SURVEY OF AMERICAN FOULBROOD IN *APIS MELLIFERA INTERMISSA* COLONIES IN MID-NORTHERN REGION OF ALGERIA

Cezayir'in Orta-Kuzey Bölgesinde Bulunan *Apis mellifera intermissa* Kolonilerinde Amerikan Yavru Çürüklüğü Hastalığının Taranması

(Genişletilmiş Türkçe Özet Makalenin Sonunda Verilmiştir)

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ABSTRACT

American foulbrood caused by *Paenibacillus larvae* is one of the most serious bacterial diseases of honeybee brood. Few data are currently available on the prevalence of this disease in Algeria. This study provides an overview of the prevalence of this disease in the Mid-North. Samples of adult bees were collected from 65 apiaries. Detection of spore samples was performed using methods bacteriological, microscopic and biochemical. Spores of *Paenibacillus larvae* were detected in 23.5 % of the apiaries examined in 2010 and 30 % in 2011. The prevalence of the wreck is different from one region to another. Many factors can possibly explain this difference in the prevalence of the disease.

Keywords: *Apis mellifera intermissa,* Algeria, *Paenibacillus larvae,* prevalence, American foulbrood

INTRODUCTION

American foulbrood is a common bacterial disease of honey bee (*Apis mellifera L*.) (Heyndrickx et al., 1996). It is found on all continents where beekeeping is common (Ellis and Munn 2005). This is the most contagious disease of the brood of the honeybee. It is among the diseases that could destroy an entire colony (Allipi et al., 2004). It causes significant economic losses as well as for the beekeeper to the farmer who needs pollinators (Hansen & Brodsgaards, 1999; Haddad *et al*., 2007)

The causative organism is a Gram positive bacterium called *Paenibacillus larvae* (Ashiralieva & Genersch, 2006). Adult bees are not attacked by the causative agent when they ingest spores (Wilson, 1971), but their digestive system is contaminated for several months (Brodsgaard & Hansen, 1999) and these bees will transmit the pathogen to young larvae (Wilson, 1971).

Paenibacillus larvae can produce over one billion spores per infected larvae (Heyndrickx *et al* 1996) Spores represent the infectious stage. If the brood absorbs spores while feeding, the spores germinate in the midgut of the larva and rods, vegetative form highly mobile, cross the intestinal wall and into the abdominal cavity. There they multiply rapidly and cause the death of the larvae (Gregorc & Bowen, 1998).

The disease can be controlled by the destruction of the brood with clinical signs and disinfection flame contaminated material (Hansen & Brodsggard 2003). The fight is also based on the use of antibiotics (oxytetracycline). But several years, strains of *Paenibacillus larvae* resistant to oxytetracycline appeared in many of the world (Miyagi *et al* 1999;

Alippi, 2000; Mussen 2000; Evans 2003; Lodesani & Costa 2005).

In Algeria, American foulbrood is legally classified as a disease contagious. There is very little about this disease. Few studies in Algeria to determine the prevalence of this disease in bee colonies. This apparent gap justifies this manuscript. The objective of this study is to determine the prevalence of this disease in the Mid-North of Algeria including agricultural areas of Algiers, Blida, Boumerdes, Tizi Ouzou and Tipaza where beekeeping is practiced intensively.

MATERIALS AND METHODS

Sampling

The sampling was conducted in 5 regions with high potential beekeeping: Tizi-Ouzou, Blida, Boumerdes, Algiers and Tipaza (Figure 1). Samples are taken in 2010 and 2011, early in the spring period (April and May). The samples, consisting of over 100 adult bees were taken from the brood frames were placed in plastic boxes containing 90% ethanol and immediately frozen. 101 and 98 samples were taken respectively on all study areas during 2010 and 2011. 65 apiaries were visited at which these samples to detect typical symptoms of AFB.

