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Mini-review

EUROPEAN FOULBROOD DISEASE –AETIOLOGY, DIAGNOSTICS AND CONTROL

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ABSTRACT

On the basis of bibliographic data and our own experience, the contemporary concepts about the aetiology, the methods of laboratory diagnosis, the control and prophylaxis of European foulbrood disease are hereby outlined. The role of secondary microflora in the aetiology of this disease is discussed.

Key words: European foulbrood disease, *Melissococcus pluton, Paenibacillus alvei*

INTRODUCTION

The European foulbrood (benign, sour) is an infectious disease prevalent among the open, uncapped brood at the age of 3–4 days. In this situation the larvae of the three honeybee castes, workers, drones and queens, are affected. The disease is not observed in the pupae and adult bees. Compared to the American (malignant) foulbrood, the course of the disease is less severe (1) but due to its wide prevalence, it causes considerable economic losses to apiculture. This condition is due rather to weakening, stunted development of colonies and reduction in honeybee products than to the death of affected bee families.

The European foulbrood occurs in all continents but is economically most important in North and South America, Europe, Japan, Australia, India and South Africa (2).

Many investigators have since identified this disease. According to some data provided by these investigators, the European foulbrood is a group of infectious diseases caused by various spore-forming and non-spore-forming micro-organisms comprising, *Melissococcus pluton, Paenibacillus alvei, Bacillus laterosporus, Achromobacter euridice, Enterococcus faecalis* and *Enterococcus faecium* (3, 4, 5), and each infection manifests similar clinical signs.

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It is presently thought that the European foulbrood is caused by *Melissococcus pluton* (6, 7, 8, 9). The other microorganisms cause secondary infections in already infected larvae, add to the virulence by impacting on the clinical signs and symptoms of the disease and finally add to the death of these hosts.

M. pluton is a Gram-positive lancet-shaped bacterium with dimensions of about 0.5 x 1 µm (**Figure 1**). It is abundant in smears from the intestine of larvae in the early stages of the infection (6). It occurs singly, in pairs or in chains. Although *M. pluton* is sensitive to dessication and UV radiation (10), it could survive up to 3 years in dry remnants of dead larvae at room temperature, and up to 65 days in wax (3). It is not resistant to high temperatures. It dies in 15 min following exposure to a 60˚C environment; it dies after 6 h in 2% phenol, in 10 min in 0.5% formaldehyde and in 30 min following exposure to 0.5% potassium permanganate.

The vegetative form of *P. alvei* is a Gram-positive rod measuring 2.0-7.0 x 0.8–1.2 µm and is a perithrix with weak motility. It forms long oval spores of 2.5–4.0 x 0.8–1.5 µm located centrally. Usually, the spores appear stacked, often with remnants of the vegetative cell on one or both poles (**Figure 2**). The vegetative cells and the spores of *P. alvei* are bigger than those of *P. larvae subsp. larvae* – the causative agent of the American foulbrood. *P. alvei* is multiplied only in rotting remnants of dead larvae and, often, its

spores prevail on other microorganisms. According to Bailey et al. (11) *P. alvei* is frequently the first indication of the presence of *M. pluton* and is almost always isolated together with the primary aetiological agent. The data of Toshkov et al. (12) show that the principal causative agent of the European foulbrood in Bulgaria is *B. alvei*. This is probably related to the cultivation characteristics of the bacillus, stated below. Unlike *M. pluton*, that is associated only with brood pathology, *P. alvei* is found in various ecological niches – it is isolated from soil, mosquito larvae, wax moths and patients with bacterial infection (13, 14, 15).

