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The spread of pathogens through trade in honey bees and their products (including queen bees and semen): overview and recent developments

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Summary

International trade in bees and bee products is a complex issue, affected by their different origins and uses. The trade in bees, which poses the main risk for disease dissemination, is very active and not all transactions may be officially registered by the competent authorities. Globally, bee health continues to deteriorate as pathogens, pests, parasites and diseases are spread internationally through legitimate trade, smuggling and well-intentioned but ill-advised bee introductions by professionals. International trade rules strengthen the ability of many countries to protect bee health while trading but also carry obligations. Countries that are Members of the World Trade Organization (WTO) should only restrict imports to protect against identifiable health risks. If imports are safe, trade should be permitted. The trading rules of the WTO have given greater importance to the international standards applicable to bee health, developed by the World Organisation for Animal Health, which aims to prevent the spread of animal diseases while facilitating international trade in animals and animal products.

Keywords

Apis mellifera – Bee product – Bee – Disease – Honey bee – International trade – Parasite – Pathogen – Pest – Regulation – Risk assessment – SPS Agreement – World Organisation for Animal Health – World Trade Organization.

Introduction

Able to survive in a wide range of climates and environments, honey bees are among the most successful organisms on the planet (19) and now inhabit most areas of the world occupied by humans. Accompanying the spread of bees globally has been a host of bee pathogens, parasites, pests and viruses. Many of these organisms, such as the small hive beetle (*Aethina tumida*), *Nosema ceranae* and many bee viruses, are only just beginning to be understood, while others, such as *Varroa destructor* and *Paenibacillus larvae*, the causative agent of American foulbrood, despite being well known, remain at the forefront of bee research (21).

Griffiths and Bowman (43) described the world distribution of the mite *V. destructor* (at that time known as *V. jacobsoni*), while preliminary world maps of *V. jacobsoni* and *Tropilaelaps clareae* were drawn up by Nixon (63). In 1988, a comprehensive review of world bee health was published by Bradbear (8) and updates have since been published at intervals (58), including one devoted to viruses (2). The latest was published by Ellis and Munn in 2005 (21). These reviews have proven a valuable source of information on worldwide bee health (21).

However, a review is limited to the worldwide distribution of pests and diseases that have been confirmed as being present in a country by reliable published information. Such a review is helpful to the competent authorities, responsible for monitoring the spread of animal diseases and for regulating international trade in bees and bee products. International trade in these commodities has been mainly responsible for distributing most of the known pests and diseases of bees (26). The reliability of any review of the international status of bee health depends on adequate and universally accepted diagnoses (75). The World Organisation for Animal Health (OIE) website (76, 77) is a source of information on the global distribution of honey-bee diseases based on the reporting obligations of OIE Members.

International trade in bees and bee products has increased considerably over the past few decades and can be expected to continue to increase, as technology makes transport easier and lowers national barriers to trade (59). It can even be argued that allowing the legal importation of bees, under controlled conditions, might reduce the threat posed by illegal importations. Nevertheless, bee diseases continue to spread throughout the world (21). The more than 160 countries which are Members of the World Trade Organization (WTO) have agreed to trading rules that limit the extent to which international trade may be restricted to protect local producers. These rules are promulgated in the Agreement on the Application of Sanitary and Phytosanitary Measures (the 'SPS Agreement') (76, 78), designed to protect human, animal and plant health. Sanitary measures are any activity which restricts trade to protect animal health (bee health, in this case), including inspection, testing, certification, heat treatment and sourcing products from particular disease-free areas, right up to an outright ban on importation (10).

Under the SPS Agreement, the OIE is recognised as the relevant international organisation for developing standards, guidelines and recommendations on animal health. The OIE publishes the *Terrestrial Animal Health Code* (the *Terrestrial Code*) (76), which is used as a basis for drafting veterinary regulations governing the import and export of animals and animal products. The *Terrestrial Code* sets out definitions and basic principles of disease control measures. Using the *Terrestrial Code* to harmonise trade requirements facilitates trade by avoiding unjustified barriers. The following honey-bee diseases are covered by the *Terrestrial Code*:

– acarapisosis (*Acarapis woodi*)
– American foulbrood (*P. larvae*)
– European foulbrood (*Melissococcus plutonius*)
– small hive beetle infestation (*A. tumida*)
– *Tropilaelaps* infestation (*Tropilaelaps* spp.)
– varroosis (*Varroa* spp.).

A number of bee diseases, such as nosemosis caused by *Nosema apis* and *N. ceranae*, ascospherosis and virus diseases, are not currently covered by the *Terrestrial Code* because they do not meet the OIE's criteria for listing diseases, as detailed in Chapter 1.2.

The purpose of this review is to assess the bee health risks posed by the trade in honey bees (*Apis mellifera*) and honey-bee products (honey, royal jelly, pollen, propolis, semen, venom). A list of the main honey-bee diseases, products that could act as vectors, preventive measures, and international regulations is given in Table I.

Honey bees

Acarapisosis

Acarapisosis is a disease of the adult honey bee, *Apis mellifera* L., and other *Apis* species. It is caused by the Tarsonemid mite, known as the tracheal mite, *A. woodi* (Rennie) (75). The mortality rate may range from moderate to high. Early signs of infestation normally go unnoticed and only when the infestation becomes heavy, usually in the early spring, does the disease become apparent. In general, only newly hatched bees under ten days old are susceptible. The mite is an internal parasite of the respiratory system, living and reproducing mainly in the large prothoracic trachea of the bee and feeding on the haemolymph. The infestation spreads by direct contact. However, to move between colonies, the mites depend upon adult bees for transport through the natural processes of drifting, robbing and swarming. Migratory beekeeping and queen bee exchange are responsible for further dissemination of the mite.

American foulbrood

American foulbrood (AFB), a fatal brood infection caused by the Gram-positive, spore-forming bacterium, *P. larvae* (36, 72), is still among the most deleterious of globally distributed bee diseases. Larval remains affected by AFB appear as a brownish, semi-fluid, glue-like colloid ('ropy' stage), containing the vegetative stage of the bacterium, which sporulates and can then be distributed in the bee colony and swallowed by the next host. The ropy stage dries down to a hard scale (foulbrood scale), tightly adhering to the lower cell wall. These scales are highly infectious since they contain millions of spores which drive disease transmission within and between colonies (6, 56). The spores remain infectious for more than 35 years and withstand heat, cold, draught and humidity (46). Contaminated containers in which bees are transported can be responsible for the transmission of AFB (64). It is the tenaciousness of the spores and the production of extremely high numbers of spores in diseased colonies that make the effective control of AFB so difficult.

