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Chapter

# American Foulbrood and the Risk in the Use of Antibiotics as a Treatment

*Enrique Mejias*

## Abstract

Honeybees (*Apis mellifera*) crucially pollinate agricultural crops and endemic species, in addition to producing various apiculture products. The most economically relevant and abundant beehive product is honey, a sweet substance made from the secretions of melliferous plants. Honey is a natural food rich in nutrients, including certain bioactive compounds inherited from floral nectar and pollen. Among the most dangerous diseases for bees is American foulbrood. Spores of the causative microorganism, *Paenibacillus larvae,* can contaminate larvae food or the operculum wax in which larval stages of honeybees are kept. Infection is further promoted by common apiculture practices, such as reusing inert material contaminated with spores, even after months of storage. American foulbrood is untreatable, and management implicates completely incinerating the infected hive and all material that could have come into contact with pathogenic spores. The purpose of such drastic measures is to decrease propagation risk for other beehives. While evidence indicates that antibiotics could effectively control and combat this disease; antibiotic use is prohibited in most honey-producing countries due to increased risks to microbial resistance. Antibiotic residues in honey can affect consumer health, since the natural biological attributes of honey can be altered.

**Keywords:** American foulbrood, *Paenibacillus larvae*, *honey*, beehive products, antibiotics residues

## 1. Introduction

Honeybees (*Apis mellifera*) and wild bees possess a number of morphological traits that facilitate pollen transport, making bees efficient pollinizers and ensuring the preservation and diversity of agricultural crops and native plant species [1]. However, the continued existence of bees and, by extension, pollinating and honey-producing activities are under threat by a range of hostile conditions. Pesticide exposure [2, 3] is one such condition, but the acute effects of climate change are among the primary drivers for decreases in and the weakening of beehive populations.

Climate-change phenomena have strongly impacted the viability of ecosystems [4]. Prolonged droughts and high temperatures due to intense heat waves have become, in recent years, determining factors in weakened [5] and decreased [6] beehive populations across Mediterranean climates, including western Australia,

southeastern Africa, central Chile, California, and the Mediterranean basin of Europe. The combination of harsh temperatures and shortened flowering periods, as associated with insufficient water, can result in reductions to fat reserves and overall body mass in bees. This status can translate into fewer pollinating and honey-producing activities [7], as well as an increased incidence of specific diseases affecting honey bees weakened by nutritional deficits [8, 9].

Addressing the aforementioned threats to sustainably preserve the apiculture industry requires compliance with strict international regulations and norms to ensure the quality and safety of export products. Sufficiently monitoring residues in honey and adequately controlling diseases affecting bee health and production are constant preoccupations for apiculturists and exporters worldwide. In this chapter, there will be listed the main issues related to the uses of antibiotics as effective treatment against *Paenibacillus larvae* and the risk of potential resistance effects over health of consumers of bee products focused mainly on honey. Also, the development of alternative strategies to control this disease is discussed briefly.

## 2. Honey and other apiculture products

Besides fulfilling the critical role of pollinizing agricultural crops, *A. mellifera* are responsible for a number of economically valuable products, including propolis, royal jelly, bee venom, beeswax, bee pollen, and honey. Pollination and these apiculture products are made possible by adaptations that facilitate the collection and transport of pollen grains to the beehive. Behaviorally, bees improve pollen-transport efficiency by wetting pollen grains with nectar, thus creating a cohesive surface that increases the amount of pollen that can be carried during flight. Due to its anatomical skills, the characteristic buzz of bees facilitates the collection of pollen grains located on floral structures [10]. Brushes of hairs present on bee legs further favor pollen collection, specifically through the formation of a special cavity known corbiculum, on the hind pair of legs. Plant pollens are first conglomerated in the corbicular structures, but once inside the beehive, flightless bees are able to move and fragment the collected material into a honeycomb cell, where it is further broken up into a powder and accumulated against the interior of the honeycomb cell [11]. This collection and conglomeration process results in bee pollen, the water content of which is between 4 and 10%. These levels ensure good preservation over time (i.e. organic components do not degrade or decompose), thus guaranteeing that the preferably polar nutritional contents of bee pollen are chemically unaltered [12].

