Journal of Invertebrate Pathology 103 (2010) S5–S9

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

European foulbrood in honey bees

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ARTICLE INFO

Article history:
Received 26 May 2009
Accepted 15 June 2009
Available online 21 December 2009

Keywords:
Apis mellifera
European foulbrood
Melissococcus plutonius

ABSTRACT

European foulbrood (EFB) is a severe bacterial brood disease caused by the Gram-positive bacterium *Melissocccus plutonius*. The disease has a worldwide distribution and is an increasing problem in some areas. Although the causative agent of EFB was described almost a century ago, many basic aspects of its pathogenesis are still unknown. This review presents both historical results and recent molecular data to synthesize present knowledge of this enigmatic honey bee disease.

1. Introduction

Honey bees (*Apis mellifera*) are not only important for the honey they produce but they are also vital as pollinators of agricultural and horticultural crops. Hence the health of honey bees has a great economic impact worldwide. In recent years, serious losses of bees from beehives and a decline in bee populations have been reported. The cause of these losses is not known, although various hypotheses have been forwarded, including pathogenic microorganisms. A large diversity of microorganisms are associated with honey bees (Gilliam, 1997; Olofsson and Vasquez, 2008). Most of them are beneficial but some are also very harmful. Honey bee brood and adults are attacked by a range of disease causing organisms such as bacteria, viruses, protozoa, fungi and parasitic mites. Two of the most economically important diseases of honey bees are bacterial diseases affecting the brood. American foulbrood (AFB) and European foulbrood (EFB) are both widely distributed and potentially lethal to infected colonies. EFB occurs in most areas where apiculture is practiced, but has not yet been reported to occur in New Zealand (Ellis and Munn, 2005; Hornitzky and Wilson, 1989). It is the most widespread bacterial brood disease in Great Britain (Wilkins et al., 2007), and of specific interest is the development in Switzerland, where the incidence of EFB has constantly risen each year since the late 1990s, even though clinically diseased colonies are eradicated (Roetschi et al., 2008).

This review aims to present up-to-date information on EFB and to synthesize present knowledge of this obscure disease of honey bees.

2. The etiological agent

The name "foulbrood" was first introduced as a descriptive name of a honey bee disease characterized by a foul smell (Schirach, 1769). The symptoms of foulbrood disease were further described in the late 19th century (Cheshire and Cheyne, 1885), but it was not until 1912 that it became clear that there were actually two honey bee brood diseases assigned the same name (White, 1912); American foulbrood caused by the spore forming bacterium *Paenibacillus larvae* (Genersch et al., 2006) and European foulbrood caused by the bacterium *Melissococcus plutonius* (see Figs. 1–3) (Bailey, 1956, 1983). The bacterium is isolated not only from the European honey bee, *A. mellifera,* but also from *Apis cerana* (Bailey, 1974) and *Apis laboriosa* (Allen et al., 1990).

EFB affects mainly unsealed brood, killing honey bee larvae usually when they are 4–5 days old. The infected larva moves in the brood cell, and instead of the normal, coiled position, the larvae dies displaced in its cell; twisted around the walls or stretched out lengthways. The color of the larvae changes from pearly white to yellow, then brown and finally, when they decompose, grayish black (Bailey, 1961). Some larvae may also die after the cell is sealed, resulting in sunken capping resembling the symptoms of AFB. If a high proportion of the larvae die, the brood pattern appears patchy and sometimes gives off a foul or sour smell.