European foulbrood

The impact of European foulbrood (EFB) on the colony is less severe than that of AFB, at least partly because the causative agent, *M. plutonius*, a non-spore-forming,

Table I
Honey-bee diseases, their products, preventive measures and international regulations (76)

Disease	Honey-bee products	Preventive measures	Regulation
Acarapisosis	Honey bee (live queen honey bees, worker bees and drones)	Come from a country free from acarapisosis (certificate)	*Terrestrial Code*
	Eggs, larvae and pupae of honey bees	Come from a country free from acarapisosis (certificate), or were examined by an official laboratory and declared free of all life stages of *Acarapis woodi*; or have originated from queens in a quarantine station and were examined microscopically and found free of all life stages of *A. woodi*	
	Semen, venom, used equipment associated with beekeeping, honey, beeswax, honey-bee-collected pollen, propolis, royal jelly	No restrictions	
American foulbrood	Honey bee (live queen honey bees, worker bees and drones)	Come from a country free from American foulbrood (certificate)	*Terrestrial Code*
	Eggs, larvae and pupae of honey bees	Come from a disease-free country or zone/ compartment; or have been isolated from queens in a quarantine station, and all workers which accompanied the queen or a representative sample of eggs or larvae were examined for the presence of *Paenibacillus larvae* by bacterial culture or PCR in accordance with the *Terrestrial Manual* (75)	
	Used equipment associated with beekeeping	Sterilisation by either immersion in 1% sodium hypochlorite for at least 30 minutes (suitable only for non-porous materials such as plastic and metal), gamma irradiation using a cobalt-60 source at a dose rate of 10 kGy, or processing to ensure the destruction of both bacillary and spore forms of *P. larvae*	
	Honey, honey-bee-collected pollen, beeswax, propolis, royal jelly	Were collected in a country or zone/compartment free from American foulbrood; or have been processed to ensure the destruction of both bacillary and spore forms of *P. larvae*	
European foulbrood	Honey bee (live queen honey bees, worker bees and drones)	Come from a country free from European foulbrood (certificate)	*Terrestrial Code*
	Eggs, larvae and pupae of honey bees	Were sourced from a disease-free country or zone/ compartment; or have been isolated from queens in a quarantine station, and all workers which accompanied the queen or a representative sample of eggs or larvae were examined for the presence of *Melissococcus plutonius* by bacterial culture or PCR in accordance with the *Terrestrial Manual* (75)	
	Used equipment associated with beekeeping	Sterilisation by either immersion in 0.5% sodium hypochlorite for at least 20 minutes (suitable only for non-porous materials such as plastic and metal), gamma irradiation using a cobalt-60 source at a dose rate of 10 kGy, or processing to ensure the destruction of *M. plutonius*	
	Honey, honey-bee-collected pollen, beeswax, propolis, royal jelly	Were collected in a country or zone/compartment free from European foulbrood; or have been processed to ensure the destruction of *M. plutonius*	
Small hive beetle infestation	A single live queen honey bee or queen bumble bee, accompanied by a small number of associated attendants (a maximum of 20 attendants per queen)	Comes from a country or zone officially free from *Aethina tumida* infestation (certificate) or the 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 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 six months; and the 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 bees is covered with fine mesh through which a live beetle cannot enter	*Terrestrial Code*

Table I (cont.)
Honey-bee diseases, their products, preventive measures and international regulations (76)

Disease	Honey-bee products	Preventive measures	Regulation
Small hive beetle infestation (cont.)	Live worker bees, drone bees or bee colonies with or without associated brood combs or for live bumble bees	The bees come from a country or zone officially free from *A. tumida* infestation; and the bees and accompanying packaging presented for export have been inspected and do not contain *A. tumida* or its eggs, larvae or pupae; and the consignment of bees is covered with fine mesh through which a live beetle cannot enter	
	Eggs, larvae and pupae of honey bees or bumble bees	The products were sourced from a country or zone free from *A. tumida* infestation; or the products have been bred and kept under a controlled environment within a recognised establishment which is supervised and controlled by the Veterinary Authority; the establishment was inspected immediately prior to dispatch and all eggs, larvae and pupae show no clinical signs or suspicion of the presence of *A. tumida* or its eggs or larvae or pupae, and the packaging material, containers, accompanying products and food are new and all precautions have been taken to prevent contamination with *A. tumida* or its eggs, larvae or pupae	
	Used equipment associated with beekeeping	Either comes from a country or zone free from *A. tumida* infestation; and contains no live honey bees or bee brood; or contains no live honey bees or bee brood; and has been thoroughly cleaned, and treated to ensure the destruction of *A. tumida*; and all precautions have been taken to prevent infestation/contamination	
	Honey-bee-collected pollen, beeswax (in the form of honeycomb)	Either comes from a country free from *A. tumida* infestation; and contains no live honey bees or bee brood; or contains no live honey bees or bee brood; and has been thoroughly cleaned, and treated to ensure the destruction of *A. tumida*; and all precautions have been taken to prevent infestation/contamination	
	Comb honey	Comes from a country free from *A. tumida* infestation (certificate); and contains no live honey bees or bee brood; or was subjected to a treatment at a temperature of –12°C or lower in the core of the product during at least 24 h	
	Honey-bee semen, honey-bee venom, packaged extracted honey, refined or rendered beeswax, propolis, frozen or dried royal jelly	No restrictions	
Tropilaelaps spp. infestation	Honey bee (live queen honey bees, worker bees and drones with associated brood combs)	Come from a country free from *Tropilaelaps* infestation (certificate)	*Terrestrial Code*
	Honey bee (queen honey bees, worker bees and drones without associated brood combs)	Held in isolation from brood and bees with access to brood for a period of at least seven days	
	Used equipment associated with beekeeping, honey-bee-collected pollen, beeswax (in the form of honeycomb), comb honey, propolis	Comes from a country free from *Tropilaelaps* infestation; or contains no live honey bees or bee brood and has been held away from contact with live honey bees for at least seven days prior to shipment; or has been treated to ensure the destruction of *Tropilaelaps* spp.	
	Honey-bee semen, honey-bee eggs, honey-bee venom, extracted honey, beeswax (not in the form of honeycomb)	No restrictions	
Varroosis	Honey bee (live queen honey bees, worker bees and drones with or without associated brood)	Come from a country free from varroosis (certificate)	*Terrestrial Code*
	Larvae and pupae of honey bees	Come from a disease-free country or zone/compartment; or have originated from queens in a quarantine station and were inspected and found free of *Varroa destructor*	
	Used equipment associated with beekeeping, honey-bee-collected pollen, beeswax (in the form of honeycomb), comb honey, propolis	Comes from a disease-free country; or contains no live honey bees or bee brood and has been held away from contact with live honey bees for at least seven days prior to shipment; or has been treated to ensure the destruction of *V. destructor*	
	Honey-bee semen, honey-bee eggs, honey-bee venom, extracted honey, beeswax (not in the form of honeycomb)	No restrictions	
Nosemosis	Honey bee, queen bee, wax, pollen	No restrictions	None
Ascospherosis	Honey bee, pollen, semen, venom,	No restrictions	None
Viruses	Honey bee, queen bee, semen	No restrictions	None

kGy: kiloGray

PCR: polymerase chain reaction

Gram-positive bacterium, is less resistant, both within the hive and in the environment. The risk of disease transmission through honey-bee trade is rather limited.

Nosemosis

Two microsporidian species infect honey bees worldwide: *Nosema apis* Zander and *N. ceranae*. The term 'nosemosis' is considered to be the infection of ventricular cells of adult honey bees by *N. apis* Zander (75). This disease is characterised, in acute forms, by:

– trembling of honey-bee workers

– bees with a dilated abdomen

– brown faecal marks on combs and the front of hives

– sick or dead bees in the vicinity of the hives

– a decrease in brood production and in the size of the colony, particularly in spring.