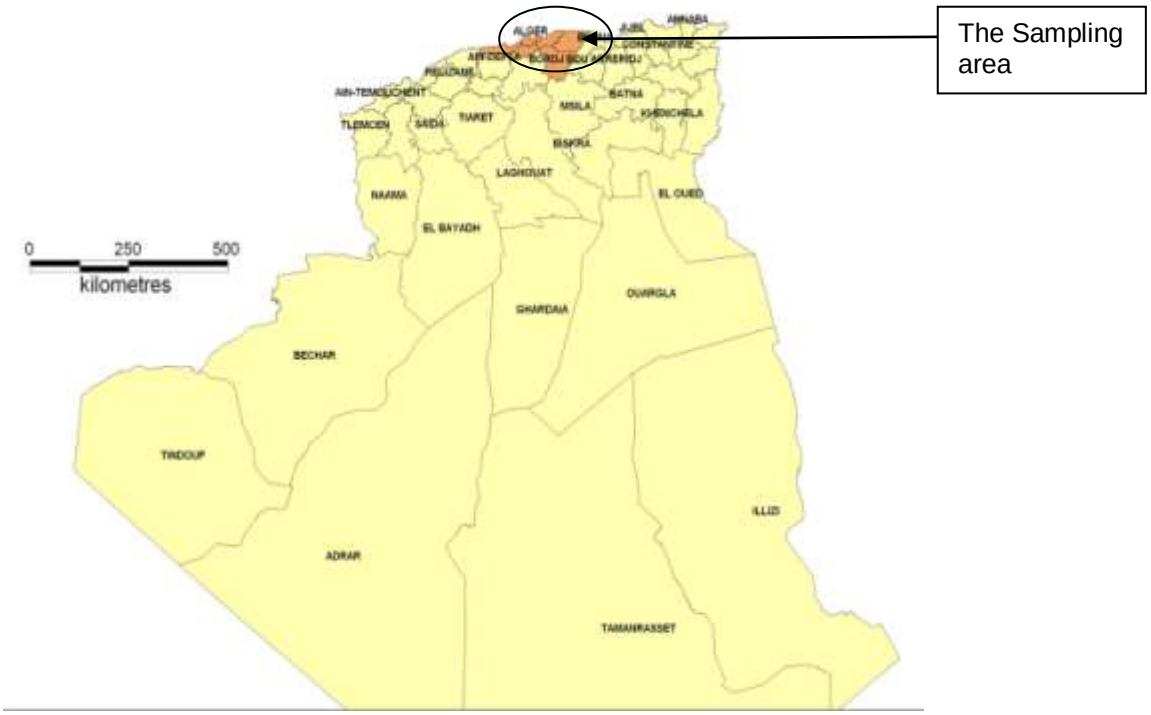

Figure 1: Study area (mid-northern region of Algeria)

Protocol Analysis

In the laboratory the spores of *Paenibacillus larvae* are detected separately for each sample by the microbiological method of Lindstrom & Fries (2005):

-Remove the intestines and thoroughly crush bee

-Place the sample in a test tube after filtration.

-Add 20 ml of physiological saline

-Centrifuge the liquid collected in a centrifuge tube of 50 ml for 10 minutes

-Replace the recovered pellet was suspended in 12ml of sterile NaCl (13 g / 1 liter)

-Incubate at 85 ° C in a water bath for 10 minutes

-Inoculate 10 ìl of the cell suspension in agar-plates MYPGP

-The plates are incubated in 5% CO2 (to stimulate the growth of the bacteria sought) at 36°C for 7 days

-And finally the identification of the causative agent *Paenibacillus larvae* MYPGP agar, colonies of the bacterium *Paenibacillus larvae* are small, regular, usually rough, flat or raised, white or beige

Biochemical tests and microscopic confirmation were performed on positive samples: Catalase test (Haynes 1972), the test of the hydrolysis of casein (Neundorf et al.,2004), and Gram staining (Murray & Aronstein 2008)

Statistics

Datas obtained from experiment were evaluated by using procedure of ANOVA of statistic package programme called SAS (1999) and difference between the groups were determined by the Newman-Keuls test (*P*=0.05)

RESULTS AND DISCUSSION

The other study areas are characterized by a frequency of less than 25%. ANOVA results registered significant difference between final prevalence in the two region of Boumerdes and Tizi ouzou, comparing with a frequency of Blida, Alger and Tipaza ($p<0.05$).

