

## Oriental Hornet (*Vespa orientalis*) as AFB Disease Vector to Honeybee (*Apis mellifera* L.) Colonies

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### ABSTRACT

The present study was carried out in the honey bee laboratory of Faculty of Agriculture, Benha University, Egypt during two successive years 2014 and 2015 at the period from October to December to prove that the oriental hornet (*Vespa orientalis* L.) was considered as AFB disease vector to honeybee (*Apis mellifera* L.) colonies. Firstly, the number of oriental wasps in two Egyptian governorates (Qalubia and Menofia) were surveyed, then these nests were checked for AFB disease infestation. Results indicated that the mean number of collected oriental wasp nests was 13.5 and 13.0 nests in 2014 and 2015, respectively. And from the collected nests, 66 and 53.4 % were infected and showed typically symptoms of AFB during 2014 and 2015, respectively. Additionally, the mean ratio of infected oriental wasp larvae with AFB was 16.7 and 21.7% in the years 2014 and 2015, respectively. The mean number of oriental wasp combs per nest was higher in 2014 with 4.3 comb/nest than 2015 with and 3.8 comb/nest. After that, four bacterial isolates were isolated from the infected larvae and identified as *Paenibacillus larvae* using the molecular techniques.

**Key words:** *Vespa orientalis*, *Apis mellifera*, AFB

### Introduction

The Oriental hornet (*Vespa orientalis* L.) is a social insect belongs to the Vespidae family, it is a member of genus *Vespa* which constitutes true hornets, their nests were build underground and consisting of caste system dominated by the queen, nests contain multiple combs in which the colony lives (Ebrahimi and Carpenter, 2012). Among insects, the oriental hornet is the most important honeybee predator in the world and particularly known to induce serious damages in apiaries (Papachristoforou *et al.*, 2008). The hornets attack honeybee hives and some species can easily destroy a bee colony (Baracchi *et al.*, 2010). The adult hornet eats nectar and fruits and scavenges for insects and animal proteins to feed to their young. Because they are scavengers, the hornets may also serve as a transmitter of disease following consumption of infected plants (Ken *et al.*, 2005). Oriental hornets can be found in Southwest Asia, the Middle East, Northeast Africa Southern Europe (Abrol, 1994), they live in seasonal colonies which are formed every year in the spring by a queen who mated during the previous fall. During the fall, the queen lays her eggs which will develop into new queens and drones. After mating, the drones die off while the fertilized queens seek hideouts in which to hibernate for the winter. The colony will grow throughout the spring and summer months until the population and activity of the colony peaks in the late summer and early fall. The peak size of the colony is several thousand individuals and a colony will contain on average 3-6 combs each containing 600-900 individual cells (Kostvfeier, 2010). *V. orientalis* is considered recently the major pest and more dangerous for Egyptian beekeeping. Also, it may be considered as a vector for honeybee diseases (Khodairy and Awad, 2013). From these diseases, American foul brood (AFB) was one of the most harmful bee diseases which causes colony collapse disorder. Honeybee larvae are most susceptible to infection in 12–36 h after hatching (Ghorbani-Nezami *et al.*, 2015). It kills not only infected larvae but also infected colonies. Dead larvae are degraded to a ropy mass which when dried creates scales, these scales contain millions of spores which can easily be transmitted across hives (Genersch, 2010). The disease spreads when spores are carried on drifting bees, hive parts and contaminated pollen or honey. When the hornets rid to the bee hive, they will feed on the honey and carry the bee larvae corpses back to their own colony to rear their developing brood (Baracchi *et al.*, 2010). The aim of this study is to prove that *V. orientalis* was vector of American foul brood (AFB) to honeybees as a first record all over the

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## Materials and Methods

### Collection of oriental hornet nests

Search for oriental wasp nests was conducted at two successive years 2014 and 2015 during the period from October to December at Qalubia and Menofia governorates. The average of temperature (T°C) and relative humidity (RH %) in these months were described in Figs (1a & b) ([www.timeanddate.com/wethear/egypt](http://www.timeanddate.com/wethear/egypt)). The attractive poisoned bait {one liter of 70% sucrose solution + 10 g Lannate (90% Methomyl)} is placed in front of the hornet nests to kill members of the colony. The nests were stored in the Honey Bee Laboratory at Plant Protection Department, Faculty of Agriculture, Benha University, Egypt. All hornet nests combs were measured (length and width) with a typical Langstroth wired frame divided into square inches.

