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American Foulbrood Disease, an Overwhelming Problem in Honey bee (*Apis Mellifera)* in Kohat and Peshawar Districts, Pakistan

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Abstract: American Foulbrood (AFB) disease caused by the bacteria *Paenibacillus larvae,* is a serious disease of honeybee throughout the world, that bring down a considerable economic loss to the beekeepers. All work was undertaken in the commercial Apiaries of Kohat and Peshawar regions that showed visual signs of AFB infection. In the investigated 32 Apiaries (containing 2100 colonies), 26 colonies were identified positive for AFB. This work was valuable for the over production of honey bees to kill the AFB bacteria which were identified in the current study.

Key words: AFB, Honey Bee, Beekeepers, Kohat, Peshawar

Introduction

Hierarchical classification shows that honey bee belongs to order hymenoptera, that generally nourish on pollen and nectar and comprises of 20,000 species worldwide (Michener, 2000). The genus *Apis* is mostly preferred for research oriented study because it plays a vital role in the formation of natural products such as propolis, honey, wax and royal jelly. Other points of interest are an agent of pollination, complex communication system and lifecycle (Nieh, 1998; Nieh and Roubik, 1995). There are four species of honey bees. (a) Oriental bee (*Apis cerana*), (b) Rock bee (*Apis dorsata*) commonly found in mountainous areas, The (c) little bee (*Apis florea*) commonly known as "Shotee Makhee" in punjabi, (d) Occidental bee (*Apis millifera*) found in western hemipshere (America and Europe).The oriental bee occurs in KPK, Punjab, Baluchistan, and Azad Jammu and Kashmir and the rock bee and little bee in the bottom of hills, plains and semi-desert areas in all the provinces. The first three species are native to Pakistan. The accidental bee was imported from Europe in 1977-1978 because of its more resourceful honey production. This species can be

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artificially reared. New colonies of this species are reared in almost all the honey producing area of Pakistan. (Ahmad, 1988; Schmidt and Hassen, 1996). Honey bees are usually invaded by several pathogens like viruses, bacteria, protozoans and fungi (Morse and Flottum, 1997).

American Foulbrood (AFB) is one of the most important burning issue regarding the morbidity and mortality of honeybees (Shimanuki and Knox, 1997). The causative agent for the infection of American Foulbrood (AFB) is the spores that are produced by the bacterium *Paenibacillus larvae* (Genersch et al., 2006). These spores affect the larval stage of honeybees (*Apis mellifera*), that become pervasive throughout the colony. The spores are infectious and show great resistance to chemical agents (Haseman, 1961). Billions of spores are present in a dead larva. (Hansen and Brødsgaard, 1999).During nourishment the spores of *P. larvae* might be transferred to young larvae. After germination, these spores march into the honey bee gut epithelium and reproduce enough spores to kill developing bees earlier than pupation. Larval bees are highly susceptible to *P. l. larvae* spores, with less than10 spores required to cause death when fed to first instars (Shimanuki, 1997).

The clinical symptoms of AFB dead brood are typically seen in the late larval or pupal stages when the cells are capped. After capping over the cells of dead brood are most often pinched and dark. The house bees are trying to clean the infected cells that are why these cells are perforated. In the uncapped cells the dead brood has pepperbox like appearance (White, 1920). The adult bees remove the infected brood which causes in the disturbance of the brood pattern. (Rothenbuhler, 1964b). Brownish, viscous mass are appeared when Larvae and pupae are killed by AFB. With a little stick it can be drawn out to a long thread that may break if pulled too far. The odour of the decaying brood is foul and then turns into dry scales. The scales stick with the cell walls and it is very difficult for the bees to remove. Dead pupae are present in the bottom of the cells and the leftovers of their tongues expand upward (Hansen and Brodsgaard, 2001).