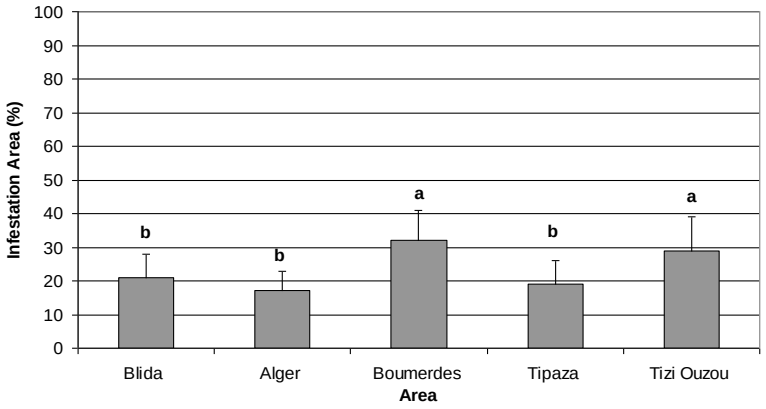

Figure 3: Prevalence of American Foulbrood in mid-northern region of Algeria in 2010

Different letters (a, b) indicate significant differences at P < 0.05 after ANOVA followed by a post hoc Newman-Keuls test.

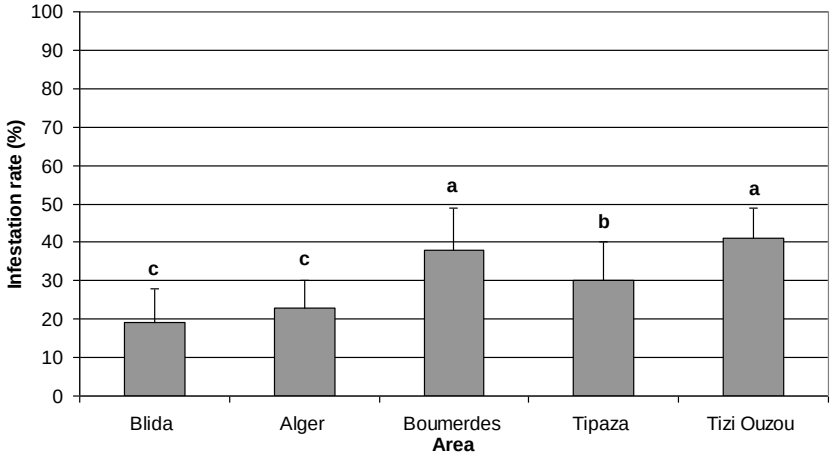

Figure 3: Prevalence of American Foulbrood in mid-northern region of Algeria in 2011. Different letters (a, b, c) indicate significant differences at P < 0.05 after ANOVA followed by a post hoc Newman-Keuls test.

In real conditions, the detection of AFB is based on the appearance of diseased brood whose larvae have a consistency typical (larva shooting). When the dead larva dries, hardened scales are formed and firmly adhere to the cell wall. For all infected hives, only 8% on average are characterized by the presence of symptoms of the disease in 2010 and 11 % in 2011 (Figure 3). The presence of *Paenibacillus larvae* in a hive was not systematically accompanied clinical symptoms (De Graaf *et al* 2001). The results of Lindstrom *et al* (2008) show that a colony can have large amounts of spores per adult bee without showing clinical signs of AFB. When clinical symptoms of the disease appear in infected colonies, in the absence of treatment, they will most likely succumb to this disease (Genersch *et al* 2005). In colonies not exhibiting clinical symptoms of disease, spores of the pathogen can be detected in samples of adult bees (Hornitzky 1988, Nordstrom *et al* 2005), or honey (Antunez *et al* 2004; Pohorecka & Boner 2008). Graff and *et al*. (2001) who found a significant relationship between the positive honey samples and the cases of AFB in Belgium, supporting the value of honey analysis for sanitary control of bee colonies.