The causative agent of the disease, *M. plutonius,* is a Gram-positive, lanceolate coccus, sometimes pleomorphic and rod-like. The bacterial cells occur singly, in pairs or in chains of various lengths. *M. plutonius* is microaerophilic to anaerobic and needs carbon dioxide for growth. Several other bacteria may be associated with EFB, and most of them have from one time to another been considered to be the primary pathogen (see below for secondary invaders). European foulbrood was originally thought to be caused by *Bacillus alvei* (Cheshire and Cheyne, 1885) but was later

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0022-2011/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2009.06.016

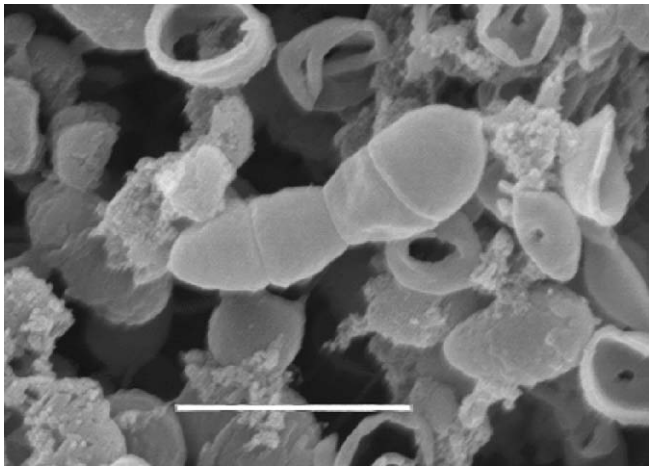

Fig. 1. Scanning electron micrograph of *Melissococcus plutonius*. The bar represents 1 μm. Image courtesy of Ingemar Fries.

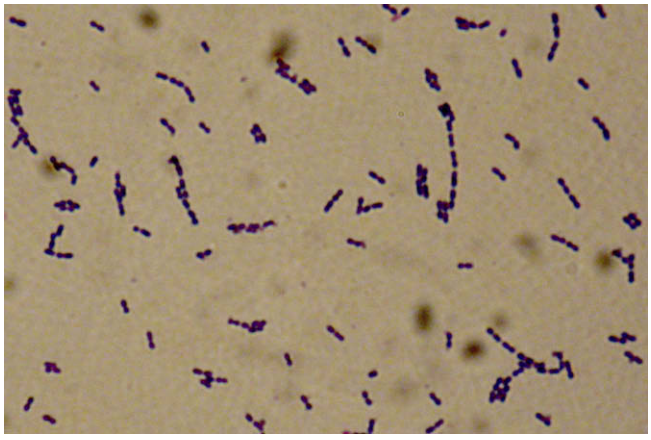

Fig. 2. Gram staining of *Melissococcus plutonius*. Photograph taken by Lena Lundgren and Karl-Erik Johansson.

Fig. 3. Symptoms of European foulbrood. Photograph courtesy of Preben Kristiansen.

described by White (1912) as *Bacillus pluton*. The organism was first cultivated and characterized by Bailey (1957) who concluded that *Streptococcus pluton* would be a more suitable name for the bacterium. Since biochemical analysis of several strains of *S. pluton* showed a low guanine + cytosine base content of its nucleic acid, its retention within the genus *Streptococcus* was not supported (Bailey and Collins, 1982a). Therefore, a new monospecific genus *Melissococcus* was described and the type species was named *Melissococcus pluton* (Allen and Ball, 1993; Bailey and Collins, 1982a,b). Later this name was modified to *Melissococcus plutonius* (Truper and dé Clari, 1998). Cai and Collins (1994) made a comparative sequence analysis revealing that *M. plutonius* is a close phylogenetic relative of the genus *Enterococcus*. Furthermore, isolates of *M. plutonius* are remarkably homogenous based on morphological, physiological and immunological (Allen and Ball, 1993; Bailey and Gibbs, 1962) as well as genetic studies (Dancer and Barnes, 1995; Djordjevic et al., 1999).

3. Secondary invaders

Achromobacter euridice commonly occurs in healthy larvae and is much more frequent in larvae infected with *M. plutonius* in the northern hemisphere, but has not been reported from Australia (Djordjevic et al., 1998). *A. euridice* is frequently isolated in mixed culture with *M. plutonius* and EFB symptoms in larvae are more easily induced with inoculate containing both of these organisms than with *M. plutonius* alone (Bailey, 1956). The source of *A. euridice* seems to be the alimentary canal of adult bees, particularly those gathering fresh pollen (Bailey, 1959b).

