies (Mumford et al. 2008; vanEngelsdorp and Meixner 2010).

Diagnosis

CCD has only been diagnosed by symptomatology. The symptoms ascribed to CCD have altered since the initial reports (Frazier et al. 2009), and Pettis and Delaplane (2010) now list five:

- the dwindling or loss of adult honey bees in the hive but with an absence of dead bees in or around the hive
- a low proportion of adult honey bees to remaining brood
- a disproportionally young workforce
- reluctance of dwindling colonies to consume food provided by the beekeeper
- reluctance of neighbouring honey bee colonies to rob the colony after death.

The symptoms that encompass the CCD syndrome have only been described in the USA despite colony losses of similar scale being reported in other parts of the northern hemisphere (Williams et al. 2010).

Control and management

Besides general advice on controlling known pests and diseases and keeping colonies strong going into winter, there is no recognised treatment or management for CCD or colony loss.

Conclusion

CCD is not listed by the OIE and has not been reported in Australia. Although honey bee colony losses have escalated in some parts of the world over the past few years, there has always been doubt that CCD presents a novel disease syndrome (Stokstad 2007). It appears increasingly likely that the colony losses being seen are due to a combination of recognised conditions—the continuing effects of *Varroa* and associated viral infections, nosema disease, pesticide exposures, and nutritional and physical stresses—rather than the introduction of a new, and as yet unknown, entity.

Therefore, DAFF concluded that based on all the available information, further assessment of CCD was not required.

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5 Risk management

Risk management measures considered in this review aim to reduce the likelihood that imported queen honey bees would lead to the release, exposure, establishment and/or spread of hazards of biosecurity concern. The method used for risk management is consistent with the OIE Code (OIE 2011b).

DAFF determined that to achieve Australia's ALOP, the biosecurity risk associated with imported queen honey bees must be at least 'very low'. In the risk assessment chapters, the unrestricted risk was estimated for each hazard to ascertain whether it achieved Australia's ALOP. If the unrestricted risk estimate was 'very low' or 'negligible', it achieved Australia's ALOP and risk management was not required. If the unrestricted risk estimate did not achieve Australia's ALOP, risk management options to reduce the risk to an appropriate level, i.e. 'very low' or 'negligible', were considered.

For the following hazards, the unrestricted risk estimate did not achieve Australia's ALOP and risk management measures were considered necessary to reduce the risk to an appropriate level:

- Acarapisosis (tracheal mites)
- *Tropilaelaps*
- Varroosis
- Africanised honey bee (*Apis mellifera scutellata* and its hybrids).

The effect of risk management measures was assessed as the change in the risk estimate and expressed as 'restricted risk'. This was derived using either a particular risk management measure or a combination of measures. Where the effect was to reduce the risk estimate to 'very low' or 'negligible', the measure, or combination of measures, was deemed appropriate. In consideration of risk management measures, equivalent measures that would achieve Australia's ALOP were considered and those that would be least trade restrictive were identified.

Risk management options

The efficacy and feasibility of risk management measures that could be applied in this review take into account that:

- the queen honey bees are alive, which rules out treatments associated with non-viable product, e.g. heat treatment
- some honey bees infected with disease agents may not show clinical signs of infection (i.e. they have a subclinical infection or latent carrier state or carry undesirable genetics that may not be immediately evident) and therefore detection of disease through visual observation and examination is unreliable
- the sensitivity of some diagnostic tests is not always optimal (i.e. infection or infestation may not be detected)
- treatments are not always reliable in preventing disease or stopping shedding of disease agents

- honey bees are essentially an uncontrolled commodity. The status of honey bee pests and diseases within a country may change and go without detection for an extended period of time.

Pre-entry measures

Approved country

For countries to be approved, Australia takes into consideration a number of criteria. These include the animal health status of the country, animal health legislation, the effectiveness of systems for control over certification of animals and products, the effectiveness of veterinary and laboratory services, and the standard of reporting of disease outbreaks to the OIE. Australia has issued *Guidelines for the approval of countries to export animals (including fish) and their products to Australia* (see AQPM 1999/62).

This review considered the importation of queen honey bees from Canada, the European Union, Japan, New Zealand and the USA, as this provided a satisfactory level of assurance of the exporting country's capacity for certifying to Australia's biosecurity requirements.

If other countries with a long history of trade with Australia wish to be added to the list of approved countries, a detailed assessment taking into account these criteria would be required to determine if Australia's import requirements could be met.

Country or zone freedom

For some hazards of biosecurity concern, queen honey bees will only be imported from countries or defined zones (in accordance with Article 4.3 of the OIE Code) free of the hazard. Determining freedom must be to a standard consistent with that recommended in Article 1.4.6 of the OIE Code (OIE 2011a), or to an equivalent standard for those hazards not listed by the OIE Code. For Australian Government authorities to be satisfied that a country or zone is free of a given agent, they must have knowledge of the relevant agricultural and/or veterinary authority (e.g. the government veterinary service or equivalent) of that country and be satisfied that the relevant agricultural and/or veterinary authority has the capacity for control, monitoring and surveillance, as appropriate for the hazard. In some cases, it might be necessary for the hazard to be subject to compulsory reporting or disease investigation.

Zone freedom will only be considered after the assessment of a submission from the relevant competent authority that provides evidence of the zone's honey bee health status with regard to the hazard in question.

Premises status

Queen honey bees will only be imported from premises where particular hazards of concern are not known to have occurred. For the status of the hazard on the premises to be certified, the relevant agricultural and/or veterinary authority may need to undertake investigations, surveillance or monitoring. Certification for hazards that are not notifiable would need to be based on a declaration from a private veterinarian or from the vendor.

Restriction of commodity

Importation will be restricted to queen honey bees with an appropriate number of escorts.

Diagnostic testing

Diagnostic testing of queen honey bees and their escorts before export may assist to reduce the likelihood of release of some hazards. Testing would need to be conducted using methods described and recommended in the OIE Manual (OIE 2011c) where such descriptions exist, and at a laboratory meeting the standards recommended by the OIE (OIE 2008b). The level of risk reduction provided by testing would depend on the availability and sensitivity of tests, and on sampling and other operational procedures. All testing must be conducted at a laboratory approved and monitored by the Veterinary Authority of the country of export, with all results attached to the health certification.

Preventative treatment

Preventive treatments, such as application of acaricides before export, may assist to reduce the likelihood of release for some hazards. Inclusion of general risk management measures such as thorough visual examination and treatment for external parasites was considered appropriate.

Treatments that could adversely affect the sensitivity of diagnostic tests used for risk management must not be administered. Details and records of treatments are to be available for inspection.

Visual inspection

Provided all other import requirements are met, only queen honey bees that are from colonies that have been inspected as follows are eligible for export:

- by an officer from the relevant agricultural and/or veterinary authority, within seven days of export, and deemed to be clinically healthy and free from visible evidence of hazards of concern and,
- by the owner of the apiary, in preparing queen honey bees and escorts for export, and deemed to be free from visible evidence of hazards of concern.

The queen honey bee and escorts in the export consignment should themselves be free of physical or behavioural abnormalities and show no visible evidence of hazards of concern.

Transport measures

Measures applied during the transport of queen honey bees and their escorts from the exporting country to the post-arrival quarantine (PAQ) facility in Australia may assist to reduce the likelihood of release for some hazards.

Queen honey bees and their escorts can only be imported by air freight in packaging that prevents the escape of hazards of biosecurity concern. On arrival in Australia, imported honey bees must immediately be taken to the PAQ facility.

Post-arrival measures

Post-arrival quarantine

Quarantine isolation of queen honey bees when they arrive in Australia may assist to reduce the likelihood of release for some hazards of biosecurity concern and the exposure of susceptible domestic honey bees to these agents. PAQ allows:

- isolation and separation of imported queen honey bees from the Australian honey bee population
- visual inspection of queen honey bees and their escorts on arrival
- monitoring for clinical signs of disease
- destruction of imported packaging materials
- testing and/or treatment for hazards of biosecurity concern
- sacrifice of imported escort bees on entry to PAQ for further testing. These escort bees can be replaced by a small number of Australian worker honey bees sourced from a local hive free of visible evidence of hazards of concern, with subsequent sacrifice and examination of these local escort bees
- establishment of a nucleus colony containing the imported queen honey bee and Australian worker honey bees sourced from a local hive free of visible evidence of disease, with subsequent examination and testing of brood and adult honey bees produced by this colony.

DAFF must be notified immediately if there is any evidence of a hazard during PAQ, and a thorough investigation must be conducted.

The PAQ facility must have restricted areas for holding the imported honey bees and material and rearing the progeny so that there is no contact with honey bees, other insects or materials from outside the PAQ facility. Genetic material, such as eggs or recently hatched larvae, must be transferred to a different restricted area for grafting of queen cells.

Biosecurity measures should be implemented in the PAQ facility to prevent the transmission of infection within, and release of hazards from, the facility. Measures include, but are not limited to, control of movement of queen honey bees and personnel in and out of the facility; hygienic operating practices to prevent transmission of infection via fomites or inadvertently via handlers or diagnostic procedures; disinfection and decontamination.

All equipment used in handling and treating honey bees must remain in PAQ or be cleaned and disinfected by an approved method before removal from PAQ. Provided all import requirements are met, only grafted queen cells that are deemed to be healthy and free from hazards of biosecurity concern will be released from PAQ.

Diagnostic testing

Diagnostic testing of honey bees during PAQ may assist to reduce the likelihood of release for some hazards of biosecurity concern. Testing would need to be conducted using methods described and recommended in the OIE Manual where such descriptions exist (OIE 2011c). The level of risk reduction provided by testing would depend on the availability and sensitivity of tests, and on sampling and other operational procedures.

Preventative treatment

Use of preventive treatments, such as application of acaricides, during PAQ may assist to reduce the likelihood of release for some hazards, and the likelihood of exposure for susceptible Australian honey bees. If possible, the chemical used should be different to that used in the country of origin before export.

Restricted post-quarantine release

Post-quarantine release of the progeny of imported queen honey bees may be restricted to certain regions in Australia to comply with internal movement restrictions for particular hazards.

Contingency measures

In the event that imported honey bees or their progeny do not meet Australia's import requirements, or during PAQ fail a test and/or show signs of disease, then affected honeybees, imported honey bees in the consignment from the same hive, any other imported honey bees deemed to have been in contact with affected honey bees and associated equipment may be destroyed without recompense.