However, the emergent disease caused by *N. ceranae*, now known to be present on all five continents (32, 48), presents a different epidemiological pattern, clinical signs and pathology. It has been present in Europe since at least 1998 (47, 65), and has long been established in the United States (13). Indeed, it is now considered a major bee health problem. Faecal marks in hive structures are usually reported in *N. apis*-infected colonies and it is commonly accepted that the infective spores of this microsporidium can be transmitted between bees by ingestion. However, these faecal marks have not been observed in colonies infected solely with *N. ceranae* (48), and so the mechanisms of transmission might be different. Contamination of material with infective spores cannot be ruled out and infected bees can contaminate beekeeping material when they are crushed during manipulation of the hives. The world trade in honey-bee products and beekeeping materials may also play an important role in the dispersal of infective spores of *N. ceranae* from apiary to apiary over different geographical areas (53). Although diagnostic techniques are described in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (75), nosemosis is not an OIE-listed disease as it does not fulfil the criteria for listing (76).

Small hive beetle (*Aethina tumida*)

The small hive beetle, *A. tumida* Murray 1867 (Coleoptera: Nitidulidae), is a parasite and scavenger of honey-bee colonies. Adults and larvae feed on the honey-bee brood, honey and pollen, causing the death of the brood, fermenting of honey and comb destruction, often resulting in the full structural collapse of the nest and absconding of the colony. The small hive beetle can be a serious problem in honey-extracting facilities, where stored comb, honey and wax cappings are all potential feeding and breeding areas. Development requires 3 to 12 weeks, depending on temperature and food availability. The beetle is able to survive at least two weeks without food and 50 days on brood combs. The flying adult beetles actively infest colonies. The small hive beetle can also parasitise bumble-bee (*Bombus terrestris*) colonies under experimental conditions and, although infestation has not been demonstrated in wild populations, *Bombus* spp. should also be considered to be susceptible to infestation (76). The small hive beetle is native to sub-Saharan Africa but has been introduced into the United States (1996), Egypt (2000) and Australia (2002) (62). It was introduced to Canada in 2002 and re-introduced in 2006, and into Mexico in 2007 (61). *Aethina tumida* can be spread by active flying from up to 6 km to 13 km from the nest site, by migratory beekeepers or by transportation of infested hive products (51, 62). Dispersal includes small hive beetles following or accompanying swarms. Spread of infestation does not require contact between adult bees. However, the movement of adult bees, honeycomb and other apiculture products, as well as used equipment associated with beekeeping, may all cause infestations to spread to previously unaffected colonies.

The larvae and eggs of *A. tumida* have been identified in cages of queens imported from the USA to Portugal (60), but all hives were immediately destroyed. Within its native range, it is usually considered a minor pest (62). However, within its new ranges it can cause considerable damage in colonies of European honey-bee subspecies. *Aethina tumida* is an exotic pest of bees in Europe. In addition to direct and microscopic examination of imported queen bees and attendants (Table I), a method has been developed to screen hive debris for the presence of the small hive beetle, using real-time polymerase chain reaction (PCR) (73). If the small hive beetle were to become established in new locations, the availability of this method would be a valuable tool to enable species identification and rapid screening of hive debris for delimiting surveys. Furthermore, laboratory investigations have demonstrated that *A. tumida* can be infected with bee viruses via food-borne transmission, and thus has the potential to become a biological vector for deformed wing virus (DWV) and sacbrood virus (SBV) (23, 24). Schäfer and colleagues (67) clearly demonstrated that larval and adult small hive beetles become contaminated with *P. larvae* spores when exposed to brood combs with clinical signs of AFB, and are vectors of *P. larvae*.

Tropilaelaps spp.

The mites of the genus *Tropilaelaps* are ectoparasites of the brood of *A. mellifera* L., *A. laboriosa* and *A. dorsata*. There are at least four species of *Tropilaelaps* (Acari: Laelapidae): *T. clareae, T. konigerum, T. thaii* and *T. mercedesae* (3). Their feeding on bee larvae and pupae causes brood malformation, death of bees and subsequent colony decline or absconding. Gravid female mites will die within two days unless they deposit their eggs (79). Development requires about one week, and the mites are dispersed on bees. *Tropilaelaps* cannot survive for periods of more than seven days away from bee brood. Their phoretic survival on bees has been defined as lasting from two to three days (79) to five to ten days (66, 74) because *Tropilaelaps* cannot pierce the integument of adult bees. However, it has been suggested that more extended survival is possible and it is now clear that *T. clareae* can easily survive even the longest of international airline flights. *Tropilaelaps* spp. are presently exotic to Europe (76).

Varroosis

The mite *V. destructor* (formerly *V. jacobsoni*) (4) is a parasite of adult bees and their brood. Its natural host is the Asian honey bee, *A. cerana*. It penetrates the intersegmental skin of adult bees between the abdominal sclera, and sometimes between the head and thorax, to ingest haemolymph. The number of parasites steadily increases with increasing brood activity and the growth of the bee population, especially late in the season when clinical signs of infestation can first be recognised. The life span of the mite depends on temperature and humidity but, in practice, can last from several days to a few months. In addition to its direct action as pathogen, the varroa mite has been proven to be an effective vector in transmitting and activating viruses.

Varroa destructor has worldwide distribution, with the exception of Australia (21).

Viruses

At least 18 viruses have been reported to infect honey bees worldwide (2, 21). Although knowledge of bee viruses is still limited, compared to that of other well-studied insect viruses, such as baculoviruses, our understanding of virus infections in honey bees has grown considerably over the last three decades (11). Viruses may attack different developmental stages and castes of bees, including eggs, larvae, pupae, adult workers, adult drones and the queen of the colony. Although bee viruses usually persist as inapparent infections, they can dramatically affect bee health and shorten the lives of infected bees under certain conditions. Six of the most common viruses identified as attacking honey bees are the subject of active research:

- DWV
- black queen cell virus (BQCV)
- SBV
- Kashmir bee virus (KBV)
- acute bee paralysis virus (ABPV)
- chronic bee paralysis virus (CBPV).

More recently, Israeli acute paralysis virus (IAPV) came to the attention of researchers after its detection in bee colonies in the United States as a possible cause/marker of colony collapse disorder (CCD) (18). Two unreported RNA viruses in North American honey bees, *V. destructor*-1 virus and Kakugo virus, and an invertebrate iridescent virus (Iridoviridae) have also been identified and associated with CCD colonies (9). Rapid and accurate diagnosis is a crucial component of virus surveillance and control programmes. The development of molecular methods has revolutionised the diagnosis of viral diseases and provided powerful tools for specific, sensitive and rapid identification. Since the varroa mite has been proven to be an effective vector for transmitting and activating viruses, timely and efficient control of the varroa population will reduce the incidence of viral diseases. In addition to controlling the vector population, effective management of viral bee diseases can be achieved by maintaining good hygiene practices, feeding bees with the proper quantity and quality of food, and replacing combs and queens when a problem is serious. However, virus infections of honey bees have not been fully characterised at the molecular level and there are many gaps in our knowledge of the key processes underlying the dynamics of transmission, epidemiology, pathogenesis and host immunity. None of the viral diseases of honey bees is listed by the OIE (35, 76).

Ascospherosis (*Ascosphaera apis*)

Ascospherosis, also named chalkbrood, is a fungal disease of honey-bee brood caused by *Ascosphaera apis*. While adult bees are not susceptible, they can transmit the pathogen within and between beehives (5). Transmission of infectious materials between adult bees in the colony appears to occur through sharing food (39). Fungal spores can be carried by foraging bees and passed onto larvae by nurse bees feeding them with contaminated food. Transmission between managed colonies is mostly beekeeper-assisted and is due to contaminated materials. Because spores can accumulate on all parts of the beehive and in all beehive products (e.g. foundation wax, stored pollen and honey), and remain viable for at least 15 years, any hive material contaminated with fungal spores will serve as a long-lasting source of infection (27, 28). Ascospherosis is not an OIE-listed disease.