Exchange of hive material such as honey, brood combs and bees between colonies is a main cause of AFB transmission within and between apiaries (Matheson & Reid 1992; Goodwin *et al.* 1994; Pfeiffer and Crailsheim, 1998). Apicultural practices also put in danger unmanaged colonies in the neighborhood of the apiaries (Fries and Raina, 2003). Infected honey provide as a reservoir of AFB spores that are dispersed when bees use these stores (Hornitzky, 1998; Fries and Camazine, 2001; Lindstrom, 2008; Lindstrom et al., 2008). Aims and objectives of our research was to investigate the detection of American Foulbrood (AFB) disease in different areas of Kohat and Peshawar districts of Khyber Pakhtunkhwa

Materials and Methods

Areas selected for sampling:

The detection of American foulbrood was carried out in the honey bee (*Apis mellifera*) colonies of various apiries at deferent regions of Kohat and Peshawar (KPK, Pakistan). At Kohat region, different areas are visited; Dhoda, Jarma, Shah Pur and Sur Gul which are situated at distance of 4 Kms, 10 Kms, 3 Kms, and 7 Kms respectively, from Kohat University of science and technology Kohat Pakistan. While at Peshawar regions, various areas visited are; Gulbahar, Latif Abad, Mathra, and Pajagi which were located at distance of 7 Kms, 12 Kms, 10 Kms, and 15 Kms respectively from Ferdos Peshawar.

Collection of samples:

During March, 2012 to May, 2012 we visited four apiaries in each region of Kohat and Peshawar, because honey bee broods were present in this duration. We collected samples randomly from the different colonies of the visited apiaries and brought the samples in sterilized bottles into Laboratory. Moreover, in each colony we checked 4 to 5 frames and approximately 10 to 15 hexagonal brood cells in each frame.

Survey Method:

For conducting the survey under the mentioned title, we prepared a questionnaire, consisting of different queries, related to the bee keeping, and then we visited to the different aperies of Kohat and Peshawar regions. We politely met with the bee keepers of the mentioned regions. Some of the bee keepers not responded to our questions that we put forward to them because they were uninterested in sharing information with us regarding the bee keeping, while some of them were very cooperative. They let we people allowed for observing American foulbrood disease in all the colonies present in their aperies. They also showed us some of the AFB affected colonies, but they were not completely aware of all the sign and symptoms of AFB, and thus we pointed out those colonies which were affected by the early attack of AFB. Some of them were educated, and the questionnaire was filled out by themselves, while some of them were illiterate and hence we collected all the relevant data regarding the AFB, like; problems faced by the them, antibiotics used for honey bee disease, honey price fluctuation, food sources, associated flora of region, weather effect and local community interference etc, and we filled out the questionnaire according to the information provided by them.

Method of Detection:

To diagnose the American foulbrood disease in honey bee (*Apis mellifera*), we followed the method of Munawar et al., (2010). Through this technique we observed several infected colonies, in which the combs structure was disturbed and shown a mottled appearance caused by a healthy capped brood, uncapped brood cells containing the residues of the diseased larvae, and emptied cells. The cells that possess an infected larva appear moist and become concave and punctured as the infection progresses. The colour of larva and pupa changes from creamy white to dark brown. By inserting a probe (match-stick) into the larval remains present in dead cell, the larva becomes sticky and threads were appeared in consistency when the probe is dragged out. For field diagnosis and survey for American foulbrood disease it was possibly the best known procedure. Sometimes the residues of larva were watery, ensuing in a Negative match-stick test. After 1 month or more the larva were becomes ropy, dry, scaly, hard and adhesive and sticks tightly to the walls of the brood cell.

Results

Percentage of AFB infected colonies in an Apiary visited in district Kohat

In the current results it was find out that APKD-II and APKD-I are more infected 2.2% and 1.6% respectively in Dhoda Kohat. While in Jarma the APKJ-II, APKJ-III and APKJ-IV were infected with AFB 2.2%, 1.5% and 1.4% respectively. In Shah Pur only APKS-IV and APKS-I were infected by AFB 1.7% and 1.2% respectively. While in Sur Gul the most prevalence rate of infection by AFB was 2% observed in APKSg-III, 1.8% in APKSg-I and 1.2% was observed in APKSg-IV as shown in Table. 1.

Percentage of AFB infected colonies in an Apiary visited in district Peshawar.

In district Peshawar the prevalence rate of AFB infection was slightly more as compared to District Kohat. In different

areas of district Peshawar APKG-I was more infected 2.8% by AFB and 2.5% in APKG-IV while 1.5% in APKG-II were observed in Gulbahar. In Latif Abad APKL-I was 1.6% infected by AFB while APKL-II was 1.3% and APKL-IV 1.5% infection by AFB in Latif Abad. APKM-I, APKM-III and APKM-IV were infected 2.4, 1.4 and 1.4% infected by AFB in Mathra, district Peshawar. Similarly APKP-III, APKP-IV and APKP-I were infected 3%, 1.8% and 1.7% infected by AFB in Pajagi district Peshawar as shown in Table 2.