Risk management options for specific hazards

Acarapisosis (tracheal mite)

The unrestricted risk for tracheal mite associated with the importation of queen honey bees was estimated to be **moderate**. Risk management options that could be applied to achieve Australia's ALOP were considered.

The likelihood of release of tracheal mite was estimated to be **high** and the likelihood of exposure of tracheal mite was estimated to be **moderate**. The following options were considered as risk management measures to reduce the likelihood of release and/or exposure for tracheal mite.

Pre-entry measures

Country or zone freedom

- tracheal mites are found throughout the world—Europe, the Americas, Africa and Asia (Matheson 1996). Tracheal mites have not been detected in Australia
- all stages of the tracheal mite—adults and brood—live exclusively within the respiratory system of the honey bee (García Fernández 1999)
- free female tracheal mites survive only a few hours, depending on temperature, relative humidity and nourishment (García Fernández 1999). The lifespan of the tracheal mite in dead honey bees is approximately a week (OIE 2008a).

Conclusion: based on this information, DAFF considered that country or zone freedom was a risk management option for tracheal mite. Country or zone freedom alone would be sufficient to achieve Australia's ALOP.

Premises status

- infestation is spread by direct contact (OIE 2008a)
- rapid dispersal of the tracheal mite is believed to be due to movements of migratory beekeepers and the selling of infested honey bees; apiary spread to neighbouring colonies is thought to be due to drifting honey bees (García Fernández 1999). Spread after its introduction into the USA was rapid (Delfinado-Baker 1985)
- all stages of the tracheal mite—adults, larvae, nymphs and eggs—live exclusively within the respiratory system (specifically the tracheae) of the honey bee (García Fernández 1999)

- early infestations are not apparent—a slow decrease in colony size may be the only indication—becoming apparent when infestation is heavy.

Conclusion: based on this information, DAFF considered that premises status was a risk management option for tracheal mite. A requirement for the queen honey bees and their escorts to have been sourced from an apiary free from visible evidence of tracheal mites at the time of sourcing was considered appropriate. However, due to the difficulty in detecting the presence of tracheal mites, premises status alone was not considered sufficient to reduce the likelihood of release.

Diagnostic testing

- tracheal mites are difficult to detect and identify due to their small size (Shimanuki 2000). No reliable method exists for detection of very low levels of infestation (OIE 2008a)
- tracheal mites can only be detected using laboratory methods (OIE 2008a)
- clinical signs of infestation are non-specific; affected honey bees crawl around in front of the hive and are unable to fly. Dysentery may be present (OIE 2008a).

Conclusion: based on this information, DAFF considered that pre-export diagnostic testing was not a risk management option for tracheal mites

Preventative treatment

- tracheal mite-free colonies can be established by removing sealed brood from affected colonies, ensuring all adhering adult honey bees have been removed from sealed brood combs (Wilson 1997)
- contact acaricides used in *Varroa* control are generally ineffective against tracheal mites due to their inability to produce sufficiently high enough doses inside the honey bees' tracheal system to kill tracheal mites (Scott-Dupree 1992; Eischen 1998)
- to keep tracheal mite levels under control, menthol crystals or oil patties made with vegetable oil and white granulated sugar can be used. Infested colonies may be treated with formic acid or menthol used as fumigants. However, fumes from formic acid and menthol can disrupt honey bee behaviour (Wilson 1997; OIE 2008a). Formic acid gel formulations are more effective than menthol treatment (Baxter 2000).

Conclusion: based on this information, DAFF considered that pre-export preventative treatment was a risk management option for tracheal mite. A requirement for the hive or nucleus colony in which queen honey bees and their escorts for export have been housed to have been treated according to the manufacturer's directions with a commercially available formic acid gel product within seven days of export for a minimum period of 24 hours was considered appropriate. However, due to preventative treatment potentially not completely eradicating tracheal mites and the potential for reinfestation, preventative treatment alone was not considered sufficient to reduce the likelihood of release.

Transportation measures

Although tracheal mites are the smallest of the parasitic mites of biosecurity concern, all life stages of tracheal mite live exclusively within the respiratory system of the honey bee (Garcia Fernandez 1999) so they are not likely to be loose in the transport box. Tracheal mites are spread by direct contact between honey bees (OIE 2008a). High security '100'

mesh (100 holes per square inch) has an aperture size of approximately 150 µm and is therefore, suitable to prevent contact between honey bees in the export consignment and those in the external population.

Post-arrival measures

Post-arrival quarantine

- all stages of tracheal mite—adults, larvae, nymphs and eggs—live exclusively within the respiratory system (specifically the tracheae) of the honey bee (García Fernández 1999)
- honey bees under four days of age are most susceptible to infestation (Gary et al. 1989)
- infestation is spread by direct contact (OIE 2008a). Rapid dispersal of the tracheal mite is believed to be due to movements of migratory beekeepers and the selling of infested honey bees and queen honey bees; apiary spread to neighbouring colonies is thought to be due to drifting bees (García Fernández 1999)
- free female tracheal mites survive only a few hours, depending on temperature, relative humidity and nourishment (García Fernández 1999). The lifespan of the tracheal mite in dead honey bees is approximately a week (OIE 2008a)
- early infestations are not apparent—a slow decrease in colony size may be the only indication—becoming apparent when infestation is heavy.

Conclusion: based on this information, DAFF considered that PAQ was a risk management option for tracheal mite. However, due to the ability of tracheal mites to survive on larval or adult honey bees, PAQ alone was not considered sufficient to reduce the likelihood of release.

Diagnostic testing

- tracheal mites are difficult to detect and identify due to their small size (Wilson 1997) and no reliable method exists for the detection of very low levels of infestation (OIE 2008a)
- tracheal mites can only be detected using laboratory methods. Tracheal mites are observed within the tracheae or removed and observed microscopically. Techniques including dissection, grinding and staining can be used to demonstrate the presence of tracheal mites. Maceration is the simplest and most reliable technique for diagnosis, allowing detection of early and light infestations using a dissecting microscope (OIE 2008a)
- honey bees under four days of age are most susceptible to infestation (Gary 1989). The female tracheal mite moves to the spiracles and into the tracheae where it lays 5–7 eggs after 2 days. Maturation after hatching takes 11–12 days for males and 14–15 days for females (García Fernández 1999).

Conclusion: based on this information, DAFF considered that post-arrival diagnostic testing was a risk management option for tracheal mites.

On arrival at the PAQ facility the consignment will be visually inspected for the presence of tracheal mites. Imported escort honey bees will be destroyed and examined by an entomologist at a government-approved laboratory. Australian-sourced escort honey bees less than four days old will then be introduced. After the queen honey bee has undergone an

isolation period for a minimum of 14 days with the Australian-sourced escort honey bees, these escorts will be destroyed and examined for tracheal mites by an entomologist. Fourteen days provides adequate opportunity for the transfer of mated tracheal mites from an infested queen honey bee to the Australian-sourced escort honey bees and after this period, tracheal mites in the later stages of development (if present) should be detectable in the Australian-sourced escort honey bees.

However, due to the potential of missing the presence of tracheal mites, diagnostic testing alone was not considered sufficient to reduce the likelihood of release.

Preventative treatment

- contact acaricides used in *Varroa* control are generally ineffective against tracheal mites due to their inability to produce sufficiently high enough doses inside the honey bee tracheal system to kill tracheal mites (Scott-Dupree 1992; Eischen 1998)
- to keep tracheal mite levels under control, menthol crystals or oil patties made with vegetable oil and white granulated sugar can be used. Infested colonies may be treated with formic acid or menthol used as fumigants. However, fumes from formic acid and menthol can disrupt honey bee behaviour (Wilson 1997; OIE 2008a). Formic acid gel formulations are more effective than menthol treatment (Baxter 2000).

Conclusion: based on this information, DAFF considered that post-arrival preventative treatment was not a suitable risk management option for tracheal mites. Acaricides are ineffective against tracheal mites and effective treatments such as formic acid or menthol are potentially damaging to the quarantine colony.

Overall conclusion

The unrestricted risk associated with tracheal mite was estimated to be **moderate**.

Other than country or zone freedom, no single risk management option reduced the unrestricted risk sufficiently to achieve Australia's ALOP of 'very low'. However, the combination of premises status, pre-export and post-arrival diagnostic testing, pre-export preventative treatment and PAQ would reduce the likelihood of release of tracheal mites from 'high' to 'extremely low'.

This would reduce the likelihood of release and exposure to 'very low' and the restricted risk to at least **very low**, thereby achieving Australia's ALOP.

Biosecurity risk management measures for acarapisosis (tracheal mites)

To achieve Australia's ALOP with respect to the risk of tracheal mites in imported queen honey bees, the following biosecurity measures are to be applied.

The queen honey bees and their escorts have been sourced from an apiary in a country or zone/region that meets the OIE Code definition of a country or zone/region free from tracheal mite.

OR

i. The queen honey bees and their escorts have been sourced from a hive or colony free from visible evidence of tracheal mite at the time of sourcing.

AND

ii. The hive or nucleus colony in which the queen honey bees and their escorts for export have been housed have been treated according to the manufacturer's directions with a commercially available formic acid product within seven days of export for a minimum period of 24 hours.

AND

iii. The queen honey bees and their escorts are to be transported in sealed packaging or boxing, and the air inlets covered with high security '100' mesh at a minimum.

AND

iv. On arrival in Australia, the imported queen honey bees and their escorts must proceed to a government approved quarantine facility. The imported queen honey bee must be removed from the imported escort honey bees and undergo an isolation period of a minimum of 14 days with Australian-sourced escort honey bees. Australian-sourced escort honey bees should be less than four days of age. Following this period, the Australian-sourced escort bees must be destroyed and examined for tracheal mites by an entomologist at a government approved laboratory.

AND

v. On arrival at the PAQ facility, imported escort honey bees must be destroyed and examined for tracheal mites by an entomologist at a government approved laboratory.

Tropilaelaps infestation

The unrestricted risk for *Tropilaelaps* infestation associated with the importation of queen honey bees was estimated to be **low**. Risk management options that could be applied to achieve Australia's ALOP were considered.

The likelihood of release of *Tropilaelaps* was estimated to be **low** and the likelihood of exposure of *Tropilaelaps* infestation was estimated to be **moderate**. The following options were considered as risk management measures to reduce the likelihood of release and/or exposure for *Tropilaelaps* infestation.