According to a study by Fries and Lindstrom (2005), 35% of infected colonies are characterized by the presence of symptoms of AFB, and 68% of the samples are characterized by the presence of spores. The clinical signs of AFB are varied and depend on the level of virulence of the strain of the bacterium (Ritter 2003), the concentration of spores in the colonies and the strength of the hive and its potential resistance to American foulbrood (Hansen & Brodsgaard 1997), and the contributions of nectar and pollen (Hansen & Brodsgaards 1999). Young larvae can be killed quickly if they are huddled in cells not capped. The workers will remove these larvae died and only one cell will remain empty (Brodsgaard *et al* 2000).

The distribution pattern of *P. l. larvae* spores in the different samples is shown in table (1). The regions were classified in 3 categories according to the mean of CFU/g of samples of honeybee. In the first category we find the Region of Tizi Ouzou showing a mean of more than 200 CFU/g. Blida, Boumerdes and Tipaza are included in a second category with a mean of CFU/g that goes from 50 to 200 UFC/g. In the third category we included Alger, with a mean below 50 UFC/g. Statistical analysis indicated that there were highly significant differences of this 3 categories ($p<0.001$).

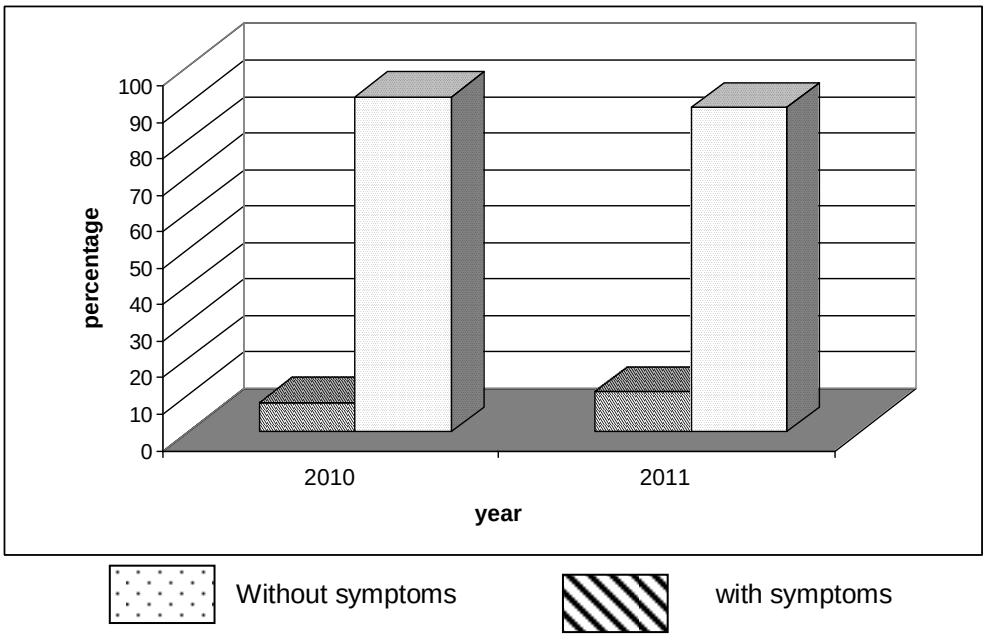

Figure 4: Frequency of symptoms of AFB in colonies infested with *Paenibacillus larvea*

Table 1: Results for the detection of *Paenibacillus larvae* spores in samples of honey bee in Algeria. Different

letters (a, b, c) indicate significant differences at P < 0.05 after ANOVA followed by a post hoc Newman-Keuls test. S.E.: standard error

Source	Samples	Mean ± S.E (CFU/g)
Alger	38	49 ± 41.85 d
Blida	43	101 ± 69.55 c
Boumerdes	37	185 ± 35.85 b
Tipaza	39	112 ± 81.52 c
Tizi ouzou	42	274 ± 78.12 a

Globally, more research has focused on the distribution of foulbrood in honey bee colonies. In Sweden, a study showed a prevalence of 70% of bees infected by foulbrood (Lindström & Fries 2005). In the Czech Republic Ryba *et al* (2009) report a rate of 12%. 5.1% of the colonies are contaminated with Iran (Yusefkhani & Lotfi 2010). A study based on the bacteriological analysis of honey samples from 1328 harvest of summer 1999 in Belgium has detected 146 samples contaminated with spores of *Paenibacillus larvae* (De Graaf *et al* 1999). In Poland 23% of honey samples are contaminated with the bacteria (Bohorecka & Bober 2008), and 50% in Argentina (Basuado *et al* 2008).