Bacteria of the genus *Enterococcus* are found in a wide variety of habitats such as soil, water, plants and in the gastrointestinal tracts of animals and humans (Franz et al., 1999). *Enterococcus faecalis* grows abundantly in larvae infected with *M. plutonius*. The bacterium can be maintained in chronically diseased colonies and may be pathogenic in heavy doses (Bailey, 1963). Increasingly pathogenic strains of *E. faecalis* may be selected in colonies where EFB is aggravated artificially, as can occur in dense beekeeping areas (Bailey, 1961, 1963).

Paenibacillus alvei is a saprophytic, aerobic bacterium which cannot grow in the gut of a normal larva, but becomes established in chronically diseased colonies growing in larval remains (Bailey, 1963). Because it is a spore producing bacterium, it survives, germinates and multiplies more readily in dead bees than other saprophytes. *Brevibacillus laterosporus* may also, in a similar way, occasionally occur in larval remains (Bailey and Ball, 1991).

Each of the microorganisms mentioned above has at one time or another been considered to be the causative agent of EFB; however no experimental data have been presented to justify such claims. In contrast, many infectivity tests causing disease in bee colonies with cultured *M. plutonius* have been reported (Bailey, 1957, 1963; Bailey and Locher, 1968) although more severe clinical symptoms were observed when using extracted naturally infected larvae (Bailey, 1960, 1963). This may partly be due to the demonstrated quick decrease of *M. plutonius* virulence after culturing (Bailey, 1963). A more recent experiment, using infectivity tests in artificially reared honey bee larvae showed a strong correlation between mortality of larvae that died from EFB and the dose of *M. plutonius* (McKee et al., 2004).

4. Pathology and pathogenesis

The first step in EFB infection is the asymptomatic colonization of the gut. Bacterial cells of *M. plutonius* are digested with contaminated food and multiply vigorously within the midgut of the honey bee larva. Larvae are susceptible at any stage, but the older they are the less they are affected by the infection (Bailey and Ball, 1991). One hundred or fewer bacterial cells are enough to cause infection in the larva (Bailey, 1960). McKee and co-workers (2004) transmitted EFB to healthy larvae at a minimum concentration of 200 bacterial cells per milliliter and reported a strong

correlation between the dose of *M. plutonius* and the larval mortality. The infection in individual larvae is not always lethal. The larvae may die before capping and be ejected from the colony, die after capping and void their infective, intestinal contents within the brood-comb cell or succeed to pupate and form normal or undersized adults (Bailey, 1959b). Even larvae experimentally fed high doses of *M. plutonius* in bioassays survived the infection to reach pupation, although pupation was delayed (McKee et al., 2004).

The mechanisms of pathogenesis and the role of secondary invaders in EFB are poorly understood. Tarr (1936) reported that EFB was purely an intestinal infection localized in the food mass and the peritrophic membrane interface. Bailey (1983) suggested that the pathogenic effect from *M. plutonius* infection results in the competition for nutrients between the infected larva and the pathogen resulting in starvation of larvae. However, in their *in vitro* infection experiments, McKee et al., 2004, provided infected larvae with food in excess and yet disease symptoms and death of infected larvae occurred. Thus, they suggested that the death of the larvae may be a result of additional pathogenic mechanisms such as invasion of the peritrophic membrane and penetration of the host tissue. The factors leading to the second step of infection – tissue damage and the phase of overt symptomatology remain enigmatic. Besides the hypothesis involving certain nutritional conditions, larval immune response (Evans, 2004), hygienic behavior (Spivak and Reuter, 2001) or interaction between *M. plutonius* and the intestinal microbiota of the honey bee larvae (Gilliam, 1997; Olofsson and Vasquez, 2008) are factors possibly linked to pathogenesis.