Pre-entry measures

Country or zone freedom

- *Tropilaelaps* are found throughout the range of the giant honey bee including mainland Asia, Indonesia and the Philippines. However, since infesting *A*. *mellifera*, *Tropilaelaps* has spread beyond the geographical range of its primary host to Afghanistan, Iran, Kenya, New Guinea and South Korea (Anderson 2007). *Tropilaelaps* has not been detected in Australia
- *Tropilaelaps* do not survive in areas where interruption of brood rearing occurs and have not become a serious problem in temperate zones except where continuous

invasion from tropical areas can occur (Woyke 1984). However, *Tropilaelaps* is considered an emerging threat to *A*. *mellifera* worldwide. Rising temperatures, causing *A*. *mellifera* to produce brood throughout the year, have been suggested as a mechanism that could lead to *Tropilaelaps* spreading into temperate regions (Anderson 2007)

- *Tropilaelaps* are parasites of *A*. *mellifera* brood (OIE 2011d)
- *Tropilaelaps* has only a short phoretic stage on the adult honey bee because it lacks the ability to feed on the mature bee (OIE 2008c). *Tropilaelaps* will survive only a short time if confined solely to adult honey bees—estimates of this period vary from as little as two days to as long as ten days (Woyke 1984; Rinderer 1994; OIE 2008c).

Conclusion: based on this information, DAFF considered that country or zone freedom was a risk management option for *Tropilaelaps* infestation. Country or zone freedom alone, using the OIE Code recommendation of a country or zone free from *Tropilaelaps* infestation, would be sufficient to achieve Australia's ALOP.

Premises status

- early signs of infestation usually go unnoticed. The *Tropilaelaps* population grows rapidly and can result in high hive mortality (OIE 2011d)
- non-specific signs such as an irregular brood pattern, cadavers partially protruding from cells and perforated cappings may be observed (OIE 2008c). Honey bees that survive infestation during development can show physical or physiological damage, including shortened lifespan, reduced body weight, shrunken and deformed wings and legs, and they may be seen crawling at the hive entrance (DEFRA 2005)
- *Tropilaelaps* spreads when adult honey bees move between colonies through the natural processes of drifting, robbing and swarming, and also spread slowly over long distances in this way. Distribution of infested combs and honey bees through usual beekeeping practices allows spread within apiaries; however, the most rapid and main method of spread is through movement of infested colonies of *A. mellifera* to new areas by beekeepers (DEFRA 2005).

Conclusion: based on this information, DAFF considered that premises status was a risk management option for *Tropilaelaps*. A requirement for the queen honey bees and their escorts to have been sourced from an apiary free from visible evidence of *Tropilaelaps* infestation at the time of sourcing was considered appropriate. However, due to the difficulty in detecting the presence of *Tropilaelaps*, premises status alone was not considered sufficient to reduce the likelihood of release.

Diagnostic testing

- adult *Tropilaelaps* are red-brown, elongated and are less than 1 mm long (OIE 2008c)
- the OIE Manual (OIE 2008c) describes methods of diagnosis of *Tropilaelaps* infestation by examination of adult honey bees, colony and brood or hive debris.

Conclusion: based on this information, DAFF considered that pre-export diagnostic testing was a risk management option for *Tropilaelaps* infestation. Queen honey bees and their escorts for export should be visually inspected for the presence of *Tropilaelaps* before export.

However, due to the difficulty in detecting the presence of *Tropilaelaps*, visual inspection alone was not considered sufficient to reduce the likelihood of release.

Preventative treatment

- given the inability of *Tropilaelaps* to survive for long periods away from brood, husbandry techniques that create breaks in the brood, such as caging queen honey bees, use of artificial swarms and comb trapping, can be used to reduce *Tropilaelaps* numbers (DEFRA 2005)
- many of the same chemicals used for *Varroa* control will kill *Tropilaelaps*, such as fluvalinate or formic acid (OIE 2008c).

Conclusion: based on this information, DAFF considered that pre-export preventative treatment was a risk management option for *Tropilaelaps*. A requirement for the hive or nucleus colony in which queen honey bees and their escorts for export have been housed to have been continuously exposed to treatment immediately prior to export, for a defined period in accordance with the manufacturer's directions was considered appropriate. The product must have demonstrated efficacy for the control of *Tropilaelaps*.

However, due to the potential for the preventative treatment not to be completely effective in eradicating *Tropilaelaps*, and the potential for reinfestation, preventative treatment alone was not considered sufficient to reduce the likelihood of release.

Transportation measures

Depending on species and gender, *Tropilaelaps* measure 600–1000 µm long and 400–550 µm wide (Anderson 2007). Normal high security '100' mesh, which has an aperture size of approximately 150 µm, is suitable to contain adult *Tropilaelaps* (the only developmental stage present on adult honey bees).

Post-arrival measures

Post-arrival quarantine

- *Tropilaelaps* has only a short and phoretic stage on the adult honey bee because it lacks the ability to feed on the mature honey bee (OIE 2008c). *Tropilaelaps* will survive only a short time if confined solely to adult honey bees—estimates of this period vary from as little as two days to as long as ten days (Woyke 1984; Rinderer 1994; OIE 2008c)
- *Tropilaelaps* are parasites of *A*. *mellifera* brood (OIE 2011d).

Conclusion: based on this information, DAFF considered that PAQ was a risk management option for *Tropilaelaps* infestation. On arrival in Australia, the queen honey bee will be removed from the imported escort honey bees and will undergo an isolation period for a minimum of 14 days with Australian-sourced escort honey bees, thereby providing a suitable period for reducing the chance of *Tropilaelaps* survival. However, due to the ability of *Tropilaelaps* to survive on larval or adult honey bees there still remains a low likelihood of Tropilaelaps survival, therefore PAQ alone was not considered sufficient to reduce the likelihood of release.

Diagnostic testing

- adult *Tropilaelaps* are red-brown, elongated and are less than 1 mm long (OIE 2008c)

- the OIE Manual (OIE 2008c) describes methods of diagnosis of *Tropilaelaps* infestation by examination of adult honey bees, colony and brood or hive debris
- *Tropilaelaps* will survive only a short time if confined solely to adult honey bees—estimates of this period vary from as little as two days to as long as ten days (Woyke 1984; Rinderer 1994; OIE 2008c). Mites return to brood cells within 1.3 days (Oldroyd 2006)
- within a colony, the female *Tropilaelaps* enters the brood cell just before they are capped (OIE 2008c).

Conclusion: based on this information, DAFF considered that post-arrival diagnostic testing was a risk management option for *Tropilaelaps* infestation.

On arrival at the PAQ facility the consignment will be visually inspected for the presence of *Tropilaelaps*. Imported escort honey bees will be destroyed and examined for *Tropilaelaps* by an entomologist at a government approved laboratory. After the queen honey bee has undergone an isolation period of a minimum of 14 days with Australian-sourced escort honey bees, the Australian-sourced escort bees will be destroyed and examined for *Tropilaelaps* by an entomologist at a government approved laboratory.

At least 14 days following the acceptance of the imported queen honey bee into the nucleus colony, a frame will be removed and all larvae/pupae from that frame will be visually inspected for *Tropilaelaps* by an entomologist at a government approved laboratory. This period provides sufficient opportunity for the imported queen honey bee to lay eggs, larvae to develop and cells to be capped.

However, due to the potential of missing the presence of *Tropilaelaps*, diagnostic testing alone was not considered sufficient to reduce the likelihood of release.

Preventative treatment

- given *Tropilaelaps*' inability to survive for long periods away from brood, husbandry techniques that create breaks in the brood, such as caging the queen honey bee, use of artificial swarms and comb trapping, can be used to reduce *Tropilaelaps* numbers (DEFRA 2005)
- many of the same chemicals used for *Varroa* control, such as fluvalinate or formic acid, will kill *Tropilaelaps* (OIE 2008c).

Conclusion: based on this information, DAFF considered that post-arrival preventative treatment was a risk management option for *Tropilaelaps* infestation. Following the acceptance of the imported queen honey bee into the nucleus colony, all honey bees in the quarantine flight room should be treated. The product should be used according to the manufacturer's directions, and must have demonstrated efficacy for the control of *Tropilaelaps*. The honey bees should be continuously exposed until grafting is allowed to commence. If possible, a different chemical agent should be used to that applied in the country of origin before export.

Due to preventative treatment potentially not completely eradicating *Tropilaelaps*, preventative treatment alone was not considered sufficient to reduce the likelihood of release.

Overall conclusion

The unrestricted risk associated with *Tropilaelaps* infestation was estimated to be **low**.

Other than country or zone/region freedom, no single risk management option reduced the unrestricted risk sufficiently to achieve Australia's ALOP. However, the combination of premises status, pre-export and post-arrival diagnostic testing and preventative treatment, and PAQ would reduce the likelihood of release for *Tropilaelaps* infestation from 'low' to 'very low'.

This would reduce the likelihood of release and exposure to 'very low' and the restricted risk to at least 'very low', thereby achieving Australia's ALOP.

Biosecurity risk management measures for *Tropilaelaps*

To achieve Australia's ALOP with respect to the risk of *Tropilaelaps* infestation in imported honey bees, the following biosecurity measures are to be applied.

The queen honey bees and their escorts have been sourced from an apiary in a country or zone that meets the OIE Code definition of a country or zone/region free from *Tropilaelaps* infestation.

OR

i. The queen honey bees and their escorts have been sourced from a hive or colony free from visible evidence of *Tropilaelaps* infestation at the time of sourcing.

AND

ii. The queen honey bees and their escorts for export have been visually inspected for the presence of *Tropilaelaps* before export and no evidence of *Tropilaelaps* were observed.

AND

iii. The hive or nucleus colony in which queen honey bees and their escorts for export have been housed have been continuously exposed to treatment immediately prior to export, for a duration in accordance with the manufacturer's directions, with a product of demonstrated efficacy for the control of *Tropilaelaps*.

AND

iv. The queen honey bees and their escorts are to be transported in sealed packaging or boxing, and the air inlets covered with high security '100' mesh at a minimum.

AND

v. On arrival in Australia, the imported queen honey bees and their escorts must proceed to a government approved quarantine facility. The imported queen honey bee must be removed from the imported escort honey bees and will undergo an isolation period for a minimum of 14 days with Australian-sourced escort honey bees. Following this period, the Australian-sourced escort honey bees must be destroyed and examined for *Tropilaelaps* by an entomologist at a government approved laboratory.

AND

vi. On arrival at the PAQ facility, imported escort honey bees must be destroyed and examined for *Tropilaelaps* by an entomologist at a government approved laboratory.