Honey

Honey is the most important product of beekeeping, both from a quantitative and an economic point of view (19). Honey is the natural sweet substance produced by honey bees from the nectar of blossoms, or from the secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature. This is the general definition of honey in the Codex Alimentarius (16), in which all commercially required characteristics of the product are described. Honey is traded primarily for human consumption and not as bee feed. The current annual world honey production is estimated to be about 1.4 million tonnes (29). In 2007, the People's Republic of China, the leader in honey production and export, produced 303 million tonnes; Argentina: 81 million tonnes; Turkey: 74; Ukraine: 68; United States: 67; Russian Federation: 55; Mexico: 54; India: 52; Ethiopia: 44; Iran: 36; Brazil: 35; Canada: 31; Spain: 31; Tanzania: 27; Kenya: 25, and the European Union: 174. The risks posed by contaminated honey are minor; leaks in shipping containers could lead to robbing of the honey by foraging bees, and discarded honey containers (whether these are barrels or consumer packs) may also be accessible to honey bees (71).

Honey as a carrier of bee diseases

American foulbrood

American foulbrood has a worldwide distribution and is the only bee disease of economic importance transmitted by honey. Only the spores of *P. larvae* are capable of causing the disease, and only young larvae are susceptible. Sturtevant (72) estimated that 50,000,000 or more *P. larvae* spores must be fed to initiate the disease in a hive of honey bees. The most extensive survey into the incidence of *P. larvae* in commercial honey was conducted by Hansen (44), who examined 131 samples of honey and found *P. larvae* spores in 56% of the samples. The average was 600,000 spores/5 g of honey, which means that it would be necessary for a colony to be fed more than 400 g of honey to initiate AFB disease (72). Furthermore, since honey is not normally fed to larvae less than three days after egg hatch, and larvae older than 53 h are no longer susceptible to AFB, the risk of transmitting the disease through honey is small. Adult bees that have ingested honey containing *P. larvae* spores and which, as a result, have contaminated mouthparts, may also transmit spores to young larvae at feeding.

The most common method of disease transmission is the interchange of bee equipment between hives. However, AFB is also vertically transmitted through swarming (34) and between colonies through robbing (56). Since honey is imported mainly for human consumption and not for bee feed (71), the risk of transmitting AFB through trade in honey is minimal. However, this risk should not be ignored by importing countries which have been shown to be free from AFB at present.

European foulbrood

European foulbrood is a severe bacterial brood disease with a worldwide distribution, which is an increasing problem in some areas. Although the causative agent was described almost a century ago, many basic aspects of its pathogenesis are still unknown (30). As with AFB, the disease only affects young larvae and is transmitted by the interchange of brood combs between colonies. Honey is commonly contaminated with *M. plutonius* (52, 57), suggesting that robbing may contribute to the spread of the bacterium between colonies and apiaries.

Other bee diseases and mites

There are no parasitic mites or other diseases of economic importance which are transmitted by honey. However, the role played by honey in the transmission and spread of *N. ceranae* has not yet been elucidated. Giersch and colleagues (37) demonstrated *N. ceranae* spores in the honey of *A. mellifera* in Australia. A PCR assay for the detection of *N. apis/N. ceranae* spores in honey samples has been developed by Granato and colleagues (42) as a reliable tool to monitor the presence and spreading of such an infection in bee colonies, even in the absence of obvious clinical signs.

Resistance of *N. ceranae* to different exposure conditions has been evaluated by using Sytox green and 4´,6-diamidino-2-phenylindole to test spore viability. High thermotolerance at 60°C and 35°C and resistance to desiccation were observed. However, a significant decrease in viability after freezing and a rapid degeneration of spores maintained at 4°C were also detected (25). The viability of *N. ceranae* spores is significantly reduced after one week in a freezer, which is not the case for *N. apis* (33). This difference in temperature sensitivity between parasite species probably has epidemiological implications and may decrease transmission opportunities for *N. ceranae*, at least on wax exposed to freezing temperatures during storage.

While adult bees are not susceptible to ascospherosis, they can transmit the disease between beehives (5). Because spores can accumulate on all parts of the beehive and in all beehive products (e.g. foundation wax, stored pollen and honey), and remain viable for at least 15 years, any hive material contaminated with fungal spores will serve as a long-lasting source of infection (27, 28).

Viruses

Shen and colleagues (69) detected KBV and SBV in colony food, including honey, pollen and royal jelly, as well as in all developmental stages of bees, suggesting the involvement of colony food in the spread of viral infections. Similar findings were reported by Chen and colleagues (12), who found BQCV and DWV in honey samples. The last two viruses were also present in over 80% of the examined brood and adult workers in the bee colonies where the colony food was collected.

Beeswax

Beeswax is a substance secreted by worker honey bees from four pairs of glands on the underside of their abdomen, and is used by them to construct honeycomb. Pure beeswax is made only of carbon, hydrogen and oxygen, all of which are readily available from the honey that bees consume. In its purest form beeswax is white, but becomes yellow because it is stained by materials in the pollen and propolis that naturally contaminate it (17). Frame-hive beekeeping produces wax almost exclusively from the cap and top part of the honey cells. Rendered but untreated beeswax comes in varying shades of yellow. The pure white beeswax on the market has always been bleached. Beeswax is imported mainly as a cake, formed by melting used honeycomb or the wax from cappings when extracting honey. In some situations, beeswax may be imported as used honeycomb or as used bee equipment.

Beeswax is primarily imported as a raw material for honeycomb foundation, candles, cosmetics and furniture polishes. It is difficult to obtain reliable figures on wax production, as the greater part of beeswax is used in beekeeping for producing comb foundations. It is estimated that its production is about 1.5% to 2.5% that of honey (19). Commercially made foundation has not been incriminated as an agent for the dissemination of any bee disease. Used honeycomb, however, when placed in a colony, poses a high risk for the transmission of disease and, to some extent, parasitic mites. To detect the agent of AFB, Shimanuki and Knox (70) used direct microscopic examination of beeswax extracts made with chloroform or boiling water, while Alippi (1) used a benzene extract of beeswax. De Guzman and colleagues (20) found the average survival of *V. jacobsoni* on beeswax honeycomb to be 37 h at 26°C and 35 h at 13°C. Nosema spores accumulate in combs and can survive in wax for one year (31), being the main source of infection with *N. ceranae*, together with latent-infected honey bees.

Royal jelly

Royal jelly is secreted by the hypopharyngeal gland of young worker (nurse) bees to feed young larvae and the adult queen bee (19). Royal jelly is always fed directly to the queen or the larvae as it is secreted; it is not stored. Royal jelly is produced and harvested through artificial queen rearing. The queen larvae cannot consume the food as fast as it is provided and royal jelly accumulates in the artificial queen cells. It is the food intended for queen bee larvae that are four to five days old. Royal jelly is not usually imported for use within bee colonies. Most, if not all, imported royal jelly is destined for use as a food supplement for humans. Furthermore, royal jelly has not been implicated as a medium for the transmission of any bee disease. However, royal jelly is frequently sold as part of a honey product and this could pose a limited risk to bees when the containers are disposed of in a landfill. Virus transmission to larvae via brood feeding has been demonstrated by detection of viruses in the thoracic gland and hypopharyngeal gland of bees (6, 7). A study conducted by Shen and colleagues (69) showed that KBV and SBV were detected in colony food, including honey, pollen and royal jelly, as well as in all developmental stages of bees, suggesting the involvement of colony food in the spread of virus infections. Although ABPV, CBPV, KBV and SBV were detected in pollen samples, the same viruses were not detected in the bees and royal jelly (12). Israeli acute paralysis virus has been detected in royal jelly samples (18).