Interesting comparisons can be made looking at the closely related bacterium *E. faecalis*, where a number of enterococcal virulence factors have been identified (Tendolkar et al., 2003). In general, these bacteria are well suited for inhabiting the mammalian gastrointestinal tract and for the most part exist as commensals in harmony with the host as well as other coresident gut flora. However, antibiotic treatment, diminished host immunity as well as acquisition of new traits that allow them to overcome host defenses and colonize new niches, can allow bacteria to deviate from their commensal behavior and become pathogenic (Tendolkar et al., 2003).

5. Persistence and transmission

In nature, *M. plutonius* multiply only within the larval gut of the honey bee. In the bee colony, pathogen transmission and persistence seem to depend on the survival of infected individuals, which deposit the bacteria along with their feces into the comb when they pupate. *M. plutonius* remain viable in these deposits, surviving long periods of desiccation (Bailey, 1959a), and although many of the bacterial cells are cleared away, some manage to infect other larvae. If the infected larva dies before it pupates, most of the bacteria in them are eliminated from the colony when they are cleaned out by adult bees. Not all individuals within the colony are colonized by the bacterium. *M. plutonius* can be detected in larvae and pupae without any clinical symptoms although mainly in diseased larvae within limited areas of the brood (Forsgren et al., 2005). Adult worker bees collected from the brood nests in diseased colonies have higher bacterial loads than bees collected from the flight entrance (Roetschi et al., 2008), probably because nest bees inhabiting the brood area are in close contact with the infected brood containing massive amounts of the bacterium. Worker bees from the brood nest in apparently healthy colonies can also contain surprisingly high levels of *M. plutonius* (Roetschi et al., 2008). Adult worker bees act as carriers of the bacterium not only within the colony, but also between colonies and apiaries (Belloy et al., 2007; McKee et al., 2003). More than a third of colonies from apparently healthy apiaries may include adult bees carrying the bacterium, depending on proximity to other apiaries with clinical cases of EFB and almost all colonies within these infected apiaries contain adults that carry the bacterium. The spatial structure of the host is important for EFB transmission because the high density of colonies and apiaries has been shown to promote pathogen transmission (Belloy et al., 2007). Honey has also been demonstrated to be commonly contaminated with *M. plutonius* (Hornitzky and Smith, 1998; McKee et al., 2003) suggesting that robbing may contribute to the spread of the bacterium between colonies and apiaries. Routine beekeeping practices may also contribute to the sometimes rapid spread of EFB.

Outbreak of the disease appears to be linked to colony stress conditions, such as lack of food or water. Genetic factors, weather and geography may also play a role (Bailey, 1961). Most larvae die within a brief period, usually around midsummer (Bailey 1981), and sudden outbreaks of the disease followed by a spontaneous recovery a few weeks later has been observed (Bailey, 1961). Severely infected colonies moved from endemically infected areas to areas free of disease can recover spontaneously and become apparently healthy (Bailey and Locher, 1968).

6. Detection and diagnosis

Diagnosis of EFB in the field is based on the visual inspection of brood-combs and detection of diseased larvae. However, symptoms of EFB may easily be confused with other diseases or abnormalities in the brood, making diagnosis difficult. The diagnosis in the field can be further verified by microscopic examination of brood smear preparations (Hornitzky and Smith, 1998; Hornitzky and Wilson, 1989), and a field test kit for the detection of *M. plutonius* in extracts of infected tissue have recently been presented (Tomkies et al., 2009). Correct sampling is important because even within the same brood frame, *M. plutonius* is mainly found in larvae with visual disease symptoms whereas healthy looking larvae can be free from the bacterium (Forsgren et al., 2005).

Although the isolation of the pathogenic agent is not always mandatory for the diagnosis of EFB, specific and sensitive detection methods may be required to ensure the absence of the bacterium from bee products, for the confirmation of the ocular diagnosis made in the field or for research purposes. There are selective media for the cultivation of *M. plutonius* involving anaerobic incubation on yeast extract medium supplemented with sugar, starch, cysteine and potassium (Allen and Ball, 1993; Bailey, 1983; Bailey and Collins, 1982a; Hornitzky and Wilson, 1989; Hornitzky and Karlovskis, 1989). Although *M. plutonius* can be isolated from honey and diseased brood by cultivation, bacterial culture methods seem to be very insensitive, detecting less than 0.2% of microscopically counted bacterial cells (Djordjevic et al., 1998; Hornitzky and Smith, 1998) and cultivation of the bacterium from honey yielded growth only in 6.2% of the samples (Hornitzky and Smith, 1998). Staining of brood smears (Hornitzky and Wilson, 1989), immunology-based tests (ELISA) (Pinnock and Featherstone, 1984) and probe based hybridization analysis (Behr et al., 2000; Dancer and Barnes, 1995) are other methods described for the detection and identification of *M. plutonius*.