AND

vii. Following the acceptance of the imported queen honey bee into the nucleus colony, all honey bees in the quarantine flight room should be treated. The product should be used according to the manufacturer's directions, and must have demonstrated efficacy for the control of *Tropilaelaps*. The honey bees should be continuously exposed until grafting is allowed to commence.

AND

viii. At least 14 days following the acceptance of the imported queen honey bee into the nucleus colony, a frame must be removed and all larvae/pupae from that frame visually inspected by an entomologist at a government approved laboratory for *Tropilaelaps*.

Varroosis

The unrestricted risk for varroosis associated with the importation of queen honey bees was estimated to be high. Risk management options that could be applied to achieve Australia's ALOP were considered.

The likelihood of release of varroosis was estimated to be **high** and the likelihood of exposure of varroosis was estimated to be **high**. The following options were considered as risk management measures to reduce the likelihood of release and/or exposure for varroosis.

Pre-entry measures

Country or zone freedom

- varroosis occurs worldwide, however Australia remains free (Ellis 2005)
- *Varroa* can survive no more than five days without honey bees or brood but can live in a comb with sealed brood for up to 30 days (Frazier 2005)
- the lifespan of *Varroa* on larval or adult honey bees varies from a few days to a few months, depending on temperature and humidity (OIE 2011e).

Conclusion: based on this information, DAFF considered that country or zone freedom was considered a risk management option for varroosis. Country or zone freedom alone, using the OIE Code recommendation of a country or zone free from varroosis, would be sufficient to achieve Australia's ALOP.

Premises status

- *Varroa* is a parasite of adult honey bees and their brood (OIE 2008d)
- the lifespan of *Varroa* on larval or adult honey bees varies from a few days to a few months, depending on temperature and humidity (OIE 2011e)
- there is little sign of damage initially and the infestation often goes unnoticed while the number of *Varroa* in a colony is low (De Jong 1997).

Conclusion: based on this information, DAFF considered that premises status was a risk management option for varroosis. A requirement for the queen honey bees and their escorts

to have been sourced from an apiary free from visible evidence of *Varroa* at the time of sourcing was considered appropriate. However, due to the difficulty in detecting the presence of *Varroa* when the number in a colony is low, premises status alone was not considered sufficient to reduce the likelihood of release.

Diagnostic testing

- adult female *Varroa* are oval, flat, pale to reddish-brown—1.1 mm long and 1.5 mm wide—and can be seen with the naked eye (Shimanuki 2000)
- the OIE Manual (OIE 2008d) describes methods of diagnosis of *Varroa* infestation by examination of adult honey bees, brood or hive debris.

Conclusion: based on this information, DAFF considered that pre-export diagnostic testing was a risk management option for varroosis. Queen honey bees and their escorts for export should be visually inspected for the presence of *Varroa* before export.

However, due to the difficulty in detecting the presence of *Varroa*, visual inspection alone was not considered sufficient to reduce the likelihood of release.

Preventative treatment

- both chemical and non-chemical treatments have been used to control *Varroa*
- hive management techniques such as drone brood removal, comb trapping, artificial swarm and the use of open mesh floors can be used to reduce *Varroa* numbers. Generally, if there is a heavy *Varroa* infestation, these methods provide insufficient control and need to be used in conjunction with acaricides (The Food and Environment Research Agency 2009)
- acaricides are applied in various ways: in feed, directly on the adult honey bees, as fumigants, contact strips or by evaporation (The Food and Environment Research Agency 2009). Synthetic pyrethroids have been used successfully but *Varroa* have developed resistance in some countries (Milani 1999). Non-pyrethroid chemicals such as amitraz, coumaphos, thymol and acids such as formic, lactic and oxalic acids have also been used with varying success (The Food and Environment Research Agency 2009).

Conclusion: based on this information, DAFF considered that pre-export preventative treatment was a risk management option for varroosis.

A requirement for the hive or nucleus colony in which queen honey bees and their escorts for export have been housed to have been continuously exposed to treatment immediately prior to export for the control of *Varroa* was considered appropriate. The product should be used according to the manufacturer's directions, and must have demonstrated efficacy for the control of *Varroa*. However, due to preventative treatment potentially not completely eradicating *Varroa* and the potential for reinfestation, preventative treatment alone was not considered sufficient to reduce the likelihood of release.

Transportation measures

Adult female *Varroa* are 1.1 mm long and 1.5 mm wide (Shimanuki 2000). Normal high security '100' mesh, which has an aperture size of approximately 150 µm, is suitable to contain adult *Varroa* (the only developmental stage present on adult honey bees).

Post-arrival measures

Post-arrival quarantine

- *Varroa* is a parasite of adult honey bees and their brood (OIE 2008d)
- *Varroa* is easily spread by direct contact with adult honey bees (The Food and Environment Research Agency 2009; OIE 2011e)
- the lifespan of *Varroa* on larval or adult honey bees varies from a few days to a few months, depending on temperature and humidity (OIE 2011e)
- *Varroa* can survive no more than five days without honey bees or brood but can live in a comb with sealed brood for up to 30 days (Frazier 2005)
- there is little sign of damage initially and the infestation often goes unnoticed while the number of *Varroa* in a colony is low; during this time *Varroa* can spread to other colonies (De Jong 1997)
- the entire lifecycle of *Varroa* occurs in the beehive.

Conclusion: based on this information, DAFF considered that PAQ was a risk management option for varroosis. However, due to the ability of *Varroa* to survive on larval or adult honey bees, PAQ alone was not considered sufficient to reduce the likelihood of release.

Diagnostic testing

- adult female *Varroa* are oval, flat, pale to reddish-brown and large—1.1 mm long and 1.5 mm wide—and can be seen with the naked eye (Shimanuki 2000)
- the OIE Manual (OIE 2008d) describes methods of diagnosis of *Varroa* infestation by examination of adult honey bees, brood or hive debris
- the mature mated female *Varroa* enters a brood cell just before the cell is capped (Oldroyd 1999). Examination of brood is done by uncapping brood and observing the dark mites against the white bodies of the brood (Shimanuki 2000)
- in their assessment of diagnostic methods for low levels of *Varroa* infestation, Fries et al. (1991) concluded that when sealed brood was present, examination of hive debris was more effective than sampling of brood, and brood sampling was more effective than sampling of live honey bees. In colonies without sealed brood, examination of hive debris and live honey bee samples were approximately equally efficient. The earliest and most precise diagnosis requires the application of medication that kills *Varroa* directly or forces them to drop off the honey bees (OIE 2008d).

Conclusion: based on this information, DAFF considered that post-arrival diagnostic testing was a risk management option for varroosis. On arrival at the PAQ facility, the consignment will be visually inspected for the presence of *Varroa*. Imported escort honey bees will be destroyed and examined for *Varroa* by an entomologist at a government approved laboratory.

The imported queen honey bee will undergo an isolation period of a minimum of 14 days with Australian-sourced escort honey bees, the Australian-sourced escort honey bees, which will then be destroyed and examined for *Varroa* by an entomologist at a government approved laboratory.

At least 14 days following the acceptance of the imported queen honey bee into the nucleus colony, a frame will be removed and all larvae/pupae from that frame will be visually

inspected by an entomologist at a government approved laboratory for *Varroa*. This period provides sufficient opportunity for the imported queen honey bee to lay eggs, larvae to develop and cells to be capped.

For the duration of treatment, hive debris will be examined for the presence of *Varroa* on a weekly basis by an entomologist at a government approved laboratory.

However, due to the potential of missing the presence of *Varroa*, diagnostic testing alone was not considered sufficient to reduce the likelihood of release.

Preventative treatment

- both chemical and non-chemical treatments have been used to control *Varroa*
- hive management techniques such as drone brood removal, comb trapping, artificial swarm and the use of open mesh floors can be used to reduce *Varroa* numbers. Generally, if there is a heavy *Varroa* infestation, these methods provide insufficient control and need to be used in conjunction with acaricides (The Food and Environment Research Agency 2009)
- acaricides are applied in various ways: in feed, directly on the adult honey bees, as fumigants, contact strips or by evaporation (The Food and Environment Research Agency 2009). Synthetic pyrethroids have been used successfully but *Varroa* have developed resistance in some countries (Milani 1999). Non-pyrethroid chemicals such as amitraz, coumaphos, thymol and acids such as formic, lactic and oxalic acids have also been used with varying success (The Food and Environment Research Agency 2009).

Conclusion: based on this information, DAFF considered that post-arrival preventative treatment was a risk management option for *Varroa* infestation. Following the acceptance of the imported queen honey bee into the nucleus colony, all honey bees in the quarantine flight room should be treated. The product should be used according to the manufacturer's directions, and must have demonstrated efficacy for the control of *Varroa*. The honey bees should be continuously exposed until grafting is allowed to commence. If possible, a different chemical agent should be used to that applied in the country of origin before export.

However, due to preventative treatment potentially not completely eradicating *Varroa*, preventative treatment alone was not considered sufficient to reduce the likelihood of release.

Overall conclusion

The unrestricted risk associated with varroosis was estimated to be **high**.

Other than country or zone/region freedom, no single risk management option reduced the unrestricted risk sufficiently to achieve Australia's ALOP. However, the combination of premises status, pre-export and post-arrival diagnostic testing and preventative treatment, and PAQ would reduce the likelihood of release for varroosis from 'high' to 'extremely low'.

This would reduce the likelihood of release and exposure to 'extremely low' and the restricted risk to at least very low, thereby achieving Australia's ALOP.

Biosecurity risk management measures for varroosis

To achieve Australia's ALOP with respect to the risk of varroosis in imported honey bees, the following biosecurity measures are to be applied.

The queen honey bees and their escorts have been sourced from an apiary in a country or zone/region that meets the OIE Code definition of a country or zone free from varroosis.

OR

i. The queen honey bees and their escorts have been sourced from an hive or colony free from visible evidence of varroosis at the time of sourcing.

AND

ii. The queen honey bees and their escorts for export have been visually inspected for the presence of *Varroa* before export and no evidence of *Varroa* was observed.

AND

iii. The hive or nucleus colony in which queen honey bees and their escorts for export have been housed have been continuously exposed to treatment immediately prior to export, for a duration in accordance with the manufacturer's directions, with a product of demonstrated efficacy for the control of *Varroa*.

AND

iv. The queen honey bees and their escorts are to be transported in sealed packaging or boxing, and the air inlets covered with high security '100' mesh at a minimum.