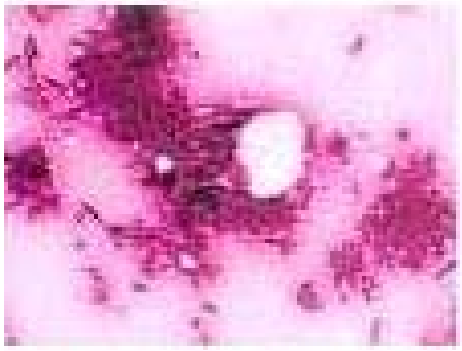

Figure 1. *M. pluton*

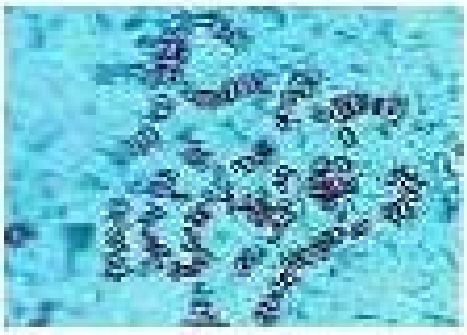

Figure 2. *P. alvei*

Achromobacter euridice is a thin Gram-negative immotile rod with dimensions of 0.2–0.4 x 0.8–2.8 μm. It does not form spores and capsules. It occurs singly or in chains. This bacterium is closely related to the life of the bee family (16). It is found in the alimentary tract of adult bees and is usually present at a lesser extent in the intestine of healthy brood. At a greater extent, *A. euridice* is observed during a *M. pluton* infection.

Bacillus laterosporus is a spore-forming microorganism with dimensions of 2.0–5.0 x 1.0–1.2 μm, and spore size of 1.2–1.5 x 1.0–1.3 μm. The spores are formed in the middle of the rod but only unilaterally. *B. laterosporus* is isolated less commonly from dead larvae bodies.

Enterococcus faecalis and *Enterococcus faecium* are similar to *M. pluton* from a morphological point of view, but have a different cultural behaviour. *Enterococci* do not grow in honeybee larvae in the absence of *M. pluton*, so their detection is considered to be an index of infection with European foulbrood (6).

Spore-forming microorganisms are much resistant to physico-chemical factors. In wax, *P. alvei* spores are killed in 2 h after heating at 120˚C and those of *B. laterosporus* are neutralised after 15 min in boiling water. *Enterococci* die in 30 min at 60 ˚C (17).

The microorganisms involved in the aetiology of European foulbrood are widely spread in nature. Often, they are detected in healthy bee colonies where they could reside for years without clinical manifestation of the disease. The disease is triggered when the resistance of the bee colony, and more precisely the bee larvae, is reduced. Various factors predispose to this event: technological mistakes during honey bee rearing, climatic conditions, infectious or parasitic diseases in adult bees, intoxication of bees with pesticides etc.

The most important technological mistakes are related to the inadequate autumn management concerned with preparing for winter. Factors contributing to early amortisation and weakening of bee colonies during the spring include the following: the wintering of weak bee colonies with deficiency of honey and pollen, the misuse of bees through the processing of large amounts of sugar syrup in the autumn, the late treatment with various drugs against varroatosis, the wintering of honeydew or sugar honey of insufficiently condensed or uncapped honey, inadequate crowding and warming of bee families and humidity in bee colonies (18).

Climatic conditions are important predisposing factors for the onset of the disease. For example, the alternating rise and fall in temperature during the spring impede the maintenance of an optimal microclimate in the hive and thus the brood in peripheral combs is reared at temperatures lower than the optimal. This weakens the resistance of larvae and creates the right background for the development of these parasitic microorganisms.

The appearance of some diseases at the end of winter/beginning of spring such as nosematosis, hafniosis, collibacteriosis and mycoses also results in the weakening of bee families and the development of European

foulbrood. Some authors emphasise the role of varroatosis in this respect (18, 19). The forms of the mite *Varroa Destructor* destabilise the resistance of bee larvae through suckling of the haemolymph and also opening an entrance door for putrid microflora.

European foulbrood appears usually in spring and the first half of summer but in recent times has not shown a clear dependence on seasons. An important cause for outbreaks is the intoxication of bee families with pesticides used in plant protection. This results in death of many flying and nest bees, ingestion of subtoxic doses during larvae feeding and decrease in their resistance to disease. Last but not the least, the changes in plant-growing technologies related to barren (without crops) periods (lack of blooming flora) are the cause for a relative starvation of bees.

The European foulbrood affects larvae at the age of 3–4 days. The younger larvae (1–2-day old) are not infected because they are fed with royal jelly that has bactericidal properties.

The microorganisms get into the gut of the larva via food. They are localised in the so-called peritrophic membrane of the mid gut, multiply and release toxic products injuring vital larval organs and tissues.

The spread of infection into the colony occurs mainly by bees responsible for removing sick or dead larvae; in the process their mouth organs are contaminated. The healthy larvae are infected by nurse bees that do the feeding process, following the cleaning of the cells. The beekeepers, the robberies, the stray bees, the infected swarms, contaminated equipment and honey bee products are also most important for spreading of infection.

Most infected colonies show visible signs of this but these signs frequently disappear during the active season. The infection remains however endemic in the different families because of the mechanical contamination of combs with resistant microorganisms.