Weather conditions can have a major effect on the frequency and distribution of pathology. According Haubruge *et al* (2006), climatic conditions are discussed as a potential cause of the wreck. Sporulation of *Paenibacillus larvae* is triggered by the high humidity in winter (Hansen & Brodsgaard 1999). Bacteria grow in the period of brood production causing clinical symptoms. In general, the AFB is detected in the spring and summer. Sampling in the case of our study was conducted in early spring, which explains the detection of the disease in almost all regions.

Shiminuki & Knox (2000) consider that the spread of the disease is closely related to the activity of beekeepers. Beekeeping practices are themselves favorable to the change in the appearance and spread of foulbrood. The exchange of brood frames containing the remains of diseased larvae is the diffusion path of the most common disease. In addition, food or raiding honey laden spores, bee bread, packets of bee wax contaminated with spores of *Paenibacillus larvae* used for the creation of new hive frames also can also disseminate disease (Faucon 2002). The frequent replacement of rays should be encouraged in the management of the colonies to fight against the disease by simple removal of contaminated rays (Graff *et al* 2001). Marketing of queens may be an element of dissemination. Indeed, viable spores have been isolated from queens from infected colonies. They can then spread the disease in healthy colonies (Wilson 1972). The sale of queens between beekeepers Algerian is a very common practice, and it is done without any sanitary control, which increases the risk of spreading the disease (Adjlane et al 2012).

Research work suggests that *Varroa destructor* can play a role in the spread of the disease (De Rycke *et al* 2002). Knowing that the apiaries in Algeria are heavily infested with varroa (Adjlane 2011). Therefore, constitutes another potential route of dissemination of the bacterium.

According Saegerman *et al* (2009) two phenomena may facilitate the spread of American foulbrood. The first is that the spores of *Paenibacillus larvae* have the ability to remain viable during a period of up to 35 years. The second is that the arrangements put in place to control American foulbrood are not always adapted to a beekeeping. The results of Lindstrom *et al* (2008) demonstrate a direct effect of the distance between the colonies clinically ill on the probability of getting a high amount of spores as well as that of developing visible symptoms of the disease. The high density of bee colonies is a factor in the spread of disease. This strong presence of the disease in bee colonies is also related to the high density of colonies in the study areas.

Looting is an important means of transmission of spores of P. larvae between bee colonies over short distances; II is evidence that contamination is particularly important at a distance of about 1 km or less (Lindström *et al* 2008).

The behavior of the bee is a factor that can determine the impact of the wreck: Fries and Raina (2003) reported in a study conducted on American foulbrood of Africanized bee colonies that hygienic behavior of this bee is responsible for weak presence of the bacteria in the colonies in Africa. In Algeria, no study has been conducted until now on the importance of this criterion on the development of the wreck in two local races of bees *Apis mellifera intermissa* and *Apis mellifera sahariensis.*

Other routes of spread of the disease are added such as the sale of the swarms that can carry spores, the movement of bee colonies (Pankiw & Corner 1966). In Algeria, beekeepers move their

hives several times a year in various locations in search of nectar resources. These movements can contribute to the spread of the disease. Contaminated hives can introduce the disease into a new place.

CONCLUSION

Paenibacillus larvae is present in the five areas studied. But bee populations are infested at different levels. Several factors can possibly explain this great variation in the distribution of the disease such as climatic factor, and especially the role of the beekeeper in the spread of the disease. The highly pathogenic nature of this disease and the inability of early detection are partly responsible for its wide dissemination. Beekeepers do not detect the disease early enough thus promoting the formation of several outbreaks of pathogens in the study areas. The presence of spores in the study areas in colonies showing no symptoms questions the apparent low frequency of AFB in Algeria.