AND

v. On arrival in Australia, the imported queen honey bees and their escorts must proceed to the PAQ facility. The imported queen honey bee must be removed from the imported escort honey bees and undergo an isolation period for a minimum of 14 days with Australian-sourced escort honey bees. Following this period, the Australian-sourced escort honey bees must be destroyed and examined for *Varroa* by an entomologist at a government approved laboratory.

AND

vi. On arrival at the PAQ facility, imported escort honey bees must be destroyed and examined for *Varroa* by an entomologist at a government approved laboratory.

AND

vii. Following the acceptance of the imported queen honey bee into the nucleus colony, all honey bees in the quarantine flight room should be treated. The product should be used according to the manufacturer's directions, and must have demonstrated efficacy for the control of *Varroa*. The honey bees should be continuously exposed until grafting is allowed to commence. For the duration of treatment, hive debris must be examined for *Varroa* on a weekly basis by an entomologist at a government approved laboratory.

AND

viii. At least 14 days following the acceptance of the imported queen honey bee into the nucleus colony, a frame must be removed and all larvae/pupae from that frame visually inspected by an entomologist at a government approved laboratory for *Varroa*.

Africanised honey bee (*Apis mellifera scutellata* and its hybrids)

The unrestricted risk for Africanised honey bees associated with the importation of queen honey bees was estimated to be **moderate**. Risk management options that could be applied to achieve Australia's ALOP were considered.

The likelihood of release of Africanised honey bees was estimated to be **moderate** and the likelihood of exposure of Africanised honey bees was estimated to be **moderate**. The following options were considered as risk management measures to reduce the likelihood of release and/or exposure for Africanised honey bees.

Pre-entry measures

Country or area freedom

- *A. m. scutellata* and its hybrids (hereafter called 'Scutellata') are now present throughout sub-Saharan Africa and Central America. In South America, Scutellata extends as far south as 34° South, where it forms a stable hybrid zone with bees of the C and M lineages (Sheppard 1991). In the USA, Scutellata has been identified in the states of Alabama, Arizona, Arkansas, California, Florida, Georgia, Louisiana, Nevada, New Mexico, New York, Oklahoma, South Carolina, Texas, Utah and Virginia (Louisiana Department of Agriculture and Forestry 2009; Department of Agriculture 2010; Utah Department of Agriculture and Food 2010; CAPS 2011)
- Scutellata are not sufficiently different from honey bees of *A*. *m*. *ligustica* origin (the majority of the commercial subspecies in Australia) to be reliably distinguishable by eye. Large colonies are extremely aggressive but this too is not reliable.

Conclusion: based on this information, DAFF considered that country or zone or compartment freedom was considered a risk management option for Africanised honey bees.

Consideration of country or zone or compartment freedom from Scutellata may be given if declaring freedom in accordance with Article 1.4.6 of the OIE Code (OIE 2011a). Supporting documentation, including evidence of adequate surveillance to demonstrate freedom, must

be provided. Factors to be considered may include but are not limited to the presence of an early detection system for Scutellata and measures to prevent introduction.

The Africanised honey bee status within a country can vary depending on factors such as prevailing weather and internal migratory beekeeping practices. The status can change and go without detection for an extended period of time. Due to the difficulty in detecting the presence of Scutellata, country or area freedom of approved countries alone was not considered sufficient to reduce the likelihood of release.

Premises status

- Scutellata are small yellow honey bees and are not sufficiently different from honey bees of *A*. *m*. *ligustica* origin (the majority of the commercial subspecies in Australia) to be reliably distinguishable by eye. Large colonies are extremely aggressive but this too is not reliable.

Conclusion: based on this information, DAFF considered that premises status was a risk management option for Africanised honey bees. Although Scutellata is not sufficiently different from other subspecies of *A*. *mellifera* to be reliably distinguishable by eye, a requirement for the queen honey bees to have been sourced from an apiary free from visible evidence, including behavioural, of Scutellata infestation at the time of sourcing was considered appropriate.

However, due to the difficulty in detecting the presence of Scutellata, premises status alone was not considered sufficient to reduce the likelihood of release.

Diagnostic testing

- the testing of mitochondria will give information on the maternal lineage of the tested honey bee. As it cannot give information on the paternal line, this method cannot give a definite diagnosis of freedom from Africanisation. Evidence of A lineage in the mitochondrial genome is sufficient for a positive diagnosis
- the use of single nucleotide polymorphisms is not recommended for diagnosis of honey bees of African descent
- there are no known DNA microsatellites that show fixed difference between honey bees of African origin and honey bees of C and M lineage (Clarke 2002). Therefore DNA microsatellites cannot be used for definitive diagnosis
- morphometrics (Daly 1978) can provide a definitive diagnosis of Africanisation. It can detect hybrids as well as purebred individuals but the sensitivity and accuracy of the method declines as the level of Africanisation decreases (Guzmán-Novoa 1994). For adult worker honey bees, the average of 10 forewing lengths is measured and the probability that the sample is Scutellata and the probability that the sample is European can be calculated (Rinderer 1986).

Conclusion: based on this information, DAFF considered that diagnostic testing was considered a risk management option for Africanised honey bees. A test of the mitochondrial lineage should be performed on a random sample of worker honey bees representing the progeny of queen honey bees for export.

However, due to the difficulty in detecting the presence of characteristics of Scutellata, diagnostic testing alone was not considered sufficient to reduce the likelihood of release.

Preventative treatment

- No preventative treatments exist for Africanised honey bees.

Conclusion: based on this information, DAFF considered that preventative treatment was not considered a risk management option for Africanised honey bees.

Transportation

Packaging sufficient to confine the imported queen honey bees and their escorts is suitable.

Post-arrival measures

Post-arrival quarantine

- *A*. *m*. *scutellata* is a subspecies of *Apis mellifera.*

Conclusion: based on this information, DAFF considered that PAQ was not considered a risk management option for Africanised honey bees, other than as required for diagnostic testing and grafting procedures to occur.

Diagnostic testing

- Scutellata are not sufficiently different from honey bees of *A*. *m*. *ligustica* origin (the majority of the commercial subspecies in Australia) to be reliably distinguishable by eye. Large colonies are extremely aggressive but this too is not reliable
- the testing of honey bee mitochondria will give information on the maternal lineage of the tested honey bee. As it cannot give information on the paternal line, this method cannot give a definite diagnosis of freedom from Africanisation. Evidence of A lineage in the mitochondrial genome is sufficient for a positive diagnosis
- the use of single nucleotide polymorphisms is not recommended for diagnosis of bees of African descent
- there are no known DNA microsatellites that show fixed difference between honey bees of African origin and honey bees of C and M lineage (Clarke 2002). Therefore DNA microsatellites cannot be used for definitive diagnosis
- morphometrics (Daly 1978) can provide a definitive diagnosis of Africanisation. It can detect hybrids as well as purebred individuals but the sensitivity and accuracy of the method declines as the level of Africanisation decreases (Guzmán-Novoa 1994). For adult worker honey bees, the average of 10 forewing lengths is measured and the probability that the sample is Scutellata and the probability that the sample is European can be calculated (Rinderer 1986).

Conclusion: based on this information, DAFF considered that diagnostic testing was considered a risk management option for Africanised honey bees. For diagnostic testing to be useful, it needs to be accompanied by isolation to prevent potential spread of Scutellata from imported honey bees to the domestic honey bee population.

During a post-arrival isolation period, honey bees should be subject to diagnostic testing for identification and visually inspected for characteristics of Scutellata before being introduced into the domestic population.

Following the acceptance of the imported queen honey bee into the nucleus colony, when appropriately aged brood become available, the mitochondrial lineage of a representative sample of at least 10 pupae will be tested for Africanisation. In addition, when adult progeny

(not less than three days of age) become available, a representative sample of at least 10 adults will be examined for morphometric traits of Africanisation.

However, due to the difficulty in detecting the presence of characteristics of Africanisation, diagnostic testing alone was not considered sufficient to reduce the likelihood of release.

Preventative treatment

- No preventative treatments exist for Africanised honey bees.

Conclusion: based on this information, DAFF considered that preventative treatment was not considered a risk management option for Africanised honey bees.

Overall conclusion

The unrestricted risk associated with Africanised honey bees was estimated to be **moderate.**

In order to achieve Australia's ALOP of 'very low', the likelihood of release and exposure needs to be reduced to 'extremely low'. For this to occur, the likelihood of release for Scutellata needs to be reduced from 'moderate' to 'extremely low'.

It is not possible to absolutely detect Africanised honey bees with currently available diagnostic tests. Therefore, the reduction of the unrestricted risk to meet Australia's ALOP can only be achieved by a combination of available diagnostic testing post-arrival and either:

A. importing from countries that are free from Africanised honey bees and where there is negligible chance of contamination from neighbouring areas infested with Africanised honey bees or

B. importing from countries or zones that are either within or adjacent to Africanised honey bee infested areas but can demonstrate absence of Africanised honey bees.

Should validated diagnostic testing methods for the presence of Africanised genes become available in the future, the risk management measures for Africanised honey bees will be reviewed.

Biosecurity risk management measures for Africanised honey bees (*Apis mellifera scutellata* and its hybrids)

To achieve Australia's ALOP with respect to the risk of Africanised honey bees in imported honey bees, either of the two following series of biosecurity measures (A **or** B) are to be applied.

A.

i. The queen honey bees have been sourced from an apiary in a country that is considered free from Africanised bees by Australia and has no land border with a country where Africanised bees are known to be present.

AND

ii. On arrival in Australia, the imported queen honey bees and their escorts must proceed to the PAQ facility. The imported queen honey bees and their escorts must be visually inspected for characteristics, including behavioural, of Africanisation. There must be no evidence of Africanisation.

AND

iii. Following the acceptance of the imported queen honey bees into the nucleus colony, when appropriately aged brood become available, the mitochondrial lineage of a representative sample of at least 10 pupae will be tested for Africanisation at a government approved laboratory. There must be no evidence of Africanisation observed.

OR

B.

The queen honey bees have been sourced from an apiary in a zone within a country where Africanised honey bees are known to be present but the zone is considered free from Africanised honey bees by Australia or in a country considered free from Africanised honey bees but the country shares at least one land border with a country where Africanised honey bees are known to be present.

AND

i. The queen honey bees have been sourced from an apiary free from visible evidence, including behavioural, of Africanisation at the time of sourcing.

AND

ii. The queen honey bees for export have been visually inspected for characteristics, including behavioural, of Africanisation before export. There must be no evidence of characteristics of Africanisation.