The clinical manifestation of European foulbrood is various and is characterised by the following clinical symptoms:

A characteristic patchy (pepperpot) brood pattern (on the same side of the wax comb, the brood is composed of younger and older larvae, eggs and empty cells)

The larvae die prior to capping but, in more severe infections, after sealing as well.

Dying larvae lose their usual pearl-white shining colour (**Figure 3**); their body becomes more transparent and the tracheal branches and the gut are visualised. Larvae change their natural position in the cell – they rise, twist up or down (**Figure 4**). Later their colour changes to yellowish, cream with tendency towards growing darker (**Figure 5**). This is the phase of the dead larva.

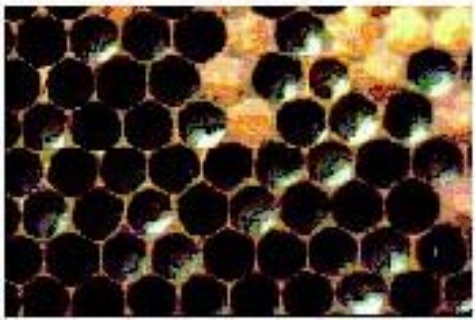

Figure 3. *Larvae with normal colour*

Figure 4. *Larvae with changed natural position in cells*

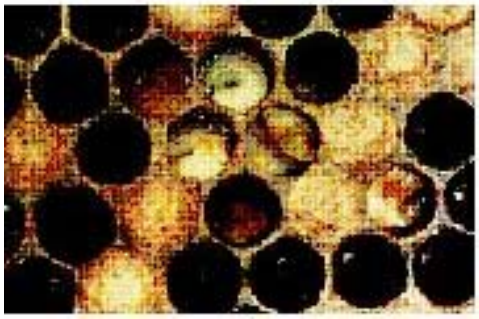

Figure 5. *Dead larvae with altered colour*

The consistency of dead brood is pasty, watery-granular, but never sticky as in the American foulbrood. After the drying of the putrid mass, brown or dark-brown scales are formed and these are easily removed from cells.

The decayed larvae give off unpleasant sour or putrid odour (when *P. alvei* and *Enterococcus spp*. are involved respectively), or odourless, depending on the type of secondarily developing microflora.

In some cases, the larvae die after sealing and the clinical picture of the disease resembles that of the American foulbrood, especially in extensive growth of *P. alvei.*

Table 1 summarises the features of the clinical manifestation of European and American foulbrood diseases.

Table 1. *Features of the primary symptoms in brood affected by European and American foulbrood*

Parameters	***European foulbrood***	***American foulbrood***
Age of affected brood	Usually uncapped brood 3-4 days of age, in C- shape.	Sealed brood
Appearance of cappings	Some cells could be with sunken, punctured cappings with altered colour	Sunken, punctured cappings with dark colour
Colour of affected brood	Yellowish, cream-coloured, later brown to almost black.	Dull white to light brown, later dark brown to black
Dead brood consistency	Pasty to granular-watery	Sticky and ropy
Odour	Sour to putrid or odourless	Unpleasant odour of glue-pot
Appearance of dead larvae	Yellowish to light brown, later dark brown scales that are easily removed from cells	Dark brown scales, tightly stuck on cell's bottom and walls, hard to remove

The European foulbrood is diagnosed on the basis of the clinical signs and is confirmed after bacteriological examination. When the European foulbrood is suspected, a 10x15 cm piece of the comb with affected brood is sent to the appropriate laboratory.

LABORATORY DIAGNOSIS

Microscopy: Preparations for microscopy are made from the mid gut of infected larvae, stained with Gram and observed under a magnification of x 1000. The presence of numerous Gram-positive lancet-shaped *cocci*, occurring singly, in chains or in groups is an almost positive diagnostic marker for European foulbrood (20). The smears made of decayed larvae suspension however exhibit various microorganisms from a morphological point of view and *M. pluton* could hardly be observed. Our experience shows that in such cases, aside the single lancet-shaped *cocci*, spore forms resembling the spores of *P. larvae subsp. larvae* are most frequently observed. When sealed cells are affected, the features are similar to those in the American foulbrood disease. In such cases, further studies are necessary in order to differentiate *M. pluton* and *Enterococcus spp*. and to accept or reject the diagnosis of American foulbrood.