Pollen

The pollen collected by honey bees is usually mixed with nectar or regurgitated honey, to make it stick together and adhere to their hind legs (19). A foraging bee rarely collects both pollen and nectar from more than one species of flower during one trip. Thus, the resulting pollen pellet on the hind leg contains only one or very few pollen species. Accordingly, the pollen pellet has a typical colour, most frequently yellow, but red, purple, green, orange and a variety of other colours occur. The partially fermented pollen mixture stored in the honey-bee combs, also referred to as beebread, has a different composition and nutritional value from the field-collected pollen pellets and is the food given to larvae and eaten by young worker bees to produce royal jelly. Bee-collected pollen is usually trapped at the entrance of beehives by a device named a pollen trap. The pollen trap literally scrapes the pollen off the hind legs of returning foraging bees. The only processing which pollen undergoes is drying (usually by warm air) and the physical removal of some debris (insects, mites and pieces of dead adults and brood). After the larger pieces of debris have been removed by hand, bee-collected pollen is cleaned by passing the pollen in front of an air-stream to separate the lighter pieces of debris from pollen pellets. In addition to pollen pellets and other pollen-formulated products intended for human consumption, beekeepers use pollen to increase the attractiveness and consumption of pollen substitutes to bees. It is unlikely that beekeepers would import pollen specifically for bee use (71).

Pollen as a carrier of bee diseases

The pollen trap design makes it difficult for house-cleaning bees to remove the remains of dead bees, scales (AFB) and mummies (ascospherosis) from the hives. As a consequence, pollen collected from diseased colonies and fed to disease-free colonies can be a means of disease dissemination (71). Since the diseased larval remains of AFB are difficult to remove from the wax comb, house-cleaning bees gnaw the AFB scales and remove the pieces, which then fall into the pollen trap. A single scale of AFB is estimated to contain 2.5×10^9 spores of *P. larvae* (45). The median lethal dose for day-old honey-bee larvae is only 35 spores, so a small piece of foulbrood scale could contaminate the mouthparts of a nurse bee feeding on pollen and initiate an infection in the larvae. For this reason, the importation of pollen for bee feed can be hazardous, particularly from those countries known to have uncontrolled AFB. Gochnauer and Corner (41) detected and identified *P. larvae* in a commercial pollen sample. Although the EFB scales are physically easier to remove than those of AFB, the honey bees also remove these scales in pieces. Consequently, pieces of EFB scales may cause contamination of bee-collected pollen (71). Infective *N. ceranae* spores have been detected in corbicular pollen of forager honey bees (50). The epidemiological consequences of the presence of Nosema spores in corbicular pollen require more study and must be considered in beekeeping practices.

In the case of ascospherosis, a dead bee larva gives rise to a 'mummy' of stone-like consistency, rather than a scale. Mummies, like scales, are removed wholly or in pieces. Thus, remnants of chalkbrood mummies can be found in pollen and may become a source of the pathogen (40). The importation of pollen is believed to have been a route of entry of this disease into the United States in the 1960s (38, 71).

There have been a number of studies on the survival of *V. destructor* on bee-collected pollen. The average survival time of *V. destructor* on pollen is 41 h at 26°C and 18 h at 13°C (20). The survival of *T. clareae* requires the presence of bee brood. Thus, if no bee brood is present, *T. clareae* cannot survive for more than 72 h (79). However, it has been suggested that more extended survival could be possible (74). Leonard and colleagues (55) examined 509 samples of pollen from 14 countries and found that they contained 71 insects and 27 mite families. This study provides a detailed list of insect and mite families found in association with pollen, their countries of origin and their frequency. No regulated arthropods were found in any of the pollen samples.

The movement of bee-collected pollen could transmit immature stages of the small hive beetle. The viruses ABPV, BQCV, CBPV, DWV, KBV and SBV have been detected in pollen samples (12). However, it appears that bees ingesting virus-contaminated food, such as pollen, might not always become infected. Importing pollen for bee feed poses a definite risk, since there are no accepted procedures for determining whether pollen is free from pathogens, insects or mites. Drying pollen would reduce the survival of mites and insects, but would not have any impact on bacterial spores.

Venom

Honey-bee venom is produced by two glands associated with the sting apparatus of worker bees. Its production increases during the first two weeks of the adult worker's life and reaches a maximum when the worker bee becomes involved in hive defence and foraging. A full venom sac holds 0.15 mg to 0.3 mg of venom (19, 68). There are no known pathogens or diseases which are transmitted by bee venom.

Propolis

Propolis is a mixture of various amounts of beeswax and resins collected by the bee from plants, particularly from flowers and leaf buds (19). It can be assumed that, in the process of collecting and modelling the resins, they are mixed with some saliva and other secretions of the bees, as well as with wax. These resins are used by worker bees to line the insides of nest cavities and all brood combs, repair combs, seal small cracks in the hive, reduce the size of hive entrances, seal off any dead animals or insects inside the hive which are too large to be carried out and, perhaps most important of all, to mix small quantities of propolis with wax to seal brood cells. These uses are significant because they take advantage of the antibacterial and antifungal effects of propolis in protecting the colony against diseases. There are no known pathogens or diseases that are transmitted by propolis.

Bee semen

Yue and colleagues (80) demonstrated, for the first time, sequences of DWV and ABPV being present in the semen of apparently healthy drones (*A. mellifera*), indicating that mating may be a route of virus transmission. As a consequence, the semen trade could be responsible for virus spreading.

Queen bees

Aethina tumida

Package bees offer the greatest potential for the transport of the small hive beetle. However, there are reports from

Florida, in the United States, of small hive beetle eggs attached to the backs of honey bees (22), suggesting that importation of queens may also be a potential entry route. Larvae and eggs of *A. tumida* have been identified in cages of queens imported from the United States into Portugal (60).

Nosemosis

The queen honey bee is susceptible to most of the diseases that attack her offspring and *N. ceranae* infection is no exception. Horizontal transmission from worker bees to queens has been confirmed in a laboratory study as a possible route of *N. ceranae* infection and pathological effects on queens have been described. These data suggest that the higher the number of infected house bees, the higher the risk of transmission to queens, and may also explain the role of house honey bees in natural queen infection (49). In this way, trade in queens and their worker bee escorts may be a source of infection in some areas (37).

Viruses

Since honey-bee queens mate with up to 28 drones (54), the chance of becoming infected by virus contained in semen is not negligible, even when only a minor proportion of drones are transmitting the virus. Virus-containing semen, therefore, is one possible source of virus-positive eggs (14, 69) and also virus-positive queens (11, 15, 80).

Conclusions

Since international rules have been established to prevent the spread of diseases of honey bees, a proper knowledge of these rules and their correct application worldwide is particularly important. Furthermore, since not all the known diseases of bees are listed by the OIE, and those that are listed could undergo changes in their spread and pathogenicity, a continuing surveillance and reporting system should be implemented and the regulatory apparatus periodically updated. This could help in tracing the trade flow of bees and bee products and consequently improve current knowledge on the distribution of bee diseases and pests.

