

MELISSOCOCCUS PLUTONIUS ISOLATION FROM HONEYBEE BROOD SAMPLES WITH EUROPEAN FOULBROOD IN SOME ITALIAN REGIONS.

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Introduction

European foulbrood is a disease of honeybee larvae caused by the bacterium *Melissococcus plutonius*. Larvae usually die 1–2 days before being sealed in their cells, or sometimes shortly afterwards, and always before transformation to pupae.

A diagnosis based on symptoms alone is not always reliable, as other causes may contribute to larval mortality. The most usual and obvious sign is the death of larvae shortly before they are due to be sealed in their cells.

Most infected colonies display few visible signs, which themselves often quickly abate spontaneously before the end of each active season. Infection remains enzootic within individual colonies because of mechanical contamination of the honeycombs by the durable microorganism. Recurrence of the disease can therefore be expected in subsequent years, though its incidence may vary.

In Italy, European foulbrood was a problem encountered only sporadically until recent times. In the past few years, however, several regions have reported recurrent outbreaks that have resulted in significant economic damage.

Material and Methods

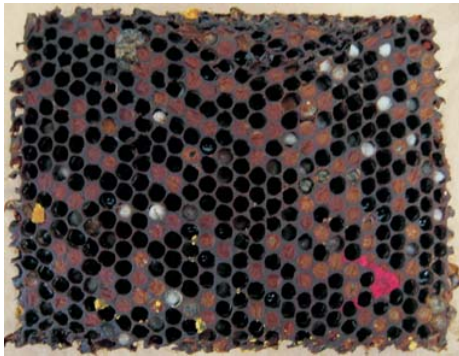
In the years 2004–2005, brood samples from hives displaying signs of likely infection with European foulbrood, weakening and, in some cases, death of families, were analysed for the presence of *Melissococcus plutonius* (Photo 1-2).

The samples examined came from several areas in the following regions: Sicily, Tuscany, Emilia Romagna, Piedmont, and Valle d'Aosta.

Before undergoing bacteriological examination several larvae were screened using the EFB diagnostic kit, specific to European Foulbrood, distributed by Vita (Europe) Ltd.

For the purpose of isolating the bacteria, aqueous suspensions of the larval gut or contents of diseased larvae were inoculated onto plates containing the medium described by Bailey. The plates were incubated for 4 days at 35°C in an anaerobic jar (Oxoid) with about 10 % (v/v) carbon dioxide using AnaeroGen System (Oxoid). Smears prepared from the same material were stained with both 5% nigrosin and 0.2% carbol-fuchsin and examined for the presence of bacteria by light microscopy at a magnification of x 1000.

Morphological characteristics were investigated by submitting small white colonies (up to 1 mm in diameter, ranging from dense to granular) to Gram's stain and staining with 5% nigrosin. Reference strains of *Melissococcus plutonius* ATCC 35311 were used in all assays.



1
Honeybee brood with signs of European foulbrood



2
Larvae of unsealed brood positive EFB kit

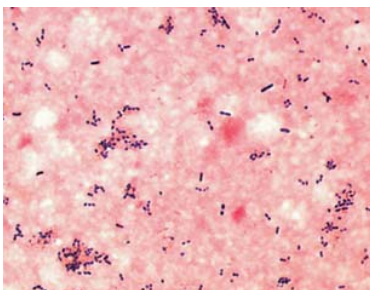
Results and Discussion

For the purpose of diagnosing European foulbrood, a direct microscopic examination as described above shows to be reliable in the majority of cases.

Most of the samples examined tested positive for *Melissococcus plutonius* and only in a few cases was it not possible to trace a specific cause for the disease.

The presence of many lanceolate cocci, occurring either singly or in pairs, or more rarely in short chains, was detected by direct staining, (photo 3) whereas cocci arranged in short and long chains were observed in cultures (photos 4-5)

The study confirms the presence of European foulbrood in several regions of Italy, with a distribution that remains localised for the time being. The infection also displays a recurrent, seasonal character and is generally characterised by a non-uniform pathological pattern.



3
Smear of mid-gut larvae unsealed brood showing: lanceolate cocci, gram+, in short chains, arranged end to end in pairs



4
Bacterial colonies growing on the agar Bailey



5
5% Nigrosin stain of *Melissococcus plutonius* from agar Bailey

References

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