Culture. The laboratory diagnosis is entirely positive after isolation of *M. pluton* on an artificial nutrient medium. M. pluton is a very exigent microorganism, except for isolates from Brazil (21) and could be cultivated on a medium described by Bailey (22) and Hornitzky and Smith (23) containing: yeast extract, D-glucose, starch, KH_2PO_4, L-cysteine and agar. The pH of the medium is corrected to 6.7 and then is autoclaved for 25 min at 121˚C. For inhibition of secondary microflora growth, the nutrient medium is supplemented with 3µg/mL nalidixic acid. The petridishes are preferably inoculated with suspension from the mid gut of diseased larvae and incubated anaerobically at 35˚C for 7 days. After 4 days, small white colonies, characteristic of *M. pluton*, appear. It should be stated that this medium is also suitable for some secondary aetiological agents such as *P. alvei* and *Enterococcus spp*. The addition of nalidixic acid inhibits their growth but not completely. Furthermore, these organisms grow faster than *M. pluton*; the colonies of *P. alvei* migrate on the surface of the medium and impede the isolation of a pure *M. pluton* culture. Nevertheless, they could be differentiated by the morphology of their colonies and the biochemical properties (23).

Other methods. A sandwich ELISA for detection of *M. pluton* is developed, allowing identification of bacteria in apparently healthy bee colonies (24). The detection of *M. pluton* could also be done by immunological methods described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (25).

During the last years, the PCR techniques are used more and more frequently as rapid and specific tests for detection of various pathogens. Specific PCR techniques for *M. pluton* are described (26, 27), allowing fast and specific identification of the bacterium directly in diseased larvae. These techniques are especially useful considering the difficult isolation of the agent at a worldwide scale.

CONTROL

In recent times, the requirements of the EU about the quality and safety of bee products have increased. In response to the new situation, the use of antibiotics and chemotherapeutics for treatment and prophylaxis of bacterial diseases in honeybees were banned (28). In countries where these drugs are still used, the European foulbrood is controlled predominantly with the tetracycline antibiotics (29, 30, 31). The countries exporting bee products to the EU are facing the challenge of finding out alternative means for the European foulbrood control. In this respect, some nutritive supplements are attractive. The alternative means of control should respond to the following requirements: effectiveness against *M. pluton* both *in vitro* and *in vivo*, a short half-life, absence of their residues in honey after treatment of bee colonies, meet approval of the EU and to be user-friendly to beekeepers.

According to the new normative acts in the Republic of Bulgaria, the control of the European foulbrood is performed without the use of antibiotics. The bee families with severe and/or advanced disease are destroyed, and those that are slightly affected and yet strong are cured via:

- removing the combs from the hive with affected brood;
- moving bee colonies in other previously disinfected hives;
- isolation of queens for a week with regard to sealing the healthy brood and sanitation of hive;
- crowding of nests in order to populate all frames with bees;
- removing the moisture from hives;
- nutrition of families with honey and pollen, honey-sugar pie or sugar syrup;
- if possible, re-queening of colonies with young, fertilised queens;
- uniting weakened families and families with few bees;
- melting the old combs and combs with affected brood for wax.

It is necessary that beekeepers observe veterinary hygienic requirements when working with bee families – personal hygiene, disinfection of hands prior to manipulation of each hive, disinfection of apicultural equipment, working with separate frame lifters etc.

When European foulbrood infection is detected, the infected apiary and all apiaries in the region located within 3 km from infected one are quarantined. The duration of quarantine lasts up to 1 month from the recovery (respectively destruction) of the last diseased family.

The prophylaxis of European foulbrood should be directed at the maintenance of strong, productive bee families and preventing the effects of favourable factors:

Observation of veterinary hygienic requirements to the apiary in course of the daily work of beekeepers with bee families should be observed.

Provision of optimal life and development conditions during the winter-spring period via *correct wintering of bee families as follows:*

- young fall bees;
- without mite infection;
- sufficiently crowded, dry and warm nests;
- nutrient stores with adequate quantity and quality,

and during the spring through:

- timely crowding and stepwise widening of bee nests;
- cleansing and disinfection of hive bottoms;
- stimulation feeding etc.

When a sudden decrease in the number of bees occurs, as in technological mistakes, disease or chemical intoxication, crowding, heating and feeding of families should be carried out. In regions with late pollen collection or during period without blooming melliferous vegetation, the families should also be fed with sugar syrup in order to maintain their active state. Bee colonies with good apiary hygiene are to be reared. A current disinfection of hives with physical or chemical means is needed.

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