To improve our knowledge of the spread of honey-bee diseases, exporting countries should establish epidemiological and health surveillance practices based on harmonised procedures and diagnostic tests. Diseases caused by viruses are not listed by the OIE and so the *Terrestrial Code* makes no recommendations on these when trading honey bees and their products. However, in recent years, molecular techniques have greatly improved diagnostic capacity and our understanding about the role of viruses as pathogens of honey bees. The role of *N. ceranae* as a pathogen has not yet been completely elucidated and more investigations are needed. While *A. tumida* and *Tropilaelaps* spp. are still exotic to Europe, it has been clearly demonstrated how *A. tumida* could be spread internationally through trade in honey bees and this could also be true for *Tropilaelaps* spp., depending on transport time and conditions. Traceability of traded honey bees and their products, movement restrictions, and controls on imported queen bees and their attendants are still the tools upon which the prevention of further spread relies.

La dissémination d'agents pathogènes lors des échanges internationaux d'abeilles mellifères et de leurs produits (reines et semence comprises) : état de la situation et évolutions récentes

F. Mutinelli

Résumé

Le commerce international des abeilles et de leurs produits pose des problèmes complexes, notamment en raison de la diversité des origines de ces abeilles et produits et des multiples usages qui en sont faits. Principal facteur de risque de dissémination des maladies, le commerce des abeilles forme un marché très dynamique, dont une partie demeure informelle. Le déclin de la santé des abeilles dans le monde se poursuit, parallèlement à la propagation d'agents pathogènes, de pestes, de parasites et de maladies due aux échanges internationaux légaux ou frauduleux, et à certaines introductions imprudentes réalisées par des professionnels non avertis. La régulation des échanges internationaux permet à de nombreux pays de protéger la santé des abeilles mais leur impose aussi certaines contraintes. Les pays membres de l'Organisation mondiale du commerce (OMC) doivent limiter les restrictions commerciales aux seules importations présentant un risque sanitaire bien identifié. Les importations qui ne comportent aucun risque sanitaire ne devraient pas faire l'objet de restrictions. Dans le domaine de la santé des abeilles, l'OMC se réfère de plus en plus aux normes internationales élaborées par l'Organisation mondiale de la santé animale, dont le but est de prévenir la propagation des maladies animales tout en facilitant les échanges commerciaux d'animaux et de produits d'origine animale.

Mots-clés

Abeille – Abeille mellifère – Accord SPS – Agent pathogène – *Apis mellifera* – Commerce international – Évaluation du risque – Maladie – Peste – Organisation mondiale de la santé animale – Organisation mondiale du commerce – Parasite – Produits des abeilles – Réglementation.

■

Propagación de agentes patógenos por el comercio de abejas mieleras y sus derivados (abejas reinas y semen inclusive): panorámica general y evolución reciente

F. Mutinelli

Resumen

El comercio internacional de abejas y derivados apícolas es una cuestión compleja, en la que influyen los distintos orígenes y usos de esos productos. El comercio de abejas, actividad muy intensa cuyas transacciones no siempre quedan registradas oficialmente, es el principal factor de riesgo de propagación de enfermedades. La salud de las abejas sigue deteriorándose en todo el mundo, a medida que patógenos, plagas, parásitos y enfermedades van cruzando fronteras a resultas de transacciones legales, de actividades de contrabando o de la introducción de abejas, tan bienintencionada como imprudente, por parte de profesionales. Las reglas del comercio internacional, si bien refuerzan la capacidad de muchos países para proteger la salud de las abejas sin interrumpir el comercio, también entrañan ciertas obligaciones. Los países que son Miembros de la Organización Mundial del Comercio (OMC) sólo deberían restringir las importaciones para protegerse de riesgos sanitarios identificables. Si las importaciones son seguras, hay que autorizar el comercio. Las reglas de la OMC han venido a otorgar mayor protagonismo a las normas internacionales aplicables a la salud de las abejas elaboradas por la Organización Mundial de Sanidad Animal, cuya misión consiste en prevenir la propagación de enfermedades animales y a la vez facilitar el comercio internacional de animales y productos de origen animal.

Palabras clave
Abeja mielera – Abeja – Acuerdo MSF – *Apis mellifera* – Comercio internacional – Determinación del riesgo – Enfermedad – Organización Mundial del Comercio – Organización Mundial de Sanidad Animal – Parásito – Patógeno – Plaga – Producto apícola – Reglamentación.

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References

1. Alippi M.A. (1997). – Background on American foulbrood in Argentina. *Bee World*, **78** (2), 92-95.

2. Allen M. & Ball B. (1996). – The incidence and world distribution of honey bee viruses. *Bee World*, **77** (3), 141-162.

3. Anderson D.L. & Morgan M.J. (2007). – Genetic and morphological variation of bee-parasitic *Tropilaelaps* mites (Acari: Laelapidae): new and re-defined species. *Experim. appl. Acarol.*, **43** (1), 1-24. E-pub.: 9 September 2007.

4. Anderson D.L. & Trueman J.W.H. (2000). – *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experim. appl. Acarol.*, **24** (3), 165-189.

5. Aronstein K.A. & Murray K.D. (2010). – Chalkbrood disease in honey bees. *J. Invertebr. Pathol.*, **103** (Suppl. 1), S20-S29. E-pub.: 11 November 2009.

6. Bailey L. & Ball B.V. (1991). – Honey bee pathology. Academic Press, London, 124 pp.

7. Bailey L. & Milne R.G. (1969). – The multiplication regions and interaction of acute and chronic bee-paralysis viruses in adult honey bees. *J. gen. Virol.*, **4** (1), 9-14.

8. Bradbear N. (1988). – World distribution of major honeybee diseases and pests. *Bee World*, **69** (1), 15-39.

9. Bromenshenk J.J., Henderson C.B., Wick C.H., Stanford M.F., Zulich A.W., Jabbour R.E., Deshpande S.V., McCubbin P.E., Seccomb R.A., Welch P.M., Williams T., Firth D.R., Skowronski E., Lehmann M.M., Bilimoria S.L., Gress J., Wanner K.W. & Cramer Jr R.A. (2010). – Iridovirus and microsporidian linked to honey bee colony decline. Doi: 10.1371/journal.pone.0013181. *PLoS ONE,* **5** (10), e13181.

10. Brown M.A., Thompson H.M. & Bew M.H. (2002). – Risks to UK beekeeping from the parasitic mite *Tropilaelaps clareae* and the small hive beetle, *Aethina tumida*. *Bee World*, **83** (4), 151-164.

11. Chen Y.P. & Siede R. (2007). – Honey bee viruses. *Adv. Virus Res.*, **70**, 33-80.

12. Chen Y.P., Evans J.D. & Feldlaufer M.F. (2006). – Horizontal and vertical transmission of viruses in the honey bee, *Apis mellifera*. *J. Invertebr. Pathol.*, **92** (3), 152-159. E-pub.: 21 June 2006.

13. Chen Y.P., Evans J.D., Smith I.B. & Pettis J.S. (2008). – *Nosema ceranae* is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States. *J. Invertebr. Pathol.*, **97** (2), 186-188. E-pub.: 6 August 2007.

14. Chen Y.P., Higgins J.A. & Feldlaufer M.F. (2005). – Quantitative real-time reverse transcription-PCR analysis of deformed wing virus infection in the honeybee (*Apis mellifera* L.). *Appl. environ. Microbiol.*, **71** (1), 436-441.

15. Chen Y.P., Pettis J.S. & Feldlaufer M.F. (2005). – Detection of multiple viruses in queens of the honey bee *Apis mellifera* L. *J. Invertebr. Pathol.*, **90** (2), 118-121. E-pub.: 7 October 2007.