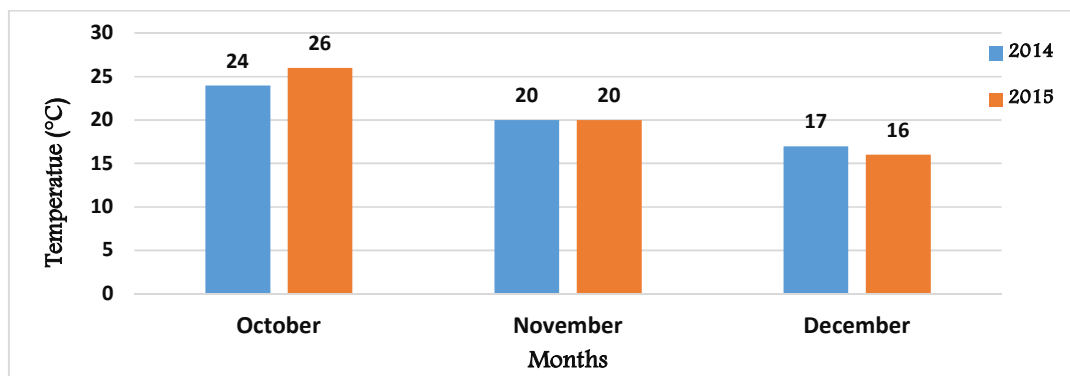


Fig. 1 a: Average of Temperature during the studying months at 2014 and 2015.

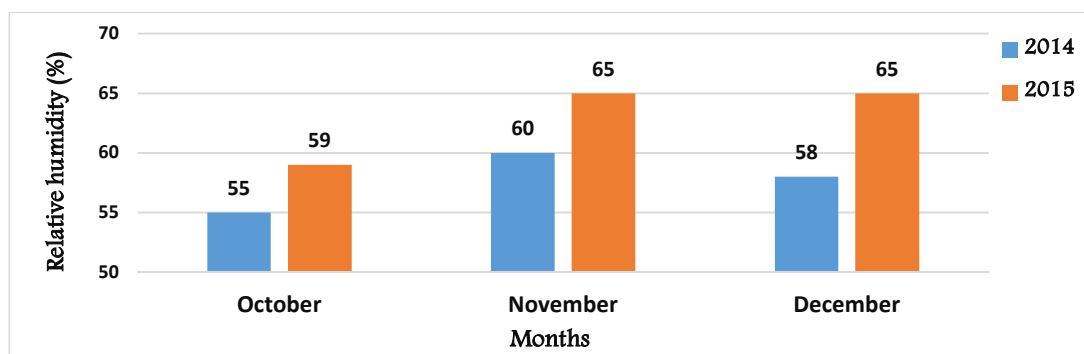


Fig. 1 b: Average of relative humidity during the studying months at 2014 and 2015.

### The infection rate

The collected nests were taken and checked in the Honeybee Lab., Plant Protection Dep., Fac. of Agric., Benha Univ., Egypt. These nests were classified as “healthy” or “infected” using phenotypic characteristics, then number of infected nests, number of infected and hygienic wasp larvae in each nest were recorded. The percent of infected nests and larvae were calculated as described by De Graaf *et al.* (2013) using the following equipment:

$$\% \text{ infection rate} = \frac{\text{Infected wasp larvae No.}}{\text{Hygienic wasp larvae No.}} \times 100$$

### Isolation and identification of AFB causative

From the obtained nests during each year, two nests were randomly selected and the infected hornet larvae remained which have typically AFB symptoms were collected from comb to isolate the causative agent of AFB (*Paenibacillus larvae*) as described by De Graaf *et al.* (2006). One bacterial isolate from each

selected nest was subjected to molecular techniques for identification of *P. larvae* as described by Abd Al-Fattah *et al.* (2010). The identification techniques were done in Molecular biology Lab., Central Research Lab., Fac., Agric., Benha Univ., Qalubia, Egypt.

## Results and Discussion

### Collecting and checking the oriental horn nests

Data in Table (1) showed that the oriental wasp nests were found in the two studying locations Qalubia and Menofia governorates. These nests were found during the two studying years 2014 and 2015. Also, data in Table (1) indicated that the mean number of collected oriental wasp nests was 13.5 and 13.0 nests in 2014 and 2015, respectively. In addition, data indicated that the number of oriental wasp nests in Qalubia governorate was higher than Menofia governorate. From points that you should be considered that the nests were collected during the first of October to the end of December. Similar results were observed by Swamy (2008) who reported that all species of hornets attained high population on the onset of monsoon rainy season in their respective habitats and high peaks in population were observed during September to October. These results may be due to the presence of large numbers of honeybee colonies in these locations and the oriental nests could occur nearby the honeybee colonies. In addition, hornets forage for food and water in areas frequented by humans. A long time ago, many researchers were demonstrated that *V. orientalis* was distributed in all continents nearly such as Europa, Asia, Africa, and America (Ken and Wang 2004; Papachristoforu *et al.*, 2008; Tan *et al.*, 2007). Additionally, Ken *et al.* (2005) reported that in China, *A. mellifera* was attacked by *V. velutin* significantly. *V. orientalis* is a common and widespread species of hornet found throughout southeastern Europe, north Africa and southwestern Asia (Archer, 1998).