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GENİŞLETİLMIŞ ÖZET

Giriş: Amerika Yavru Çürüklüğü *Paenibacillus larvae* tarafından sebep olan balarısı yavrularının en önemli bakteri hastalığıdır. Tüm ülkelerde görülen en bulaşıcı hastalıktır. Gram pozitif bakteri tarafından neden olan bu hastalık yetişkin arıları etkilememekte ancak sindirim sistemlerinde uzun sure kalmakta ve daha sonra genç larvalara bu patojeni bulaştırmaktadır. Bu bakteri bir milyondan fazla spor üretmekte, yavrular sporu aldığında spor larvanın midesinde gelişimine başlamaktadır. Daha sonra çoğalmakta ve larvanın ölümüne neden olmaktadır. Hastalığın kontrolü farklı şekillerde yapılabilmektedir; yavruların yok edilmesi, yakılması, antibiyotik tedavisi gibi. Ancak uzun yıllar bu bakteri oksitetrasikline karşı direnç geliştirmiştir. Cezayir'de bu hastalık salgın hastalık olarak kabul edilmektedir. Bu konuda çok az çalışma bulunmaktadır. Bu çalışma bu açığı kapatmak için yapılmış ve hastalığın Orta-Kuzey Cezayir'de hastalığın yaygınlığını tespit etmek için yapılmıştır.

Materyal ve Metot:

Örnekleme: Örnekleme arıcılığın en fazla olduğu 5 bölgede yapılmıştır. 2010-2011 yıllarında erken ilkbaharda (Nisan-Mayıs) yavru çerçeveleri üzerinden 100 yetişkin arı %90 alkole alınmış ve hemen dondurulmuştur. 2010-2011 yıllarından sırası ile 101 ve 98 örnek toplanmıştır. Bu hastalığı gösteren 65 arılık ziyaret edilmiştir.

Analiz Protokolü: Laboratuvarda *Paenibacillus larvae* sporları her örnek için ayrı ayrı Lindstrom ve Fries (2005) metoduna gore yapılmıştır. Pozitif örnekler üzerinde ise biyokimyasal ve mikroskopik teyitler gerşekleştirilmiştir; bunlar Katalaz testi (Haynes 1972), Kasein hidroliz testi (Neundorf ve ark., 2004) ve gram boyama (Murray ve Aronstein, 2008) testleridir.

İstatistik: Deneyler sonucunda elde edilen veriler SAS (1999) adı verilen paket program kullanılarak varyans analizi (ANOVA) yapılmış ve gruplar arası farklılık Newman-Keuls testi (P=0.05) ile belirlenmiştir.

Bulgular ve Tartışma: 2010 ve 2011 yıllarında 5 farklı bölgeden toplanan örneklerdeki hastaklık etmeni çalışılmış ve bulgular tablo ve şekiller ile makale içerisinde gösterilmiştir.

Paenibacillus larvae sporları 2010 yılında kolonilerin %23.5'inde 2011 yılında ise %30'unda belirlenmiştir. Farklı bölgelerde farklı yaygınlık belirlenmiş ve bunlar arasındaki anlamlılık istatistiksel olarak gösterilmiştir. Hastalığın yaygınlığı bölgeden bölgeye farklılık göstermektedir. Hastalığın yaygınlığındaki farklılığı açıklamada bir çok olası faktör bulunmaktadır. Bunlar arasında iklim koşulları, arıcılık uygulamaları, *Varroa destructor* tartışılmış ve diğer çalışmalar ile karşılaştırılmıştır. Bunlar arasındaki bakterinin çok patojenik olması ve hastalığın erken teşhisinin arıcı tarafından yapılamaması son derece önemlidir. Çalışma alanındaki kolonilerdeki spor varlığı ve herhangi bir semptom görülmemesi Cezayir'de düşük Amerikan Yavru Çürüklüğünü sorgulamalıdır.

Anahtar kelimeler: *Apis mellifera intermissa,* Cezayir, *Paenibacillus larvae,* yaygınlık, Amerika Yavru Çürüklüğü.