16. Codex Alimentarius Commission (CAC) (2001). – Revised Codex standard for honey. Adopted in 1981. Revisions 1987 and 2001. CAC, Rome, 1-8. Available at: www. codexalimentarius.net/web/more_info.jsp?id_sta=310 (accessed on 13 August 2010).

17. Coggshall W.L. & Morse R.A. (1984). – Beeswax. Wicwas Press, Kalamazoo, Michigan, 192 pp.

18. Cox-Foster D.L., Conlan S., Holmes E.C., Palacios G., Evans J.D., Moran N.A., Quan P.L., Briese T., Hornig M., Geiser D.M., Martinson V., van Engelsdorp D., Kalkstein A.L., Drysdale A., Hui J., Zhai J.H., Cui L.W., Hutchison S.K., Simons J.F., Egholm M., Pettis J.S. & Lipkin W.I. (2007). – A metagenomic survey of microbes in honey bee colony collapse disorder. *Science*, **318** (5848), 283-287. E-pub.: 6 September 2007.

19. Crane E. (1992). – Bees and beekeeping. Heinemann Newnes, Oxford, 614 pp.

20. De Guzman L.I., Rinderer T.E. & Beaman L.D. (1993). – Survival of *Varroa jacobsoni* Oud. (Acari: Varroidae) away from its living host *Apis mellifera*. *Experim. appl. Acarol.*, **17** (4), 283-290.

21. Ellis J.D. & Munn P.A. (2005). – The worldwide health status of honey bees. *Bee World*, **86** (4), 88-101.

22. Elzen P.J., Baxter J.R., Westervelt D., Randall C., Cutts L., Wilson W., Eischen F.A., Delaplane K.S. & Hopkins D.I. (1999). – Status of the small hive beetle in the US. *Bee Cult.*, **127**, 28-29.

23. Eyer M., Chen Y.P., Schäfer M.O., Pettis J.S. & Neumann P. (2009). – Honey bee sacbrood virus infects adult small hive beetles, *Aethina tumida* (Coleoptera: Nitidulidae). *J. Apic. Res.*, **48** (4), 296-297.

24. Eyer M., Chen Y.P., Schäfer M.O., Pettis J.S. & Neumann P. (2009). – Small hive beetle, *Aethina tumida*, as a potential biological vector of honeybee viruses. *Apidologie*, **40** (4), 419-428.

25. Fenoy S., Rueda C., Higes M., Martín-Hernández R. & del Aguila C. (2009). – High-level resistance of *Nosema ceranae*, a parasite of the honeybee, to temperature and desiccation. *Appl. environ. Microbiol.*, **75** (21), 6886-6889. E-pub.: 4 September 2009.

26. Fléché C. (1997). – Risques de dissémination des maladies apiaires par les mouvements internationaux des abeilles et de leurs produits. *In* Contamination of animal products: prevention and risks for animal health (P. Sutmoller, ed.). *Rev. sci. tech. Off. int. Epiz.*, **16** (1), 177-186.

27. Flores J.M., Gutiérrez I. & Espejo R. (2005). – The role of pollen in chalkbrood disease in *Apis mellifera*: transmission and predisposing conditions. *Mycologia*, **97** (6), 1171-1176.

28. Flores J.M., Spivak M. & Gutiérrez I. (2005). – Spores of *Ascosphaera apis* contained in wax foundation can infect honeybee brood. *Vet. Microbiol.*, **108** (1-2), 141-144. E-pub.: 26 April 2005.

29. Food and Agriculture Organization of the United Nations (FAO) (2005). – FAOSTAT. FAO, Rome. Available at: faostat.fao.org (accessed on 17 August 2010).

30. Forsgren E. (2010). – European foulbrood in honey bees. *J. Invertebr. Pathol.*, **103** (Suppl. 1), S5-S9. E-pub.: 21 December 2010.

31. Fries I. (1988). – Comb replacement and Nosema disease (*Nosema apis* Z.) in honey bee colonies. *Apidologie*, **19** (4), 343-354.

32. Fries I. (2010). – *Nosema ceranae* in European honey bees (*Apis mellifera*). *J. Invertebr. Pathol.*, **103** (Suppl. 1), S73-S79. E-pub.: 11 November 2009.

33. Fries I. & Forsgren E. (2009). – *Nosema ceranae* fungerar inte som *Nosema apis*. *Nosema ceranae* does not function as *Nosema apis* [in Swedish]. *Bitidningen*, **107**, 20-21.

34. Fries I., Lindström A. & Korpela S. (2006). – Vertical transmission of American foulbrood (*Paenibacillus larvae*) in honey bees (*Apis mellifera*). *Vet. Microbiol.*, **114** (3-4), 269-274. E-pub.: 18 January 2006.

35. Genersch E. & Aubert M. (2010). – Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). Doi: 10.1051/vetres/2010027. *Vet. Res.*, **41** (6), 54. E-pub.: 29 April 2010.

36. Genersch E., Forsgren E., Pentikäinen J., Ashiralieva A., Rauch S., Kilwinski J. & Fries I. (2006). – Reclassification of *Paenibacillus larvae* subsp. *pulvifaciens* and *Paenibacillus larvae* subsp. *larvae* as *Paenibacillus larvae* without subspecies differentiation. *Int. J. syst. evolut. Microbiol.*, **56** (Pt 3), 501-511.

37. Giersch T., Berg T., Galea F. & Hornitzky M. (2009). – *Nosema ceranae* infects honey bees (*Apis mellifera*) and contaminates honey in Australia. *Apidologie*, **40** (2), 117-123.

38. Gilliam M. (1990). – Chalkbrood disease of honey bees, *Apis mellifera*, caused by the fungus, *Ascosphaera apis*: a review of past and current research. *In* Proc. & Abstr. 5th International Colloquium on Invertebrate Pathology and Microbial Control, incorporating the 23rd Annual Meeting of the Society for Invertebrate Pathology, 20-24 August, Adelaide, Australia, 398-402.

39. Gilliam M. & Vandenberg J.D. (1997). – Fungi. *In* Honey bee pests, predators, and diseases (R. Morse & K. Flottum, eds), 3rd Ed. AI Root, Ohio, 81-110.

40. Gilliam M., Taber S., Lorenz B.J. & Prest D.B. (1988). – Factors affecting development of chalkbrood disease in colonies of honey bee, *Apis mellifera*, fed pollen contaminated with *Ascosphaera apis*. *J. Invertebr. Pathol.*, **52** (2), 314-325.

41. Gochnauer T.A. & Corner J. (1974). – Detection and identification of *Bacillus larvae* in a commercial pollen sample. *J. Apic. Res.*, **13** (6), 264-267.

42. Granato A., Caldon M., Colamonico R., Boscarato M., Falcaro C., Stocco N., Gallina A. & Mutinelli F. (2009). – Detection of *Nosema apis/Nosema ceranae* spores in honey samples. *In* Proc. 41st Apimondia Congress, 15-20 September, Montpellier, France. Apimondia, Rome, 163.

43. Griffiths D.A. & Bowman C.E. (1981). – World distribution of the mite *Varroa jacobsoni*, a parasite of honey bees. *Bee World*, **62** (4), 154-163.

44. Hansen H. (1984). – The incidence of the foulbrood bacterium *Bacillus larvae* in honey retailed in Denmark. *Dan. J. Plant Soil Sci.*, **88**, 329-336.