AND

iii. Testing of mitochondrial lineage has been performed on a randomly selected sample of at least 10 worker honey bees representing the progeny of each of the queen honey bees for export. There must be no evidence of Africanisation.

AND

iv. On arrival in Australia, the imported queen honey bees and their escorts must proceed to the PAQ facility. The imported queen honey bees and their escorts must be visually inspected for characteristics, including behavioural, of Africanisation. There must be no evidence of Africanisation.

AND

v. Following the acceptance of the imported queen honey bee into the nucleus colony, when appropriately aged brood become available, the mitochondrial lineage of a representative sample of at least 10 pupae will be tested for Africanisation at a government approved laboratory. There must be no evidence of Africanisation.

AND

vi. A representative sample of at least 10 adult (not less than three days of age) progeny of the imported queen honey bees will be examined for morphometric traits of Africanisation by an entomologist at a government approved laboratory. There must be no evidence of Africanisation.

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6 Proposed biosecurity measures

Requirements for the importation of queen honey bees

1. Eligibility

a) Importation under these conditions is restricted to queen honey bees from approved countries only.
b) Importation of honey bees is restricted to queen honey bees with 6–12 escort worker honey bees.

2. Documentation

a) A written application to import honey bees must be lodged with DAFF prior to any import proceeding.
b) At least 48 hours prior to the expected arrival of the honey bees, DAFF must be provided with written advice of the flight number and expected arrival time.
c) Each consignment of queen honey bees must be accompanied by:
    i. a valid import permit
    ii. a declaration from the owner of the exporting apiary (requirements of this declaration are specified in Attachment 1)
    iii. an original international veterinary health certificate consistent with the OIE Code, signed by a Government Apiary Officer or an Official Veterinarian of the country of export (requirements of this certification are specified in Attachment 2).
d) Any inadequacies in certification may result in the consignment being returned to the country of origin at the importer's expense or the destruction of the queen honey bees and escorts without recompense.

3. Hazards

For the purposes of these requirements, the word 'hazard' refers to the pests and pathogens listed below:

- acarapisosis (tracheal mite)
- Africanised honey bees (*Apis mellifera scutellata* and its hybrids)
- *Braula* fly
- Cape honey bees (*A. m. capensis*)
- *Tropilaelaps* (*Tropilaelaps* spp.)
- varroosis (*Varroa destructor* and *V. jacobsoni*)

4. Transport

Queen honey bees can only be imported by air freight in packaging that prevents the escape of hazards of biosecurity concern and meets the IATA requirements for the transport of such organisms. At a minimum, the honey bees are to be transported in

queen cages contained in sealed packaging or boxing where air inlets are covered with '100' mesh[8] or equivalent.

5. Post-arrival quarantine

a) On arrival in Australia, imported honey bees (queen/s and escorts) will immediately be taken to the PAQ facility where the PAQ procedures at Attachment 3 will be followed.
b) If at importation or any stage of PAQ imported honey bees are found not to meet Australia's biosecurity requirements, the honey bees may be returned to the country of origin at the importer's expense or be destroyed without recompense. Any progeny from the imported honey bees, and other colonies and equipment that DAFF considers to have come into contact with affected honey bees may be destroyed without recompense.
c) No liability will be accepted by the Director of Animal and Plant Quarantine for deaths of imported honey bees subject to quarantine or failure of any larval graft.
d) Removal from PAQ will be restricted only to grafted queen cells produced by the nucleus colony. At the end of the period of time that the imported queen honey bee is to be maintained in quarantine, the imported queen, adult honey bees, brood and all other components comprising the nucleus colony will be destroyed.

6. Importer's/agent's responsibilities

a) The importer is required to enter into a written agreement with DAFF for use of the quarantine facilities and is responsible for all prescribed fees associated with importation of queen honey bees, including for the duration of PAQ.
b) During the PAQ period the importer is responsible for the provision of beekeeping equipment (frames, boxes etc), local honey bees as escorts for imported queen honey bees and for establishment of nucleus colonies, and other resources as required.

7. Review

The requirements of importation may be reviewed if there are any changes in the animal health situation and/or the import policy of the exporting country or at any time at the discretion of the Director of Animal and Plant Quarantine.

[8] '100' mesh has 100 openings per linear inch. This gives an aperture size of approximately 150 µm.

Attachment 1

Declaration by the owner of the hives or colonies from which the honey bees are destined to be exported to Australia

- This declaration must accompany the consignment and be signed and dated by the owner and witnessed by the Government Apiary Officer or Official Veterinarian.
- It must be headed by a statement detailing the name of the owner of the source apiary from which the queen honey bees and escort honey bees to be exported to Australia are to be sourced.
- It must contain details of the consignment—number of queen honey bees consigned and the breed/race of the honey bees.
- It must contain a declaration to assure the Government Apiary Officer or Official Veterinarian of the country of export that:

1. The owner has knowledge of the Australian import requirements.

2. In preparing all queen honey bees and escorts for export to Australia, the owner has complied with the following requirements:
    a) The queen honey bee and escorts in the export consignment are from colonies with no visible signs of disease
    b) The queen honey bee and escorts in the export consignment are from colonies with no visible evidence of the hazards listed in clause 3 of the Australian import requirements.
    c) The queen honey bee and escorts in the export consignment are themselves free of physical or behavioural abnormalities and show no visible evidence of the hazards listed in clause 3.
    d) Before export the colonies from which the honey bees for export to Australia were sourced were continuously treated, according to the manufacturer's directions, with a product of demonstrated efficacy for the control of *Varroa* and *Tropilaelaps*. Details of this product must be provided, including:
        i. the product name and manufacturer's details
        ii. dates of application
        iii. the number of brood frames per miticidal strip
    e) In countries that are not free of tracheal mites (*Acarapis woodi*) the colonies from which the honey bees for export to Australia were sourced were treated according to the manufacturer's directions with a commercially available formic acid product within seven days of export for a minimum period of 24 hours. Details of this product are to be provided, including:
        i. the product name and manufacturer's details
        ii. dates of application
    f) The escort honey bees accompanying each queen honey bee originated from the same hive/colony as the queen honey bee in each instance.

Attachment 2

Health Certificate for honey bees exported to Australia

- This certificate must accompany the consignment of honey bees.
- The Government Apiary Officer or Official Veterinarian must sign, date and stamp with the official government stamp each page of the certificate.
- The certificate must include a statement showing the exporting country and the name of the Government Apiary Officer or Official Veterinarian and contain a declaration as to the following with respect to the source of honey bees for export to Australia:

1. After due enquiry, the signee has no reason to doubt the Owner's Declaration providing assurances that the owner has knowledge of the Australian import requirements and has complied with the requirements in the Owner's Declaration.

2. The colony has been examined by a Government Apiary Officer or Official Veterinarian within seven days of export and found to be clinically healthy and free from visible evidence of the hazards listed at clause 3.

3. *The country or region of export is free from tracheal mite (*Acarapis woodi*)

OR

*It has been declared to the signee, and they have no reason to doubt, that the colony was treated according to the manufacturer's directions with a commercially available formic acid product within seven days of export for a minimum period of 24 hours.

**One of these two statements to be included*

4. It has been declared to the signee, and they have no reason to doubt, that the colony was continuously exposed to treatment according to the manufacturer's directions during the period prior to export, with a product of demonstrated efficacy for the control of *Varroa* and *Tropilaelaps*.

5. The country of export is free from Cape honey bees (*A. mellifera capensis*).

6. *The country of export is free from Africanised bees (*A. mellifera scutellata* and its hybrids) and the country does not share a land border with a country where Africanised honey bees are known to be present.

OR

*The honey bees for export have been sourced from an apiary in a zone considered free from Africanised honey bees within a country where Africanised honey bees are known to be present or from an apiary in a country free from Africanised honey bees but the country shares at least one land border with a country where Africanised honey bees are known to be present. AND

The apiary has been visually inspected for characteristics, including behavioural, of Africanisation before export and no evidence of Africanisation was observed. AND

Testing of mitochondrial lineage has been performed on a randomly selected sample of at least 10 worker honey bees representing the progeny of each of the queen honey bees for export and no evidence of Africanisation was present.

** One of these two statements to be included*

Attachment 3

PAQ procedures

1. All routine maintenance procedures are carried out by DAFF officers (or delegated persons) familiar with beekeeping management. For all honey bee management procedures, personnel (including the nominated grafter) must change into clean, protective clothing which remains in the PAQ facility.

2. On arrival at the PAQ facility, the documentation is checked to ensure that it corresponds to the honey bees imported and to ensure compliance with the import permit conditions.

3. If the documentation is in order, consignment cages are opened and all imported honey bees visually inspected for external parasites and characteristics of Africanisation.

If evidence of parasites and/or Africanisation is detected, the consignment is destroyed.
If not:

4. Imported escort honey bees remain in the original cage, are euthanised and examined externally and internally (by an entomologist) for the presence of parasitic mites (tracheal mite*, Tropilaelaps, Varroa*). After inspection, the imported cage and escort material are destroyed.

If evidence of parasites is detected, the consignment is destroyed.
If not:

5. The imported queen honey bee is placed in a new cage and enters an isolation period with Australian escorts within the PAQ facility for a minimum of 14 days. Australian escorts must not be older than four days of age and sourced from a colony provided by the importer or their representative and have been inspected by a DAFF officer or another person approved by DAFF for this purpose and found free of visible disease.

6. At completion of the isolation period the Australian escort honey bees are euthanised and examined externally and internally (by an entomologist) for the presence of parasitic mites (tracheal mite, *Tropilaelaps, Varroa*).

If evidence of parasites is detected, the consignment is destroyed.
If not:

7. The imported queen honey bee is introduced into a nucleus colony in a quarantine flight room of the PAQ facility.
    a) Local honey bees and equipment for nucleus colonies are provided by the importer or their representatives and sourced from a colony that has been

inspected by a DAFF officer or another person approved by DAFF for this purpose and is free of visible evidence of disease.

b) Each nucleus colony provided includes a frame of comb containing larvae prior to capping stage.
c) The preparation of the nucleus colony and the introduction of the imported queen honey bee are done by a DAFF officer or another person approved by DAFF for this purpose.

8. Following acceptance of the imported queen honey bee into the nucleus colony, all honey bees in the quarantine flight room are continuously exposed to treatment, according to the manufacturer's directions, with a product of demonstrated efficacy for the control of *Varroa* and *Tropilaelaps* until grafting is allowed to commence. This treatment will cease once the permission to commence grafting is given.