In addition to pollen collection, young melliferous bees secrete a liquid from wax glands that, when exposed to air, hardens and forms small flakes that collect on the underside of the bee. This economically valuable natural substance is known as beeswax and is used by bees to construct hexagonal alveoli into honeycombs. The rigid structure of honeycomb cells serves to conserve honey and pollen. Likewise, alveoli serve as a place for the queen bee to deposit eggs and for larvae or pupae to develop [13]. Beeswax contains carbohydrates (present in pollen and nectar) that have transformed into fats due to the presence of enzymes and enzyme precursors secreted by bees. More specifically, beeswax is constituted by water and minerals (1–2%), mono-esters and hydroxyl mono-esters, complex wax esthers, hydrocarbons, and free wax acids [14].

Despite the importance of bee pollen and beeswax, honey is the primary apiculture product. The global honey trade is valued at 2.4 billion dollars annually and involves the movement of approximately 630 thousand tons of honey. Chile accounted for 0.6% of total exports in 2017 and is ranked 30th among export countries. In 2017, the main import markets of honey were the United States and Germany.

Honey has been described as a naturally sweet mixture produced by *A. mellifera* bees from the nectar of flowers and the secretions of melliferous plants. These components are mixed with bee-produced substances and are deposited, dehydrated, and stored in honeycomb cells until later use [15]. The composition of this natural food includes sugars, mostly glucose and fructose, the ratio of which determines the degree of granulation for a honey [16]. Disaccharide and maltose sugars are also present [17]. Components found in lesser quantities include organic acids, amino acids, proteins, enzymes, minerals, lipids, vitamins [18, 19], and hydroxymethylfurfural, which is used as an indicator of freshness [20, 21].

Status as a natural functional food means that honey is the best-characterized apiculture product. Bees selectively use floral resources available in proximity to beehives [22–24]. This is important to consider as the traits of apiculture products, including honey and bee pollen, are inherited through secondary plant metabolites transferred in nectar [25]. Consequently, the attributes of melliferous species are directly related to the biological properties of resulting honeys [26]. Notable among the biologically active components of honey are phenolic compounds [27] and flavonoids [28, 29]. Phenolic compounds and flavonoids have antioxidant capacities, acting through routes complementary to enzymatic antioxidants identified in honeys, such as glucose oxidase and catalase [30–32]. Antibiotic activity, also as related to phenolic acids and flavonoids, has been reported in some honeys globally [33–36].

In addition to affecting biological properties, plant origin also directly influences the market value of honey. Quantitative and qualitative melissopalynological analyses can be used to classify honeys as monofloral, bifloral, or polyfloral. The highest demand is for monofloral honeys, which are primarily constituted (>45%) by pollen grains of the same melliferous species. Therefore, honey quality depends on the presence and concentration of specific chemical compounds and on the botanical origin of said compounds [37].

The elaboration of the aforementioned apiculture products can, under certain conditions, concurrently occur with the production of live material. More specifically, rearing queen bees and colonies are diversification options for national apiculturists [38]. There is a demand for bee packages, nucleus colonies, and, particularly, queen bees in countries such as Canada, France, Mexico, and Italy. This point has driven industry growth in Chile, which, over the last 3 years, has doubled in size, going from more than 10,000 exported queen bees in 2015 to more than 20,000 in 2017 [39].

## 3. American foulbrood

There are two groups of diseases that can affect beehives—exotic and endemic diseases. Exotic diseases include parasites such as the small hive beetle (*Aethina tumida*) and Tropilaelaps mites (*Tropilaelaps clareae*). Endemic diseases include pathologies that more frequently affect bees, such as nosemosis (caused by *Nosema apis*), varroosis (caused by *Varroa destructor*), and acarapisosis (caused by *Acarapis woodi*). This same group of diseases also includes two Gram-positive microorganisms that cause American (*Paenibacillus larvae*) and European (*Melissococcus plutonius*) foulbrood [40].

