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Comparative Study Between Techniques for the Diagnosis of American Foulbrood (*Paenibacillus* larvae) in Honeybee Colony

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Abstract: American foulbrood is a bacterial disease caused by the Gram-positive bacterium *Paenibacillus* larvae and is the most dangerous disease of the honeybee. The objective of this study is to compare the methods of detection of this bacterium in different products and samples from the hive. The samples of honey bees, wax, pollen and debris were taken from the hive during Spring period of 2013. Different microbiological methods were used for detection of the bacteria. MYPGP the culture medium is used during all the identifications of the bacterium. The results show that the diagnosis of the disease is more effective in samples of honey and bees than the detection of the bacteria in the wax, pollen and debris from the hive. Even in the absence of symptoms of the disease, the bacteria can be detected in honey or bees.

Key words: *Paenibacillus* larvae, microbiological detection, samples, honey bee, diagnosis

INTRODUCTION

The Apidae predominantly involved in the pollination of many plants. The majority of these plants could not complete their development cycle without the intervention of bees. Bees have a crucial role in the environment, agro and maintaining biodiversity. For several years, the populations of these pollinators are experiencing a sharp decline. The loss of pollinators is an important issue both for the plant species that are dependent on these insects for their reproduction and animals that feed on these plants (Neumann and Carreck, 2010).

In Algeria, many cases of bee colony mortality have been observed since 2007. The presence of pathogens in these colonies and their health status is the main cause of this lethality. The varroa mite, Nosema and American foulbrood are the most dominant diseases in the hives (Adjlane *et al*., 2012). American foulbrood is a common bacterial disease in bees (*Apis mellifera* L.) (Heyndrickx *et al*., 1996). It is found on all continents where beekeeping is practiced (Elus and Munn, 2005) and considered as the most contagious brood disease of the honey bee (Hansen and Brodsgaard, 1999) which can destroy an entire colony (Alippi *et al*., 2004). The causative agent of American foulbrood is a Gram-positive bacterium *Paenibacillus* larvae (Ashiralieva and Genersch, 2006). The latter can produce more than one billion spores (infective stage) in each infected larva (Heyndrickx *et al*., 1996). If the larva absorbs spores while feeding, these spores germinate in the midgut of the larva and rods, vegetative form highly mobile, cross the intestinal wall and enter the abdominal cavity. At this level, the spores multiply rapidly and cause death of the larva (Gregorc and Bowen, 1998). Further, samples brood detection of bacterial spores can be carried in honey (Shimanuki and Knox, 1988; Hornitzky and Clark, 1991), pollen (Gochnauer and Corner 1974), wax (Gochnauer, 1981), adult workers (Lindstrom and Fries, 2005) and hive debris (Titera and Haklova, 2003).

This research proposes to compare the methods of detection of *Paenibacillus* larvae in bee L depending on the nature of lechantillon (honey bees, pollen, wax and debris from the hive) colonies.

MATERIALS AND METHODS

The study was conducted in the laboratory of Regional Veterinary Draa Ben Kheda in Tizi-Ouzou (Algeria). Sampling was carried out on two apiaries:

- An AFB contaminated apiary located in the region of Bougara (Blida) which is used to isolate sick hives in this area
- A healthy supposed apiary located in the region of Baba Ali (wilaya of Algiers)

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Samples were taken during the spring period of 10 colonies at both apiaries. The samples were taken on:

- The worker bees collected directly from frames
- Honey stored in cells
- Wax taken directly from frames
- From pollen collected within the colony
- The remains of the hive collected at the bottom of the hive

MYPGP the culture medium is used during all the identifications of the bacterium. Protocol, Lindstrom and Fries (2005) is used for the diagnosis of bacteria in samples of bees. The search for the bacteria in honey is inspired by the method by Nguyen. The identification of the wreck on the samples of wax and debris from the hive is based methods by Hornitzky and Wilson (1989). For pollen, the method used is that by Gochnauer and Corner (1974).

RESULTS

For the contaminated apiary, the detection rate of AFB obtained in samples of bees, honey and debris from the hive is 80 and 70% contamination. For debris from the hive, the detection rate is 70%. For pollen, the rate of contamination by bacteria is 60%. In the wax, the average obtained is lower at 40%. The apiary considered to be healthy. One positive sample is detected on bees and debris from the hive of honey and two (10 and 20% contamination). For other categories (wax, pollen) no positive samples were detected (Fig. 1).

The results obtained from the AFB contaminated apiary, the percentage of the highest contamination is detected on bees and honey. This result confirms that the search for the bacteria on these two samples is more effective than the detection of the bacteria in the wax, pollen and debris from the hive. This study also points out that even in the absence of symptoms; it is possible

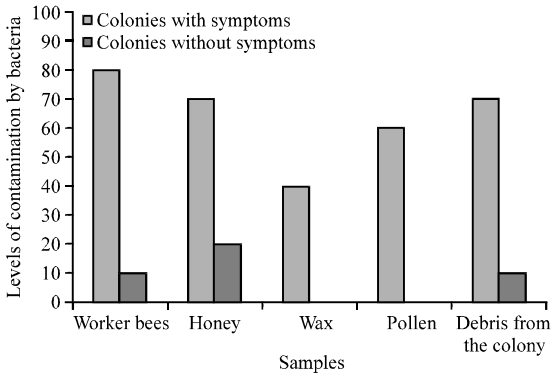

Fig. 1: Rates of detection of AFB in different products of the hive

to detect bacteria in honey or bees or debris from the hive. This is done in the results obtained through the second apiary or microbiological tests have detected two hives contaminated with the bacteria.