Table 1: Percentage of AFB infected colonies in an Apiary visited in district Kohat.

S/no	Location	Apiary codes	No. of colony present	No. of AFB infected colonies	No. of AFB non-infected colonies	% of AFB infected colonies	% of AFB non-infected colonies
1	Dhoda	APKD-I	60	1	59	1.6	98.3
		APKD-II	90	2	88	2.2	97.7
		APKD-III	85	0	85	0	100
		APKD-IV	65	0	65	0	100
2	Jarma	APKJ-I	70	1	69	1.4	98.5
		APKJ-II	90	2	88	2.2	97.7
		APKJ-III	65	1	64	1.5	98.4
		APKJ-IV	45	0	45	0	100
3	Shah Pur	APKS-I	80	1	79	1.2	98.7
		APKS-II	63	0	63	0	100
		APKS-III	50	0	50	0	100
		APKS-IV	57	1	56	1.7	98.2
4	Sur Gul	APKSg-I	55	1	54	1.8	98.1
		APKSg-II	45	0	45	0	100
		APKSg-III	50	1	49	2	98
		APKSg-IV	80	1	79	1.2	98.7

APKD= Apiaries Kohat Dhoda, APKJ= Apiaries Kohat Jarma, APKS= Apiaries Kohat Shah Pur, APKSg= Apiaries Kohat Sur Gul, AFB= American Foulbrood, P=0.0000 <0.05, Significant

Table 2: Percentage of AFB infected colonies in an Apiary visited in district Peshawar.

APPG= Apiaries Peshawar Gulbahar, APPL= Apiaries Peshawar Latif Abad, APPM= Apiaries

S/no	Location	Apiary codes	No. of colony present	No. of AFB infected colonies	No. of AFB non-infected colonies	% of AFB infected colonies	% of AFB non-infected colonies
1	Gulbahar	APPG-I	70	2	68	2.8	97.1
		APPG-II	65	1	64	1.5	98.4
		APPG-III	55	0	54	0	100
		APPG-IV	80	2	78	2.5	97.5
2	Latif Abad	APPL-I	60	1	59	1.6	98.3
		APPL-II	74	1	73	1.3	98.6
		APPL-III	50	0	50	0	100
		APPL-IV	66	1	65	1.5	98.4
3	Mathra	APPM-I	82	2	80	2.4	97.5
		APPM-II	68	0	68	0	100
		APPM-III	70	1	69	1.4	98.5
		APPM-IV	80	1	79	1.2	98.7
4	Pajagi	APPP-I	57	1	56	1.7	98.2
		APPP-II	52	0	52	0	100
		APPP-III	68	2	66	3	97.0
		APPP-IV	53	1	52	1.8	98.1

AFB= American Foulbrood, APPG=Apiaries Peshawar Gulbahar, APPL= Apiaries Peshawar Latif Abad, APPM= Apiaries Peshawar Mathra, APPP= Apiaries Peshawar Pajagi, P=0.0000 <0.05, Significant

Discussion

The research findings of Pernal and Melathopoulos (2006) revealed that for the diagnosis of *Varroa destructor, Acarapis woodi* and *Nosema apis* in Canada adult honey bees samples were used. They have verified that with presented samples of adult bees collected in 70% ethanol, the recognition of AFB were achievable. These samples were collected at the same time that colonies were inspected for infection and are more highly associated with innate disease levels within beekeeping operations as a whole. Their findings show that 5 out of 9 adult bees' apiaries were infected with AFB in (2004).

According to Munawar et al., (2010), American foulbrood is the most severe disease that without proper treatment results not only in death of affected bee colonies but also in death of entire apiaries. Lately the disease is becoming a problem in the world. The traditional methods of control through killing and burning of affected bee families those were in use until several years ago the treatments of the other bee families with antibiotics and sulfonamides were a real hazard with regard to the accumulation of drug residues in honey bee foodstuff. That is why the use of antibiotics and sulfonamides in most European countries is prohibited by law (law on Apiculture 2003). It is therefore very important to develop and implement the alternative methods for the control of AFB that exclude the use of antibiotics.

The final result of our investigation about AFB in different regions of Kohat and Peshawar (Pakistan) showed that out of 32 Apiaries (containing 2100 colonies), 26 colonies were identified positive for AFB and 12 colonies were infected in Kohat region (Table 1) while 14 colonies in Peshawar region (Table 2) were reported positive for AFB disease.

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