The first report of American foulbrood in Chile was in 2001, whereas the first case of European foulbrood was in 2009. According to protocols for the management of apiculture diseases issued by the Chilean Ministry of Agriculture, both foulbrood diseases are classified as endemic and with low prevalence in the country. Nevertheless, the management of European foulbrood is less complex and involves

less drastic sanitary measures than American foulbrood. Indeed, the incidence of European-foulbrood outbreaks has consistently declined since initial detection, with only one incident reported in 2016.

By contrast, American foulbrood is difficult to manage and eradicate. This pathogen has been detected in most regions of Chile, but the number of reported cases has varied since 2005. Notwithstanding, a worrying 44 outbreaks were reported in 2018, and an additional 61 outbreaks have been reported as of June 2019. Most cases have been reported in the Atacama, O'Higgins, and Maule Regions of Chile [41]. Given that antibiotic treatment of this disease is prohibited [42] and that sanitary control measures include the incineration of all live material, it is believed that American foulbrood outbreaks are underreported in Chile out of fear for the total loss of infected beehives. In this way, according to the World Organization for Animal Health (OIE), there are cases reported in the first half of 2019 in Europe (with declared infection in Finland), South Africa, North America, South America and Australia. Despite those data, many countries have no information available for knowing the real state of this disease around the world as it shows **Figure 1**.

The infectious pathway of *P. larvae* is through spores that can survive in the environment for many years, contaminating beehive materials and apiculture products. These spores are particularly resistant to heat and a number of chemical compounds. Once bee larvae have ingested food contaminated with spores, the bacteria, in a vegetative state, proliferate without damaging the stomach lining of the larva. During this infectious stage, bacteria obtain nutrients from food ingested by the larva [43]. American foulbrood affects larvae in any of the three honey-bee castes. The most susceptible, however, are immunosuppressed bees due to exposure to environmental contaminants (e.g. pesticides, metals) or that have suffered any of the aforementioned diseases. During outbreaks, *P. larvae* spores can be found in the honey and beeswax, and pillaging from sick hives, the use of contaminated beekeeping materials, and poor beehive management, among other factors, can contribute to the spread of disease [44].

Bee colonies present a coexistence mechanism with *P. larvae.* This host-etiological agent relationship has existed for more than 2400 years and is a highly specific infection, with germination possible only in bee larvae aged 1 or 2 days [45].

