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DETECTION OF *PAENIBACILLUS LARVAE* SPORES IN HONEY SAMPLES FROM BEEKEEPERS OF THE CENTRAL REGION OF ALGERIA

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ABSTRACT

The American foulbrood in one of the most serious diseases that may affect brood of larvae and pupae stages, which cause economic losses and biological hazards in a large beekeeping sector in several countries across the world in general and Algeria in particular. The causative agent of this disease is a bacterium called *Paenibacillus larvae* that target bees *Apis mellifera* the latter are often present in honey. The aim of this project is studying the spread of this disease in the northern region of Algeria through the analysis of honey obtained from these areas. Microbiological, microscopic and biochemical methods were used in this study. The results obtained have shown that the prevalence rate varies from region to region, several factors may explain this variation in the prevalence of the disease. The average infection rate for all regions is 32%. To prevent the spread of this disease in Algeria must be taken is mandatory and means of prevention into account.

Keywords: Algeria, american foulbrood, detection, honey, *Paenibacillus larvae*, spread



INTRODUCTION

American foulbrood is a bacterial disease of common bees (*Apis mellifera* L) (Heyndrickx et al. 1996). It is found on all continents where beekeeping is (Ellis and Munn, 2005). This is the most contagious disease of honeybee brood. It is among the diseases that can destroy an entire colony (Alippi et al., 2004). It generates significant economic losses for both the beekeeper for the farmer who needs pollinators (Hansen and Brodsgaards, 1999). The causative organism is a Gram positive bacterium *Paenibacillus larvae* (Ashiralieva and Genersch, 2006). Adult bees are not affected by the causative agent when they ingest spores (Wilson, 1971), but their digestive system is contaminated for several months (Hansen and Brodsgaards, 1999) and these bees will transmit the pathogen to young larvae (Wilson, 1971). The diagnosis of American foulbrood is based on visual inspection of hives. This procedure has limitations because it depends on the observation of clinical symptoms are not always easy to recognize. Diagnostic confirmation requires visual culture rag and characterization morphological, biochemical and physiological bacterial isolates (Martinez et al., 2010). In Algeria, American foulbrood is legally classified as a disease contagious. There is very little information on this disease. Few studies in Algeria to determine the prevalence of this disease in honey bee colonies. This apparent gap justifies the present work. The objective of this study was to determine the prevalence of this disease in some regions of Algeria where beekeeping is practiced intensively. The diagnostic method used is based on sampling of honey and detection of bacteria using microbiological methods, microscopic and biochemical.

MATERIAL AND METHODS

Sampling of Honey

Experimental work was performed in the bacteriology laboratory of Algerian Centre of Quality Control and Packaging (CACQE) in El-Harrach (Algiers, Algeria). The study was conducted on 56 samples of honey of various origins, harvested directly from the hive in 2012 and from different parts of northern Algeria

Preparation of the culture medium

MYPGP agar culture medium was used in this test. MYPGP agar was prepared according to Dingmann and Stahly (1983) consisted of 10 g of Mueller-Hinton broth (Oxoid), 15 g of yeast extract, 3 g of potassium phosphate (K_2PO_4), 2 g of glucose, 1 g of sodium pyruvate ($C_3H_3NO_3$) and 20 g of agar. Ingredients were weighed and placed into a 1000 cm³ flask and distilled water was filled to the 1000 cm³. After preparation, medium was boiled at 100 °C and poured into the flasks. Vials of cultivating medium were autoclaved at 120 °C for 1 hour on agar and paste boxes Petrie and allowed to cool.

Methods for microbiological diagnosis of the causative agent

The method of Lindstrom and Fries (2005) was used in the detection of the bacterium. The Petri dishes were incubated at 37 °C for 4 days under aerobic conditions and the number of viable spores and colony forming (CFU) were counted. If the number of colonies was too high, dilutions 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} were performed. A sample was considered as positive if *Paenibacillus larvae* microscopic observations put Highlight bacilli rod-like rounded, straight and curved sometimes.

Confirmatory tests

The bacterium *Paenibacillus larvae* is a Gram-positive with certain characteristics that distinguish it from other bacilli contaminating bees and hive products. After positive results on samples, biochemical confirmation tests (Test of catalase, presence hydrolysis of casein) and microscopic (Gram stain) are used in order to verify the accuracy of the initial results.