DISCUSSION

Lindstrom *et al.* (2008) showed that a colony can have large amounts of spores per adult bee without clinical signs of American foulbrood. In colonies showing no clinical symptoms of the disease, the spores of the pathogen can be detected in samples of adult bees (Hornitzky, 1988; Nordstrom *et al.*, 2002). Thus, spore loads on adult bees allow quantification of pathogen transmission between colonies (Fries *et al.*, 2006). The results presented by Lindstrom (2008) strongly suggest that the samples of adult bees individual colonies are very effective detect clinically diseased colonies. The same researcher in 2005 has already noted the importance of honey as a reservoir of *Paenibacillus* larvae spores inside the colony. Pernal and Currie (2000) reported that the detection of bacteria in honey is the most effective for the diagnosis of the disease method. Unlike Nguyen who reported only a 20% rate of detection of AFB in colonies with symptoms in samples of honey. In the results presented by Fries and Nordstrom (2001) reported that bee samples collected on the frames of brood are more sensitive than the use of honey samples collected from the same region.

In a study conducted in the colonies without symptoms of American foulbrood there was a lot more bee samples that tested positive for *Paenibacillus* larvae compared to samples of honey from the same colonies (Fries and Nordstrom, 2001). According to Lindstrom (2008), the number of bees required for efficient detection of AFB in a colony must be >200 bees. The same study reports that there is a strong correlation between the number of colony-forming and the proportion of positive bees units (Shimanuki and Knox, 1988; Hornitzky and Clark, 1991). The results by Bassi *et al.* (2010) reported that the PCR protocol used is culturally sensitive with MYPGP for the detection of *Paenibacillus* larvae spores in honey. Concerning the method of detecting spores of the bacteria in the wax, Bzdil (2007) reported that the use of toluene can kill a spore of micro-organisms in one of the stages of sporulation. It is also possible that residues of toluene on the plates of agar culture media negatively influence the activity of germination and growth of microorganisms. These effects can decrease the chances of detecting the bacteria in samples of wax.

Over time, new culture media have been developed or adapted for use in the diagnosis of American foulbrood: J-agar (Gordon *et al.*, 1973). Agar (Bhit) heart-brain supplemented with thiamine (Gochnauer, 1973) (MYPGP)

Mueller-Hinton broth, yeast extract, potassium phosphate, glucose, sodium pyruvate and agar (Dingman and Stahly, 1983), Columbia blood agar (Plagemann, 1985) agar sheep blood (Loyd, 1986) and more recently (PLA) *Paenibacillus* larvae agar (Schuch *et al*., 2001).

The MYPGP and *Paenibacillus* Larvae Agar (PLA) (Schuch *et al*., 2001) have shown high efficiency in detecting the bacterium. *Paenibacillus* larvae agar has the additional advantage that the majority of micro-organisms normally present in the hive and bee products are inhibited (Hornitzky, 1988). It is known that different strains of *Paenibacillus* larvae may show varying levels of virulence (Genersch *et al*., 2005) and that different larval physiological traits can lead to different degrees of sensitivity (Rothenbuhler and Thompson, 1956).

At the colony, the hygienic behavior of adult bees may affect the development of the disease (Spivak and Reuter, 2001). Fries and Raina (2003) reported in a study conducted on American foulbrood in colonies of Africanized bees that hygienic behavior of the honeybee is responsible for the low presence of the bacteria in the colonies in Africa. In addition, environmental factors such as the availability of pollen and nectar intensity may also play an important role. The appearance of the clinical form of the disease depends on several factors: the level of contamination, the virulence of the strain of *Paenibacillus* larvae (Crailsheim and Riessberger-Galle, 2001; Ritter, 2003).

Kilic *et al*. (2010) have studied 100 samples of honey and beeswax by both microbiological and PCR Method, *Paenibacillus* larvae was identified in (7%) samples by culture method and (8%) of the samples by the PCR Method. Nordstrom *et al*. (2002) reported that among 20 samples of honey collected from Sweden clinically sick colonies, a honey sample was negative for *Paenibacillus* larvae and 19 were positive (95%) among 162 samples of honey collected from healthy colonies, 11 were positive for *Paenibacillus* larvae (6.7%). Titera and Haklova (2003) report comparing two methods of detection of AFB in honey and debris from the hive that the number of positive cases is 38 against 29 in the debris in the honey.

CONCLUSION

In light of the results, several conclusions can be drawn:

- Even in the absence of symptoms of the disease, the bacteria can be detected in honey or bees
- Detection methods on honey bees are the most effective in the diagnosis of pathology techniques

The results have detected the bacteria in the debris from the hive. This result tells us about the importance of cleaning these colonies by the beekeeper to limit the spread of the disease to other hives and apiaries. Moreover, the presence of AFB in honey is a danger in the case of excessive comb exchange between colonies or hives. It is necessary in the future to test other culture media in order to determine the most reliable and easiest method of detection. The calculation of the number of spores in each sample is very important to determine the limit of detection of the disease.

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