**Table 1:** Number of collected oriental wasp nests and its infection rate by AFB during the two years of the study 2014 & 2015.

Year	Location	No. of oriental horn nests	% of infected nests by AFB	% of infected larvae by AFB in each infected nest
2014	Qalubia	20.0	75.0	26.5
	Menofia	7.00	57.0	6.80
Mean		13.5	66.0	16.7
2015	Qalubia	21.0	66.7	24.2
	Menofia	5.00	40.0	19.2
Mean		13.0	53.4	21.7

Regarding the percent of infection by AFB in the collected nests, Photo (1) showed the AFB infection symptoms on the collected horenst nests. Additionally, data in Table (1) indicated that 66 and 53.4 % of the collected nests during 2014 and 2015, respectively were infected and showed typically symptoms of AFB. Moreover, data showed that the mean ratio of infected oriental wasp larvae with AFB was 16.7 and 21.7% in the years 2014 and 2015, respectively. Although, the number of the collected nests during 2014 was higher than 2015 and the infection percentage by AFB in nests was higher during 2015 than 2014. Also, data showed that the collected nests from Qalubia governorate showed higher infection percentage than Menofia governorate. These results may be because the hornets cause damages in many fruits especially grapes and dates before and after harvesting. AFB spores could be translated from hunted honey bee workers (as a carrier for the spores) and from attacked honey bee infected colonies. During its feeding on these fruits, AFB spores were translated to the fruit surface where honeybee were also feeding. Also, hornets were fed on sugary substances which located in apiaries and left AFB spores on these substances. It was clearly that the collection of nests was during October and December, the environmental conditions during these months were optimum to AFB prevalence. In this respect, Al-Ghazawi *et al.* (2009) reported that oriental wasp, is one of the most distributed pests which cause considerable damage for honey bee colonies all over the world. Also, Baracchi *et al.* (2010) who reported that when the hornets rid to the bee hive, they will feed on the honey and carry the bee larvae and adult corpses back to their own colony to rear their developing brood. Moreover, *V. orientalis* is known to be a serious worldwide apicultural pest. Khodairy and Awad (2013) reported that *V. orientalis* was considered recently the major pest and more dangerous for Egyptian beekeeping. On reverse, Khater *et al.* (2001) mentioned that a gradual increase took place until mid-June and then a more rapid increase with the maximum rate of occurrence during early September, followed by gradual decrease and disappearance at the end of December in Ismailia governorate.



**Photo 1:** *Vespa orientalis* L. infected comb with AFB.

### Diameters of the collected oriental wasp nests

Data presented in Table (2) and Photo (2) clearly indicated that the mean number of oriental wasp combs per nest was higher in 2014 with 4.3 comb/nest than 2015 with and 3.8 comb/nest. This trend of results may be due to the difference in weather conditions during the two studying years. The nests collection was in the first of October to the end of December during the two studying years. In this respect, Villemant *et al.* (2008) reported that mean number of oriental horn combs/nest was 8-10 combs. To confirm this interpretation of results, similar results were observed by Shoreit (1998) who indicated that *V. orientalis* queens were observed from January to May. Also, Khater *et al.* (2001) revealed that *V. orientalis* adults started to appear in the apiaries as early as the beginning of March, but in low numbers. Concerning to the diameters of *V. orientalis* L. combs, data in Table (2) and Photo (2) indicated that the length and width of the collected combs during the two years of study. Data also showed that the size (length x width) were higher in the collected combs during 2014 than 2015. This may be because the weather conditions were suitable during 2014 than 2015.