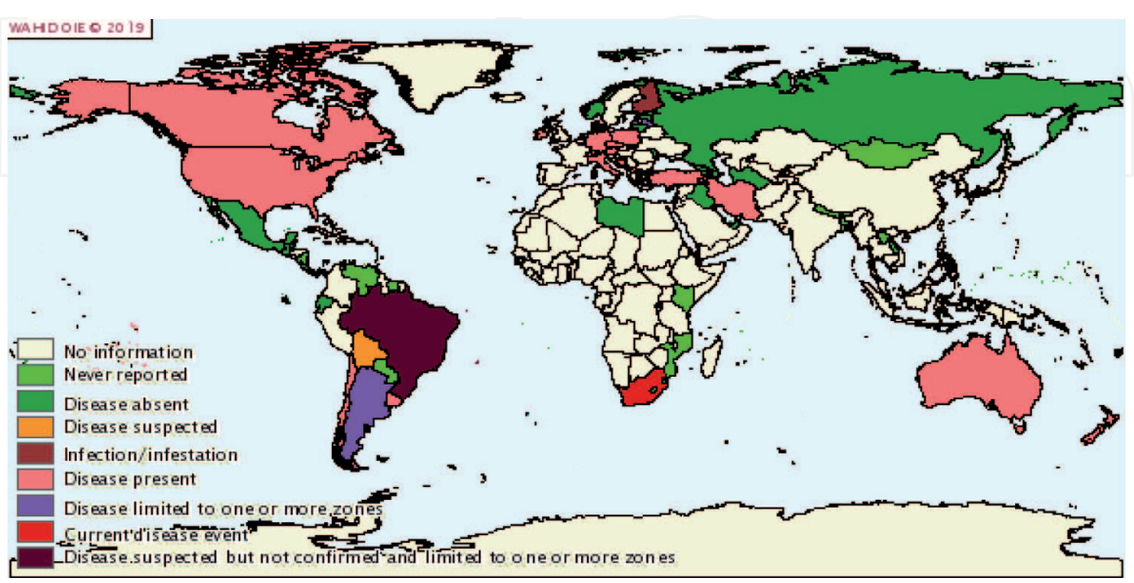


**Figure 1.**
*Dynamic maps showing the presence or absence of American foulbrood at the national and sub-national levels. Information based on 6-monthly reports (first half of 2019) according to the data base taken from World Organization for Animal Health (OIE).*

Nevertheless, this microorganism has at least four distinct genotypes (ERIC I-IV) that modulate infection with different degrees of pathogenicity. The ERIC I and ERIC II genotypes were found to be the most aggressive through repetitive-element PCR analyses performed with primers amplifying enterobacterial repetitive intergenic consensus elements [46]. Therefore, American foulbrood infection can, in some cases, mean a total loss of colony larvae. In other cases, hives can survive with the spores and, even, never show visible clinical symptoms [47]. Inadequate management by beekeepers can result in a disease outbreak, specifically by unbalancing the internal equilibrium of the beehive and provoking a violent increase in the load of spores within larvae nests [48].

The symptoms and effects of American foulbrood manifest slowly in beehives and occur while larvae receive contaminated food. In this stage, disease is not visible, but the first signs include the presence of dark, sunken, and greasy cappings that may be perforated by bees removing brood already in the process of putrefaction [49]. Finally, hive death occurs due to the lack of new, live brood and the aging and death of adult bees. The weakened hive then become an easy target for pillaging by bees from stronger hives seeking food reserves. Such pillaging serves to propagate the disease in nearby beehives and, consequently, the entire apiary [43, 50].

## 3.1 Control strategies

### *3.1.1 Antibiotic treatments and the analytical methods for detecting residues in honey*

The need to control American foulbrood is principally driven by damage caused by infection, which can include the loss of beehives and compromised honey and queen-bee exports. The use of tetracycline prophylactics is widespread in large animals and is allowed for bees in some honey-producing countries. In most countries, however, *P. larvae* expansion is controlled through the total incineration of hives with active infections [51]. The application and uses of veterinary antibiotics have been restricted primarily due to the appearance of antibiotic-resistant *P. larvae* strains. Such resistance could partially be due to the frequent application of veterinary drugs to prevent and control potential infestations, even in the absence of disease diagnosis [52]. In addition to antibiotic-resistance in *P. larvae,* the presence of antibiotics represents a health risk for consumers of contaminated honeys.

Where antibiotic use is allowed, maximum residual limits range between 10 and 50 ppb. These limits are intended to minimize the presence of antibiotic compounds in end-products, such as honey [53]. Antibiotics can, undoubtedly, affect the properties, quality, and, finally, export price of honey. Additionally, some purchasing countries regulate against the presence of antibiotics in beehives, thus impacting beekeepers that export honey [54–56]. This is a particularly relevant point for Chilean beekeepers as the primary export market is Europe, which has zero tolerance for antibiotics in imported honey (**Table 1**) [42]. These strict regulations require the determination of each compound in honey through highly sensitive analytical methods.