RESULTS AND DISCUSSION

The results of the research *Paenibacillus larvae* at our study have shown notable variability between samples of honey from different regions examined (Table 1). In the region of Tizi Ouzou 9 honey samples we found, six positive samples *Paenibacillus larvae* with the highest frequency (66.66%), with an average of 39 CFU/g. Six samples of honey from the Boumerdes region from 11 samples were positive, the infection rate recorded is 54.54% with an average of 33 CFU/g. For Tipaza region, two positive samples over the entire 5 is an infection rate of 40%, the number of which is 45 CFU/g. Of 10 samples analyzed in the region of

Blida, 3 were positive (30%) with an average of 107 CFU/g. For regions of Algiers, Medea and Bouira, only 1 sample was positive among the 4 samples with average 33, 4 and 42 CFU/g respectively. In Jijel, the infection rate is 20% with an average of 146 CFU/g. It is only the Djelfa region which is characterized by negative samples. The average contamination in all samples was 32%. American foulbrood is a serious disease that affects our bees. Beekeepers have expressed that this bacterium is the second most serious threat after the mite *Varroa destructor*. Under the direction of veterinary services in Algeria, the evolution of bee disease outbreaks from 2003 to 2009, the American foulbrood was detected in some homes beekeeping but lesser degree against *Varroa*. There are many factors that may explain this difference in the rate of contamination of bee colonies. The weather can be a major effect on frequency and distribution of disease. In temperate climates, the sporulation of *Paenibacillus larvae* is triggered, this can lead to heavy loads during the spring spores during brood-rearing and thus probably increases the risk of infection in this period since there are many spores available (Lindstrom et al., 2010). In Iran, Yusufkhani and Lotfi (2010) reported in a study of 650 hives, an infestation rate of colonies (17.3%) and (11%) of the samples in May and June respectively. Sporulation of *Paenibacillus larvae* is triggered by the high humidity in winter (Hansen and Brodsgaard, 1999). The bacteria multiply during the brood production causing clinical symptoms. In general, American foulbrood is found in spring and summer. Sampling in the case of our study was carried out in early spring, which explains the detection of the disease in almost all regions.

Table 1 Prevalence of *Paenibacillus larvae* spores in honey samples of different region

Sampling region	Positives samples (%)	Average number and range of spores CFU/g
Boumerdès	54.54	33 (2 - 75)
Algiers	25	55 (1 - 55)
Blida	30	107 (75 - 155)
Tizi Ouzou	66.66	39 (10 - 82)
Tipaza	40	45 (15 - 78)
Bouira	25	4 (1 - 4)
Djelfa	0	0
Jijel	20	146 (1-146)
Médéa	25	42 (1-42)

The development and spread of American foulbrood can be fostered by bad beekeeping practices that promote the spread of the disease: the anarchic management frameworks (frameworks transfer), the use of contaminated wax and honey dietary or contaminated pollen. The exchange of brood frames containing the remains of diseased larvae is the way of the spread of the disease more common. Hives executives who received infected can also become sources of contamination in the apiary then weaken and die. In addition, food or loaded plunder honey spores, bee bread, packets of beeswax, contaminated with spores of *Paenibacillus larvae* used for the creation of new hive frames can also disseminate disease (Faucon, 1992). Frequent replacement of rays should be encouraged in the management of colonies to fight against the disease by simply removing contaminated rays (De Graff et al., 2001).

Müller et al (2011) found that infection *Paenibacillus larvae* occurs when queens infected hives are introduced into healthy hives and beekeepers use when beekeeping equipment contaminated or improperly cleaned during harvesting or maintenance. In Algeria, beekeepers move their hives several times a year in different locations in search of nectar resource. These movements can contribute to the spread of the disease. Contaminated hives may introduce the disease into a new place, or hives may attack healthy displaced from hives found in the new neighborhood. The exchange of queens may be an element of dissemination indeed viable spores was isolated from queens from infected colonies, then they can spread the disease in healthy colonies. However, the characteristics of the races of bees could affect the probability of outbreak and spread of American foulbrood (Fries et al. 2001). Sale of queens between beekeepers Algerian is a very common practice, and it is done without any sanitary control which increases the risk of the spread of the disease.

According to Nguyen et al (2009), there are two factors that facilitate spread of American foulbrood. The first is that the spores of *Paenibacillus larvae* have the ability to remain viable for a period up to 35 years. The second is that the arrangements put in place to control American foulbrood were not and are still not adapted to a beekeeping still very marginal. So if a beekeeper suspected colony be infringing or to be contaminated, it is required to make a statement immediately and then send a veterinary assistant, which collects samples and sends them to study by center and veterinary research to treat and minimize the risk of infection. This practice is generally absent in most beekeepers in Algeria, who treat their hives directly with antibiotics.