**Table 2:** Diameters of *Vespa orientalis* L. combs during the two years of study.

Year	Mean no. of oriental horn combs/nest	Mean of oriental horn combs diameter (inch <sup>2</sup> )	
		Length	Width
2014	4.3	16.4	10.3
2015	3.8	12.1	9.7



**Photo 2:** Big *V. orientalis* L. nest

### AFB symptoms on *V. orientalis* infected larvae

Photo (3) showed the hygienic immature stages of *V. orientalis* L., it was clearly that the hygienic larvae had a shiny, creamy color, normal in size and normal segmentation. On contrast, Photo (4) showed the infected *V. orientalis* larvae that appear as a brown to black in color, wrinkled body shapes, semi-fluid and glue-like colloid (ropy stage). These symptoms were typically to AFB symptoms in infected honeybee larvae as demonstrated by Ghorbani-Nezami *et al.* (2015) who indicated that larval remains affected by *P. larvae* appear as a brownish, semi-fluid, glue-like colloid, containing the vegetative stage of this bacterium, which sporulates and can then be distributed in the bee colony and swallowed by the next host. The dead larvae are degraded to a ropy mass which when dried creates scales, these scales contain millions of spores which can easily be transmitted across hives (Genersch, 2010).

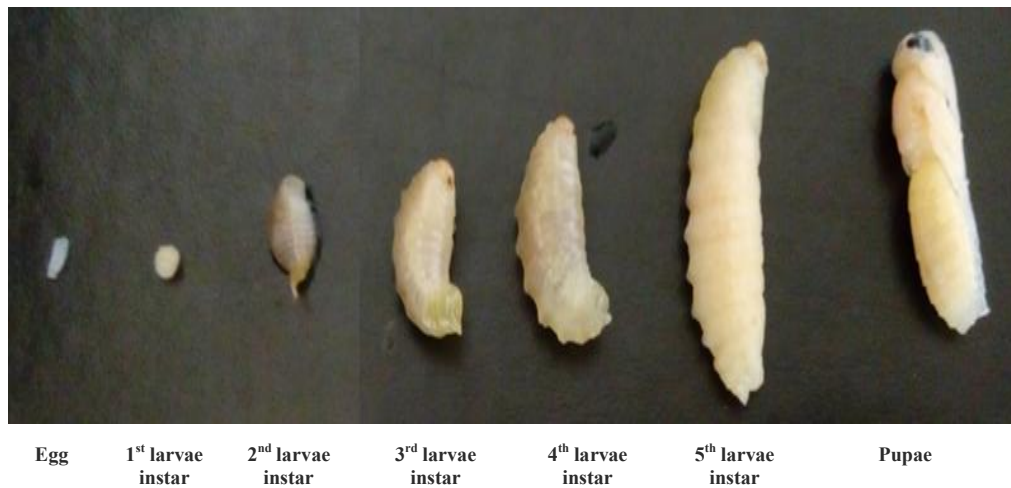


Photo 3: Hygienic immature stages of *V. orientalis* L.



Photo 4: *V. orientalis* L. infected larvae with AFB.

### Isolation and identification of *P. larvae* from *V. orientalis* infected larvae

All infected nests were identical in AFB symptoms, from these collected nests during each year, two nests were randomly selected for isolation and identification of *P. larvae*. One bacterial isolate from each selected nest were subjected to molecular technique for identification of *P. larvae*. Genotypic identification was performed by amplification and partial nucleotide sequencing of the 16S ribosomal RNA (16SrRNA) of four bacterial isolates from infected nests. The identification of bacterial isolates based on 16SrRNA sequences is presented in Table (3). Molecular analysis based on 16SrRNA confirmed all four isolates as *Paenibacillus larvae*. In this respect, Govan *et al.* (1999) reported that molecular techniques were exploited for identification of *P. larvae* which isolated from apiary sources. Dobbelaere *et al.* (2001) also used PCR for identification of *P. larvae*, the primers derived from gene regions encoding 16SrRNA. Additionally, Abd Al-Fattah *et al.* (2010) used the phenotypic identification of the AFB pathogen (*P. larvae*) as well as genotypic identification adopting the 16srRNA gene analysis technique.

**Table 3:** Genetic characterization of selected bacterial isolates to gene level

Bacterial isolates	Description *	Identity (%)
OH1	<i>Paenibacillus larvae</i>	97%
OH 2	<i>Paenibacillus larvae</i>	98%
OH 3	<i>Paenibacillus larvae</i>	100%
OH 4	<i>Paenibacillus larvae</i>	98%

\* Based on partial sequencing of 16S rDNA gene and comparison with the National Center for Biotechnology Information database.

### Conclusion

From the current study, it was concluded that *V. orientalis* nests were widely spread during the two studying years from October to December. AFB disease symptoms were observed in the collected nests and the bacterial isolates from these nests were identified as *P. larvae*. These results proved that *V. orientalis* was one of the important and dangerous vector for AFB to honeybee colonies which considered as a reason for colony Collab's disorders.

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