Several studies have aimed to develop reliable methods for detecting and quantifying the presence of antibiotics in complex organic matrixes, such as honey. Despite the ban of antibiotics in beekeeping, these substances have been detected in various European honey samples [57]. Liquid chromatography with UV–Vis detection resulted in the isolation of tetracycline, oxytetracycline, chlortetracycline, doxycycline, minocycline, and methacycline in different fortified honey samples cleaned by solid-phase extraction [58]. A more recent methodology with good

| Antibiotic | Maximum residual limit |
|---|---|
| Oxytetracycline | Forbidden |
| Tylosin | Forbidden |
| Lincomycin | Forbidden |
| Streptomycin | Forbidden |
| Sulfonamides | Forbidden |
| Chloramphenicol | Forbidden |
| Nitrofurans | Forbidden |

**Table 1.**
*Maximum residual limits for antibiotics in the European Union.*

results is QuEChERS solid-phase extraction followed by liquid chromatography tandem mass spectrometry [59].

Antibiotic resistance against tetracyclines by American and European foulbrood strains has led to research of other antibiotics. Sulfonamides have been widely used, but specific methods of determining and detecting these compounds in honey are needed since toxic collateral effects in association with allergies have been observed in humans [60]. To this end, high performance liquid chromatography paired with time-of-flight mass spectrophotometry has detected trace amounts of these compounds through direct injection [61].

Tylosin, a macrolide antibiotic active against many Gram-positive bacteria, has been increasingly used instead of tetracyclines and sulfonamides in beekeeping. Nevertheless, American foulbrood also presents resistance against macrolides. The best methodology for detecting macrolides in honey samples is solid-phase extraction followed by liquid chromatography tandem mass spectrophotometry [62]. Another type of antibiotic used against American and European foulbrood is streptomycin. This aminoglycoside can potentially control foulbrood disease in beehives. Traditional methods of detection include high-performance liquid chromatography with different strategies of solid-phase extraction [63, 64]. The adverse effects to consumers of honeys contaminated by streptomycin include acute otitis and allergic dermatitis [65].

Finally, a number of antibiotics have been fully banned in the control of American foulbrood due to adverse effects to human health. For example, nitrofurans are associated with possible carcinogenic effects while chloramphenicol can cause aplastic anemia, in addition to evidencing possible carcinogenic risks [59, 66].

### *3.1.2 Nuclear irradiation*

One reliable and traceable treatment for efficiently eliminating the highly resistant *P. larvae* spores is the gamma irradiation (15 kGy) of structural components in beehives [67]. Effective treatment would reduce the significant economic losses caused by the destruction of all material contaminated with *P. larvae.* An important advantage of this methodology is that the same procedure can be used to control various diseases at once; i.e., fungal, viral, and bacterial diseases affecting bees can be effectively eliminated through gamma irradiation [68]. Nevertheless, the use of gamma irradiation to control apiculture diseases is restricted only to the elimination of spores in honey, beeswax, and inert material in the hive. Irradiation cannot be used on live individuals within the hive due to previously reported adverse effects [69].

### *3.1.3 Antimicrobial peptides*

An alternative strategy for controlling and combating *P. larvae* has been through peptides that an act as natural antibiotics against this microorganism. Some peptides evidencing infection resistance have already been isolated from adult melliferous bees [70]. More recent studies have established which peptides with antibiotic activity originate from symbiont bacteria present in bees, such as lactic acid bacteria and *Brevibacillus laterosporus* [71, 72].

## 4. Conclusions

American foulbrood has been present since the beginning of beekeeping and has evolved over time. Nevertheless, the apiculture industry today faces a complex situation. The effects of climate change have modified the availability of nutrients and food for bees, ultimately weakening hive health. Food availability for bees has been further decreased by the use of agrochemicals and the occurrence of extensive, devastating forest fires. These situations have provoked a resurgence of American foulbrood outbreaks, which need to be controlled to mitigate population and economic losses. Researchers specializing in apiculture should focus efforts on the search for new, environmentally friendly control strategies against this disease. Such efforts will help prevent the use of antibiotics, which in addition to inducing *P. larvae* resistance can lead to adverse effects in individuals who consume honeys contaminated by veterinary-use drugs.

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## Conflict of interest

The author declares no conflict of interest.

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