45. Hansen H. & Brødsgaard C.J. (1999). – American foulbrood: a review of its biology, diagnosis and control. *Bee World*, **80** (1), 5-23.

46. Hasemann L. (1961). – How long can spores of American foulbrood live? *Am. Bee J.*, **101**, 298-299.

47. Higes M., Martín-Hernández R. & Meana A. (2006). – *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. *Experim. appl. Acarol.*, **92** (2), 93-95.

48. Higes M., Martín-Hernández R. & Meana A. (2010). – *Nosema ceranae* in Europe: an emergent type C nosemosis. *Apidologie*, **41** (1), 375-392.

49. Higes M., Martín-Hernández R., García-Palencia P., Marín P. & Meana A. (2009). – Horizontal transmission of *Nosema ceranae* (Microsporidia) from worker honey bees to queens (*Apis mellifera*). *Environ. Microbiol. Rep.*, **1** (6), 495-498.

50. Higes M., Martín-Hernández R., Garrido-Bailón E., García-Palencia P. & Meana A. (2008). – Detection of infective *Nosema ceranae* (Microsporidia) spores in corbicular pollen of forager honeybees. *J. Invertebr. Pathol.*, **97** (1), 76-78. E-pub.: 20 January 2007.

51. Hood M.W. (2004). – The small hive beetle, *Aethina tumida*: a review. *Bee World*, **85** (3), 51-59.

52. Hornitzky A.Z. & Smith L. (1998). – Procedures for the culture of *Melissococcus pluton* from diseased brood and bulk honey samples. *J. Apic. Res.*, **37** (4), 293-294.

53. Klee J., Besana A.M., Genersch E., Gisder S., Nanetti A., Tam D.Q., Chinh T.X., Puerta F., Ruz J.M., Kryger P., Message D., Hatjina F., Korpela S., Fries I. & Paxton R. (2007). – Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis Mellifera*. *J. Invertebr. Pathol.*, **96** (1), 1-10. E-pub.: 12 March 2007.

54. Kraus F.B., Neumann P. & Moritz R.F.A. (2005). – Genetic variance of mating frequency in the honeybee (*Apis mellifera* L.). *Insect. Soc.*, **52** (1), 1-5.

55. Leonard F.W., Reichelderfer C.F. & Shimanuki H. (1983). – Pollen importation – a possible route for pest introduction. *Apidologie*, **14** (4), 303-307.

56. Lindström A., Korpela S. & Fries I. (2008). – Horizontal transmission of *Paenibacillus larvae* spores between honey bee (*Apis mellifera*) colonies through robbing. *Apidologie*, **39** (5), 505-522.

57. McKee B.A., Djordjevic S.P., Goodman R.D. & Hornitzky M.A.Z. (2003). – The detection of *Melissococcus pluton* in honey bees (*Apis mellifera*) and their products using a hemi-nested PCR. *Apidologie*, **34** (1), 19-27.

58. Matheson A. (1996). – World bee health update 1996. *Bee World*, **77** (1), 45-51.

59. Matheson A. (2000). – Managing risks in world trade in bees and bee products. *Apiacta,* **35** (1), 1-12.

60. Murilhas A.M. (2004). – *Aethina tumida* arrives in Portugal. Will it be eradicated? *Eurbee Newsl.*, **2**, 7-9.

61. Neumann P. & Ellis J.D. (2008). – The small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): distribution, biology and control of an invasive species. *J. Apic. Res.*, **47** (3), 181-183.

62. Neumann P. & Elzen P.J. (2004). – The biology of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae): gaps in our knowledge of an invasive species. *Apidologie*, **35** (3), 229-247.

63. Nixon M. (1982). – Preliminary world maps of the honeybee diseases and parasites. *Bee World*, **63** (1), 23-42.

64. Pankiw P. & Corner J. (1966). – Transmission of American foulbrood by package bees. *J. Apic. Res.*, **5** (2), 99-101.

65. Paxton R., Klee J., Korpela S. & Fries I. (2007). – *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. *Apidologie*, **38** (6), 558-565.

66. Rinderer T.E., Oldroyd B.P., Lekprayoon C., Wongsiri S., Boonthai C. & Thapa R. (1994). – Extended survival of the parasitic honey bee mite *Tropilaelaps clareae* on adult workers of *Apis mellifera* and *Apis dorsata*. *J. Apic. Res.*, **33** (3), 171-174.

67. Schäfer M.O., Ritter W., Pettis J.S. & Neumann P. (2010). – Small hive beetles, *Aethina tumida*, are vectors of *Paenibacillus larvae*. *Apidologie*, **41** (1), 14-20.

68. Schumacher M.J., Schmidt J.O. & Egen W.B. (1989). – Lethality of ‘killer’ bee stings. *Nature*, **337** (6206), 413.

69. Shen M., Yang X., Cox-Foster D. & Cui L. (2005). – The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. *Virology*, **342** (1), 141-149. E-pub.: 18 August 2005.

70. Shimanuki H. & Knox D.A. (1991). – Diagnosis of honey bee diseases. Handbook No. AH-690. United States Department of Agriculture, Washington, DC, 53 pp.

71. Shimanuki H. & Knox D.A. (1997). – Bee health and international trade. *In* Contamination of animal products: prevention and risks for animal health (P. Sutmoller, ed.). *Rev. sci. tech. Off. int. Epiz.*, **16** (1), 172-176.

72. Sturtevant A.P. (1932). – Relation of commercial honey to the spread of American foulbrood. *J. agric. Res.*, **45**, 257-258.

73. Ward L., Brown M., Neumann P., Wilkins S., Pettis J. & Boonham N. (2007). – A DNA method for screening hive debris for the presence of small hive beetle (*Aethina tumida*). *Apidologie*, **38** (3), 272-280.

74. Wilde J. (2000). – How long can *Tropilaelaps clareae* survive on adult honeybee workers? *In* Proc. Euroconference on Molecular Mechanisms of Disease Tolerance in Honeybees (MOMEDITO), 17-19 October, Kralupy, Czech Republic. Bee Research Institute, Dol, Czech Republic, 217-221.

75. World Organisation for Animal Health (OIE) (2008). – Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 6th Ed. OIE, Paris. Available at: www.oie.int/eng/normes /mmanual/A_summry.htm (accessed on 13 August 2010).

76. World Organisation for Animal Health (OIE) (2010). – Terrestrial Animal Health Code, 19th Ed. OIE, Paris. Available at: www.oie.int/eng/normes/mcode/en_sommaire.htm (accessed on 13 August 2010).

77. World Organisation for Animal Health (OIE) (2010). – OIE World Animal Health Information Database (WAHID) Interface. OIE, Paris. Available at: www.oie.int/wahis/public.php?page=trade_status (accessed on 17 August 2010).

78. World Trade Organization (WTO) (1995). – The Agreement on the Application of Sanitary and Phytosanitary Measures. WTO, Geneva. Available at: www.wto.org/english/tratop_e/sps_e/spsagr_e.htm (accessed on 25 August 2010).

79. Woyke J. (1987). – Length of stay of the parasite *Tropilaelaps clareae* outside sealed honeybee brood cell as a basis for its effective control. *J. Apic. Res.*, **26** (2), 104-109.

80. Yue C., Schröder M., Bienefeld K. & Genersch E. (2006). – Detection of viral sequences in semen of honeybees (*Apis mellifera*): evidence for vertical transmission of viruses through drones. *J. Invertebr. Pathol.*, **92** (2), 105-108. E-pub.: 21 April 2006.