Transmission and virulence of *Paenibacillus larvae* in honeybees present a special case because the vitality of *Paenibacillus larvae* depends not only on its ability to infect and spread among the colony, but also its ability to spread from one colony to another. The transmission can be done either horizontally e.g drift

bees entering temporarily in other colonies to steal honey) or vertically (when the colony reproduces by seeding) (Fries et al. 2001).

Looting is generally considered a major factor in the horizontal transmission of American foulbrood. During the looting, the bees attack the weakest colonies and attempt to plunder their stock of honey. If colonies are weakened, eg due to illness, pathogens can be transmitted to colonies marauding through the contaminated honey (Lindstrom et al., 2010). It is easy for the bees to rob honey from hives infected situated 1-2 km or more from their own hive, especially in the absence or in case of poor honey. Besides looting, horizontal transmission of spores may be caused by the drift between foreign colonies or mechanical transmission in its exchange operations or parts of frames between hives settlements made by the beekeeper (Lindstrom et al., 2010). Hygienic behavior and physiological sensitivity of bees may affect the incidence of the disease; it is well documented that the bees with the ability to protect and remove diseased brood can be completely resistance to American foulbrood (Fries et al. 2001).

In 2003, Fries and Raina reported in a study conducted on American foulbrood of colonies of Africanized bees that bee hygienic behavior that is responsible for the low presence of bacteria in the colonies in Africa. Globally, many research focused on the distribution of the wreck in the honey samples. The results regarding the prevalence of infection of the bacteria in apiaries abroad are very varied. Steinkraux and Morse (1992) reported an 8.5% contamination *Paenibacillus larvae* in 82 samples of honey harvested from the USA and Canada; There is *Paenibacillus larvae* range of 10-25 CFU/g of 3 honey samples, 320-440 CFU/g of honey in on sample of 560-3000 CFU/g of honey in 2 samples. In Belgium, a study based on bacteriological analysis of 1328 samples of honey harvest of the summer of 1999 provided 146 samples contaminated with spores *Paenibacillus larvae* (De Graaf et al. 2001). In 2001, 39 samples of honey collected in sub-Saharan Africa, among them no sample shows signs of contamination *Paenibacillus larvae*. By cons, of the 14 samples of honey imported and sold in Africa, 6 were positive for *Paenibacillus larvae* (42.85%) (Fries et al. 2001).

Nordström et al (2002) reported that among 20 samples of honey from Sweden harvested from clinically diseased colonies, a honey sample was negative for *Paenibacillus larvae* and 19 were positive. Among 162 samples of honey harvested from healthy colonies, 11 were positive for *Paenibacillus larvae* (6.7%). Antunez et al (2004) found 52 of the 101 samples of honey from apiaries located in 19 provinces of Uruguay, were positive for *Paenibacillus larvae* (51.5%). In Argentina, the bacterium is present in 17% of samples of honey sold in the local market and 60% of harvested honey directly from the hive (Lurlina and Fritz, 2005). Lurlina et al (2006) found that among 70 samples of honey analyzed in Argentina in 2006, 27 samples were positive (26 were harvested honey straight from the hive) that is to say, a contamination rate of 38%. In Switzerland, among the 142 samples tested in 2005, the presence of *Paenibacillus larvae* is detected in 33 samples (23%) and 100 samples tested in 2007, the presence of the bacterium is detected in 23 samples (Pohorecka and Bober, 2008). Nguyen et al (2009) in Belgium have detected the presence of spores of *Paenibacillus larvae* in 20% of the honey samples tested. On the other hand Turkey Kilic et al (2010) have studied 100 samples of honey and beeswax by both the microbiological method and PCR, was identified in *Paenibacillus larvae* (7% of the samples culture method and (8% of the samples by the method of PCR. Martinez et al (2010) have shown that among 14 samples of honey from Chile, 4 were positive for the real-time PCR, the number of spores varies between 63 and 135 spores / g honey, and 3 honey samples were positive for both methods (culture and PCR) and have found that the use of PCR is a sensitive and specific method for detecting and quantifying vegetative cells and spores *Paenibacillus larvae*.

CONCLUSION

The results obtained in this study show a strong presence of the disease in apiaries Algerian mastery of this disease must always go through a better understanding of it (including beekeepers), but also a greater awareness of these last.

Further work is needed to :

- Determine the percentage of the presence of bacteria in the other hive products (wax and pollen).
- Identify depending on the season and bioclimatic zones, the prevalence of the disease in apiaries Algerian.

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