9. For the duration of treatment, hive debris is examined for the presence of *Varroa* on a weekly basis by an entomologist.

10. At least 14 days following the acceptance of the imported queen honey bee, a frame of the nucleus colony containing the brood is removed and all larvae/pupae from that frame is inspected by an entomologist at a government approved laboratory for the presence of *Varroa* and *Tropilaelaps*.

If evidence of parasitic mites is detected, the consignment is destroyed.
If not:

11. When pupae derived from the imported queen honey bee becomes available in the nucleus colony, a representative sample of at least ten pupae is tested at a government approved laboratory for the presence of mitochondrial DNA of Africanised honey bees.

If evidence of Africanisation is detected, the consignment is destroyed.
If not:

12. The following additional testing applies for imports from zones or countries that have been approved by DAFF as being free from Africanised honey bees but are within or adjacent to countries that contain Africanised honey bees:-
    a) A random sample of at least ten adult (at least three days of age) progeny of the imported queen honey bee is morphometrically examined for Africanisation by an entomologist at a government approved laboratory.

If evidence of Africanisation is detected, the consignment is destroyed.
If not:

13. Should all testing and inspection of imported honey bees and honey bees of the nucleus colony provide no evidence of hazards of biosecurity concern then DAFF allows grafting, by a nominated grafter approved by DAFF, to commence.

14. All grafting is under the supervision of a DAFF officer or another person authorised by DAFF for this purpose.

15. The importer must provide DAFF with written notice at least 48 hours before grafting is scheduled to take place.

16. A frame of suitable eggs/larvae is removed from the nucleus colony in the quarantine flight room by the DAFF officer or another person authorised by DAFF for this purpose and provided to the grafter in the grafting room.

17. Grafting must be into new, pre-polished plastic queen cells supplied by the importer.

18. Only grafted queen cells may be removed from the PAQ facility.

19. The importer must supply details (name and address of owner and initial location of apiaries) of where all honey bee material released from PAQ is sent in case follow-up is required.

20. The importer must notify DAFF in writing when all grafting is completed. When notification is received, the imported queen honey bees, all remaining adult honey bees, brood and other components comprising the nucleus colony are destroyed. Quarantine fees for an imported queen honey bee cease with the destruction of that queen honey bee and the associated colony.

7 Appendix A

7.1 Conditions for the importation of honey bees (1996)

AQIS AUSTRALIAN QUARANTINE AND INSPECTION SERVICE

DEPARTMENT OF PRIMARY INDUSTRIES AND ENERGY

T 96/1630

12 August 1996

CONDITIONS FOR THE IMPORTATION OF HONEY-BEES – suspended 25 August 2008 due to possible disease risks associated with colony collapse disorder.

(Country list amended 8 December 2000)

General Information

1. Under current legislation the import of the honey-bee (*Apis mellifera*) into Australia is permitted from:

   Austria, Canada, Canary Islands, Czech Republic, Slovakia, France, Germany, Italy, Israel, Denmark, New Zealand, Norfolk Island, Poland, the United Kingdom, the USA, the Newly Independent States (NIS) of the former Soviet Union, Croatia, Slovenia, Former Yugoslav Republic of Macedonia, Bosnia and Herzogovina, Federal Republic of Yugoslavia.

NOTE: The importation of bees from countries in which the Africanised strain (*Apis mellifera scutellata*) of honey-bee is known to occur is permitted under certain conditions [see Appendix 2, Item 6 (ii)] and is subject to approval of the Director of Quarantine.

2. (a) An application to import bees must be lodged with the Australian Quarantine and Inspection Service (AQIS) in New South Wales. The address for applications is:

Att: PRINCIPAL QUARANTINE OFFICER

Australian Quarantine and Inspection Service (NSW)

Quarantine Services Building

Locked Bag 6

MASCOT NSW 2020

(b) For bees from the countries listed above under Item 1, applications will be assessed by AQIS and may be approved by the Manager, Animal Programs Section, AQIS. Applications to import bees from countries other than those listed under Item 1 will be considered on a case by case basis.

(c) As time and space are limiting factors, approval to import will only be granted for a specified time period. Allocations will be made on a first come, first served basis. The facility at Eastern Creek Quarantine Station has 12 cages each capable of being occupied by 2 nucleus hives. It is therefore preferable that queens be imported in multiples of two for efficient utilisation of the space available.

3. (a) The Principal Quarantine Officer, AQIS (NSW) must receive at least 48 hours prior notice of the expected arrival of the bees and their mode of transport.

(b) Each consignment must be clearly addressed on the outside to:

AUSTRALIAN QUARANTINE AND INSPECTION SERVICE

"CAUTION: LIVE BEES"

PLEASE CONTACT PRINCIPAL QUARANTINE OFFICER

AQIS NSW Ph. 02 9364 7222

(c) In exceptional circumstances, application may be made to the Manager, Animal Programs Section, AQIS, to allow import consignments of bees to be transported to Australia as personal effects/items (ie other than by mail or air freight). Subject to approval being granted, import consignments transported as personal effects must be securely packaged in mite-proof material (in accordance with specifications available from the Principal Quarantine Officer) and are to be delivered to a Quarantine Officer at the port of entry.

(d) Each consignment is to be accompanied by the original of the "Permit to Import" and a completed certified Health Certificate from:

(i) Owner of the Apiary of Origin (Appendix 1); and

(ii) Government Apiary Officer or Veterinary Officer (Appendix 2); the latter to be officially stamped with the seal of the appropriate government authority.

(e) Any inadequacies in certification may result in the consignment being returned to the country of origin at the importer's expense or the destruction of the queen(s) and escorts.

4. On arrival in Australia the imported bees (queen/s and escorts) will be taken to the quarantine station at Eastern Creek by a Quarantine Officer where the post-arrival quarantine procedures in Appendix 3 will be followed.

5. Importers should be aware that if any disease detailed in Appendix 2 or undesirable germplasm is detected in the imported queens, escorts, or larvae, the Principal Quarantine Officer, AQIS (NSW) may order destruction of the affected bees, imported bees in the consignment from the same hive and (in the case of infectious disease or parasites) any other imported bees deemed to have been in-contact with the affected bees. Compensation will not be paid for bees destroyed.

6. Because of the many and varied circumstances associated with the success of:

. (i) establishing a queen in a nucleus hive

. (ii) grafting

no liability will be accepted by the Director of Quarantine, AQIS, for deaths of imported queens in quarantine or for failure of any larval graft.

7. Importers will be responsible for all prescribed fees associated with the importation of the queen bee as well as the maintenance fee for the queen whilst in quarantine.

8. At the end of the period of time that an imported queen bee is to be maintained in quarantine, as formally agreed between the importer and the Principal Quarantine Officer, AQIS (NSW), the queen bee, adult bees, brood and all other components comprising the nucleus colony will be destroyed.

9. The importer will be required to enter into a written agreement with AQIS for use of the quarantine facilities and for payment of the quarantine fees.

DAVID WILSON

A/g Assistant Director

Animal Quarantine Policy Branch

APPENDIX 1

DECLARATION BY THE OWNER OF THE APIARY IN THE

COUNTRY OF ORIGIN

I, (name) ..... of

(locality, state/province) .....

hereby declare that:

1. the queen bees and escorts described in the schedule below originated from progeny that were bred and reared in:

   (country of origin) .....

   and are from an apiary located at:

   (address) .....

   .....

   of which I am the registered owner.

2. the queen bees and escorts are from a hive free from visible evidence of the following diseases affecting honey-bees:

   American foul brood (*Bacillus larvae*)

   European foul brood (*Melissococcus pluton*)

   External acariasis (*Acarapis externus, A. dorsalis, A. vagans*)

   Tracheal mite (*Acarapis woodi*)

   mite (*Varroa spp.*)

   Tropilaelaps mite (*Tropilaelaps spp.*)

   Bee Lice (*Braula spp.*)

   Half-moon disorder

3. the queen bees and escorts in the export consignment do not exhibit any physical or behavioural abnormalities and are free from any visible evidence of the parasitic diseases listed above under item 2.

4.(a)**** during the 56 days prior to export, the hive(s) or nucleus colony(ies) in which the bees (queen/s and escorts) for export have been housed has/have been continuously exposed to treatment with a product of proven efficacy for the control of parasitic bee mites as per the following details:-

Product Name: _____

Manufacturer : _____

Contact details of manufacturer: ph: _____

fax.: _____

(i) no.of brood frames per acaricidal strip: _____

(ii) no.of acaricidal strips applied: _____

(iii) date of application: _____

****delete that which does not apply**

APPENDIX 1

OR

(b)******the health certification accompanying this consignment confirms country freedom from the following parasitic mites of bees; *Varroa* mite (*Varroa spp.)*, *Tropilaelaps* mite *(Tropilaelaps spp*.), and Tracheal mite (*Acarapis woodi*) and therefore no pre-export acaricidal treatment has been applied.

5. the escort bees accompanying the queen bee are daughters of that queen bee in each instance and each queen is accompanied by not less than 6 escort bees.

NOTE: A minimum number of six (6) escorts is required for each queen consigned.

Schedule

Details of Consignment:

No.of queen bees consigned: .....

Breed and/or strain: .....

Person to Whom Bees Exported (Australian Importer):

Name: .....

Address .....

Declared at ..... on .....//.....

Signature: .....

Witnessed by:- **Government Apiary Officer or **Government Veterinary Officer

Name: .....

Signature: .....

Date: ...../...../.....

**** delete that which does not apply**

APPENDIX 2

OFFICIAL CERTIFICATION OF THE GOVERNMENT APIARY OFFICER OR GOVERNMENT VETERINARY OFFICER OF THE COUNTRY OF ORIGIN

I, (name) ..... of

(town and state/province) .....

am a full-time ******Government Apiary Officer / ******Government Veterinary Officer whose duties relate to apiculture and hereby certify in relation to the honey-bees (*Apis mellifera*) described in the owner's declaration, that:

1. after due enquiry I have no reason to doubt the Owner's Declaration (Appendix 1)

2. the queen bees and escorts originated from an apiary subject to government registration and periodic government inspection

3. ******(country of origin) .....

   is the country of origin of bees in the export consignment and is free of the following parasitic mites of honey bees:

   Varroa mite (*Varroa spp.*)

   Tropilaelaps mite (*Tropilaelaps spp.*)

   Tracheal mite (*Acarapis woodi*)

   (NOTE: Delete Item 3 if it does not apply)

4. within twenty-one (21) days of export, the hive(s) belonging to:-

   Owners Name: .....

   and located at:

   Address:.....