DNA amplification using the polymerase chain reaction (PCR) has been used in a number of different applications, including detection of *M. plutonius*. In the late 1990s, a PCR for detection of *M. plutonius* in diseased larvae was published (Govan et al., 1998). At the same time, Djordjevic et al., 1998 presented a hemi-nested PCR assay for detection of *M. plutonius*. This method was further developed for the detection of *M. plutonius* in larvae,

adult bees, honey and pollen (McKee et al., 2003). Recently, a real-time PCR assay for the quantification of *M. plutonius* was developed (Roetschi et al., 2008), making quantification of the bacterium possible. Molecular diagnostics tools such as PCR make it possible to detect infection before any clinical signs of disease are visible in the colony. Hence, analyzing pooled samples of worker bees from the brood nests of several colonies within an apiary have been suggested as an alternative to routine visual brood control (Roetschi et al., 2008).

7. Prevention and control

Oxytetracycline hydrochloride (OTC) is a bacteriostatic antibiotic that inhibits the multiplication of *M. plutonius* and is used in many countries (Thompson and Brown, 2001). American beekeepers have used the antibiotic for prevention of both EFB and AFB since the 1950s, and OTC resistance of *P. larvae* has been reported (Miyagi et al., 2000). No resistance to OTC has been found in *M. plutonius* and studies in the UK showed continued susceptibility of the bacterium (Waite et al., 2003). In Australia, another area where EFB is controlled with OTC, isolates of *M. plutonius* are still sensitive to this antibiotic (Hornitzky and Smith, 1999). EFB infected colonies in Great Britain may be treated with either OTC or the shook swarm method (shaking the bees onto new comb foundation and destroying the infected comb), but heavily infected colonies are destroyed (Wilkins et al., 2007). The shook swarm method in combination with OTC lowered the colony level recurrence rate of clinical symptoms (Waite et al., 2003). However, recently the shook swarm method without antibiotic treatment was recommended for EFB control (Thompson et al., 2006; Waite et al., 2003) because clinical signs of disease may be suppressed by the use of the bacteriostatic and beekeepers unconsciously may spread the infection between colonies. In Switzerland, another endemic EFB location, the sanitation procedure is quite similar to Great Britain although the use of antibiotics is restricted. Colonies with clinical symptoms must be destroyed although Roetschi et al., (2008) showed that the sanitation procedure applied in Switzerland is not sufficient to prevent new EFB outbreaks in the same apiaries the following year.

8. Concluding remarks

European foulbrood is a bacterial disease of honey bees that appears to be benign in some areas and more severe in other parts of the world. Apiculture may locally agglomerate extremely high densities of honey bee populations thus promoting transmission of the pathogen. This may partly explain the situation in Switzerland where EFB at the moment is a major problem. However, the differences and variation in severity of the disease may also be due to other factors such as climatic variations, variation in nutritional qualities of food and genetic variations of the host as well as variations in virulence in the pathogenic agent. Since *M. plutonius* seems to be a genetically homogenous pathogen irrespective of geographic origin, differences in virulence between isolates may be relatively minor. If differences in pathogenicity are responsible for the variation in severity of EFB, then these differences would likely be reflected as variations in expression of virulence factors. By employing new techniques, future studies may be able to unravel some of the mechanisms whereby *M. plutonius* produce infection and provide knowledge for the development of improved strategies for the control of EFB.

Conflicts of interest

There are no conflicts of interest to be declared.

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