   Country of Origin: .....

from which bees for export to Australia will be sourced, was/were inspected by me and was/were found to be free of visible evidence of the following diseases (including parasitic infestation) affecting honey-bees:

American foul brood (*Bacillus larvae*)

European foul brood (*Melissococcus pluton*)

External acariasis (*Acarapis externus, A. dorsalis, A. vagans*)

Tracheal mite (*Acarapis woodi*)

Half moon disorder

Varroa mite (*Varroa spp.*)

Tropilaelaps mite (*Tropilaelaps spp.*)

Bee Lice (*Braula spp*.)

NOTE: Please stamp each page of this certificate with official government seal

****delete that which does not apply**

5.** I confirmed that the hive(s) or nucleus hive(s) in which the bees to be exported have been housed was/were undergoing treatment with a product of proven efficacy for the control of parasitic bee mites:

Product Name: .....

Manufacturer: .....

(NOTE: Delete Item 5 if country is free from Tracheal mite *Varroa* mite and *Tropilaelaps* mite)

6.(i)**the African strain of bee (*Apis mellifera scutellata*) is not known to occur in:

(country of origin) .....

and a visual inspection of random samples within the apiary of origin has not indicated the presence of behavioural or anatomical evidence of the African strain of honey bee or its hybrids

OR

(ii)** the African strain of bee (*Apis mellifera scutellata*) is known to occur in:

(country of origin) .....

but is not known to occur in:

(state/province/territory) .....

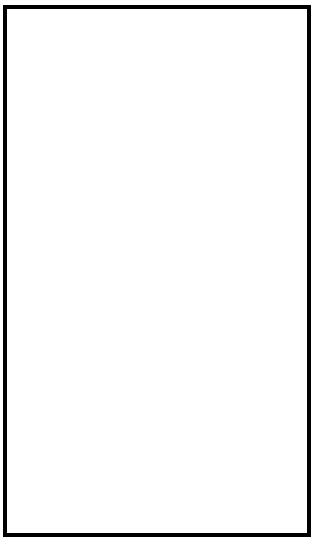
from which the bees are being exported.

A visual inspection of random samples within the apiary of origin has not indicated the presence of behavioural or anatomical evidence of the African strain of honey bee or its hybrids.

Dated at ..... on ...../...../.19.....

Signature: .....

Designation: ..... Official Seal



****delete that which does not apply**

APPENDIX 3

POST ARRIVAL QUARANTINE PROCEDURES

1. Entry of the bees into Australia cannot occur until an import permit has been issued by the Australian Quarantine and Inspection Service (AQIS).

2. The original permit is to accompany the bees together with the required health certification from the country of origin.

3. Queen bees with escorts can travel to Australia as follows:(i) by mail (ii) by air freight or (iii) in mite-proof material as "personal effects" (subject to prior approval being obtained from The Manager, Animal Programs Section, AQIS).

   The bees should be consigned as follows:

   AUSTRALIAN QUARANTINE AND INSPECTION SERVICE

   "CAUTION: LIVE BEES"

   PLEASE CONTACT PRINCIPAL QUARANTINE OFFICER

   AQIS NSW Ph. 02 9364 7222

4. The imported bees will be collected by a Quarantine Officer at the Sydney Mail Exchange or Sydney International Airport depending on the mode of importation. Apiarists returning with imported bees as "personal effects" are to deliver bees and accompanying documentation to a Quarantine Officer at the port of entry.

5. The Quarantine Officer will deliver the imported bees to the Eastern Creek Animal Quarantine Station bee facility. The consignment(s) and the attached certification will be checked to ensure compliance with the import conditions.

6. If the documentation is in order and corresponds to the bees imported, an Australian Quarantine Apiary Officer will open the consignment(s) and visually inspect the queen(s) and her escorts for external parasites. If no visible evidence of parasites is detected, the imported queen will be placed in a new cage and undergo an isolation period with Australian escorts (not older than 4 days) for not less than fourteen (14) days.

7. All imported escort bees will remain in the original cage, be killed and then examined internally and externally by an entomologist for the presence of:

   - Tracheal mite (*Acarapis woodi*)
   - *Varroa* mite (*Varroa spp*.)
   - *Tropilaelaps* mite (*Tropilaelaps spp.*)

   The imported cage and escort material will be destroyed by incineration.

8. At the end of the isolation period, the Australian escort bees will be killed and examined internally and externally by an entomologist for the presence of:

   - Tracheal mite (*Acarapis woodi*)
   - *Varroa* mite (*Varroa spp*.)
   - *Tropilaelaps* mite (*Tropilaelaps spp.*)

   The cage and escort material will be destroyed by incineration.

9. Provided that all tests have been completed to the satisfaction of the Principal Quarantine Officer, AQIS, (NSW), the imported queen will be introduced into a nucleus colony in a quarantine flight cage.

   Bees in the nucleus colony are to be sourced from a resident hive at the Eastern Creek quarantine facility which has been inspected by an Australian Quarantine Apiary Officer and is free of visible evidence of the diseases listed in Appendix 2 (for which quarantine inspection and/or testing is required).

10. The introduction and preparation of the nucleus colony will be done by an Australian Quarantine Apiary Officer. An acceptance period of up to ten (10) days will be allowed following the introduction of each imported queen into a nucleus colony.

11. Following acceptance of an imported queen by the nucleus bee colony, all bees in the quarantine flight cage are to be continuously exposed (in accordance with the product manufacturers directions) to acaricidal treatment with a product of proven efficacy for the control of parasitic bee mites for the remaining duration of the quarantine period. If possible, treatment is to be applied through exposure of a different chemical agent to that employed in the country of origin prior to export.

12. All routine maintenance procedures will be carried out by AQIS officers (or nominees) familiar with beekeeping management. For all bee management procedures, personnel will be supplied with clean, protective clothing which will remain on the quarantine station. Personnel will shower before entering and leaving the facility.

13. The nucleus bee colony will include a frame of comb (removed from a resident hive at the Eastern Creek quarantine facility) containing young larvae. At an interval of

not less than 10 days following introduction of the imported queen, that frame will be removed and all larvae/pupae (from that frame - ie 100% sampling) will be examined at a government approved laboratory for the presence of:

- *Varroa* mite (visual inspection)
- *Tropilaelaps* mite (visual inspection)

14. When appropriately aged brood of an imported queen first become available in the quarantine flight cage following introduction of the queen into a nucleus colony, a representative sample (as specified below) will be tested at a government approved laboratory for traits associated with the Africanised strain of honey-bee:

    . not less than ten (10) pupae

    • Africanised gene testing (DNA analysis)

15. For imports from those countries where the Africanised strain of honey-bee is known to occur, the following additional testing requirements will apply:

    when adult progeny (not less than 3 days of age) of an imported queen first become available in a quarantine flight cage a representative sample (as specified below) will undergo examination at a government approved laboratory for morphometric traits of the Africanised strain of honeybee:

    . not less than ten (10) adults

    • Africanised gene testing (morphometric analysis)

16. For all bee imports, the Principal Quarantine Officer, AQIS, (NSW) will allow grafting to commence only after all required testing and inspection of brood for undesirable genetic traits, infectious diseases and/or parasites has been completed with negative results.

17. If either a queen or her attendants or brood are found to be infected with organisms associated with the diseases listed in Appendix 2 of the import conditions, the Principal Quarantine Officer , AQIS, (NSW) may order destruction of the cage, affected bees and all other components comprising the colony to which affected bees belonged. Compensation will not be paid for bees destroyed.

18. If a queen bee or her progeny are found to exhibit traits associated with Africanised strain of honey-bee, the affected queen bee and her brood will be destroyed.

19. The nominated grafter, who must be approved by AQIS, will then be allowed to commence grafting.

20. The grafter must advise the Principal Quarantine Officer, AQIS (or nominee), in advance, the days on which grafting is to take place and the number of cells required for grafting.

21. The grafter will be supplied with clean, protective clothing which will remain on the station. He/she will only be allowed into the grafting room and will shower before entering and prior to leaving the facility.

22. A frame of larvae of a suitable age for grafting will be removed from the nucleus in the flight cage by an Australian Quarantine Apiary Officer and given to the grafter (in the grafting room) for grafting into new pre-polished plastic queen cells supplied by the Quarantine Station.

23. All grafting will be supervised by an Australian Quarantine Apiary Officer or a nominated Quarantine Station staff member.

24. Only grafted queen cells may be removed from the Quarantine Station.

    The importer, must inform the Principal Quarantine Officer, AQIS (NSW), in writing, of the name and address of the owner and the location of the apiaries in which the grafted queen cells are inserted in case follow-up action is required.

25. The importer is to notify the Principal Quarantine Officer, AQIS (NSW), in writing, when grafting has been completed. The imported queen(s) will be destroyed and examined for the parasitic diseases listed in Appendix 2.

    All remaining adult bees, brood and all other components comprising the nucleus colony will be destroyed by incineration.

    Quarantine fees for an imported queen bee will cease on the death of that bee.

26. Post-quarantine release of the grafted queen cells derived from any importation under these conditions is only permitted into Queensland, New South Wales, South Australia and Victoria.

APPENDIX 4

EXAMINATION OF BEES BY AUSTRALIAN GOVERNMENT

APIARY OFFICER OR VETERINARY OFFICER

1. Country of origin of consignment : .....

2. Apiary of origin: .....

   .....

3. Consigned to: .....

   .....

4. Import permit no.:- .....

5. Is the documentation accompanying the import consignment in order?

   ******YES/******NO If "NO" please detail deficiencies:-

   .....

   .....

5. Date consigned to Australia: ...../...../.....

6. Arrival date in Australia: ...../...../.....

7. Mode of Transport to Australia:******air freight /****** mail /****** personal effects

8. Number of Bees in Consignment:

	Live	Dead	Total
A.	Queens		
B.	Escorts		

9. An initial inspection of bees in the import consignment found no visible evidence of disease, parasitism, or genetically undesirable traits.

10. The required samples for laboratory testing were forwarded to the following government approved laboratory/(ies):-

    .....

    Completed testing results were received on ...../...../..... and:-

    ******(i) confirmed the absence of diseases, parasites and germplasm of quarantine concern

    OR

******(ii) identified diseases, parasites and/or germplasm of quarantine concern as outlined below:-

.....

Name: .....

Signature: .....

Designation: .....

Date: ...../...../.....

**** delete that which does not apply**