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THE FIRST RECORDS OF THE PARASITE ZOMBIE FLY (*APOCEPHALUS BOREALIS* BRUES) ON HONEYBEE, *APIS MELLIFERA* IN EGYPT

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ABSTRACT

During the last year, there have been large-scaleunexplained losses of honey bee (*Apismellifera* L.) colonies in Egypt. This phenomenon is characterized by severe shortages in the population numbers of adult honeybee workers. *Apocephalus borealis* fly is suspected of contribution to the decrease in the honeybee population, the infected foragers left their hives at night and dying shortly thereafter. Honey bee colonies are infected by numerous pathogens and parasites, the interaction among multiple pathogens and parasites is the proposed cause of the phenomenon represents a recent host shift and an emerging problem for honeybee Colony Collapse Disorder (CCD), Phorid fly (Zombie fly) *A. borealis*, previously known to parasitize bumble bees.Based on that, many samples of adult bees were collected from different Governorates in Egypt, the infection with Phorid fly appeared in all collected samples with different infection ratios. The highest rate of infected colonies was recorded in Qalubia Governorate at 37% but the less incidence of Zombie fly was found in Minya Gov.at 12%.This is the first record of Zombie fly, *Apocephalus borealis* appearance on Honeybee, *Apismellifera* L. in Egypt. This research was carried out during the period from October 2013 to May 2014.

KEYWORDS: Honeybee, Phorid Fly, Zombie Fly, Apocephalus Borealis, CCD, Egypt

INTRODUCTION

Honeybees (*Apismellifera*) are obviously the most valuable crop pollinators to agriculture because they can be easily transported to pollinator-dependent crops **(Williams *et al,* 2010)**. Yet, despite an almost 50% increase in world honey bee stocks over the last century, beekeepers have not kept velocity with the >300% increase in pollinator-dependent crops. This has led to great uncertainty surrounding the recent large scale die-offs of honey bee colonies around the world, and has sparked enormous interest from both scientists and the general public **(Aizen and Harder, 2009)**. These phenomenon losses are called Colony Collapse Disorder (CCD), which is characterized by the rapid disappearance of adult bees from colonies containing brood and food stores **(Engelsdorp*et al,* 2009)**. Pathogens and other environmental stresses, including pesticides, have been linked to CCD, but a causal relationship has not yet been demonstrated **(Johnson *et al,* 2009)**.

Beginning in fall 2006, the American apiculture industry experienced catastrophic losses of unknown origin. The phenomenon, called colony collapse disorder (CCD), was identified by a set of distinctive characteristics, including the absence of dead bees in or near the colony and the presence of abundant brood, honey, and pollen despite vastly reduced numbers of adult workers**(Engelsdorp*et al,* 2007)**.No simple explanations for the cause of CCD emerge from the microarray analysis. However, considerable geographic variation existed among CCD bees, with west coast bees seemingly more severely affected **(Johnson *et al,* 2009)**.But, it well known that Honeybees suffer from numerous parasites

and pathogens including viruses, bacteria, parasitic fungi and ectoparasitic mites **(Core *et al,* 2012)**.Parasitoid Phorid flyattack bumble-bees (Bombus), inserting eggs on or within a bee's body. Such attacks probably occur while bees or foraging at flowers **(Otterstatter*et al,* 2002)**. The Phorid fly, *Apocephalus borealis*Brues 1924 (Diptera, Phoriadea), known to broad distribution of *A. borealis* throughout North America, virtually nothing is known about its biology and association with bumble-bees. The aim of this research is to record the first time of the parasite Phroid fly (Zombei fly) appearance on Honeybee in Egypt and the prevalence study.

Identifying Phorid fly, (Brues, 1924)

Table 1

Kingdom	**Animalia**
Phylum	Arthropoda
Class	Insecta
Order	Diptera
Family	Phoridae
Tribe	Metopininae
Genus	*Apocephalus*
Species	***Apocephalus borealis***

Apocephalus borealis is a species of North American parasitoid Phorid fly that parasitizes bumblebees, honey bees and paper wasps. These flies are colloquially known as zombie flies and the bees they infect are colloquially known as zombees **(Figard, 2012)**. The association with honey bees has so far only been documented from California, South Dakota, Oregon, and Washington**(Doughton, 2012)**; elsewhere, they are primarily associated with bumblebees and paper wasps, but most recently this species has changed host and has begun to attack the European honey bee. A case was confirmed in October 2013 of an infestation of honeybees in Vermont (**Gittleson, 2014).**

History

The infection of European honey bees in North America by *Apocephalus borealis* was first discovered by Dr. John Hafernik, who noticed disoriented honey bees at a light at night on San Francisco State University's campus. He picked some up in a vial, forgot about them, and about a week later noticed that fly larvae had emerged from the dead bees **(Talk, 2012).** There is insufficient information as to why the parasitic fly jumped to its new host, but there is concern that this new host provides an opportunity for the fly to thrive and further threaten the decreasing honey bee population **(Arce and Pedraza, 2012).** *A. borealis* has been suggested as a possible vector promoting the spread of the pathogens responsible for colony collapse disorder **(Core*et al,* 2012)** and **(Coghlan, 2012)**.

Life Cycle

Female flies lay their eggs in the bees, and as the larvae develop, they attack the bees' brains and cause them to become disoriented. Infected bees can be found walking in circles as well as losing the ability to stand. The honey bees will also remain inactive during the daytime until death occurs. The infected bees are also known to fly at night and exhibit other unusual behaviors such as hive abandonment in unusual weather conditions such as cold rainy nights when most insects remain inactive **(Castro,2012)**.These behaviors eventually result in the death of the bees, but increase the survival and spread of the Phorid flies. Laboratory results show that female Phorid flies immediately attack honey bees when put together. Females attack and pursue host honey bees until they land on their abdomens and insert the ovipositor for about

two to four seconds, injecting eggs. Development of larvae takes about an average of a week; the larvae feed on the honey bees' muscles and nervous system. Mature fly larvae typically emerge from the host between the head and thorax (but rarely result in decapitation), and pupate outside the host body. It usually takes about 28 days for the entire life cycle to take place **(Core*et al*, 2012).**

MATERIALS AND METHODS

Honeybee Worker's Samples

The honeybee worker's samples were collected from many regions in sexGovernorates in Egypt namely; Qalubia, Minya, BeniSuef, Alexandria, Behira and Sharkiato survey the prevalence of Zombei fly parasite, *Apocephalus borealis*.

Samples were collected randomly from the Apiaries using light traps or from the hives, and then every fifty insects placed in glass jar one kg tied with thin wire to follow up all parasite stages. After that, jars were put under Lab. conditions, and all samples were received candy to make them alive. After seven days the infection ratio was calculated.

Calculation of the Infection Ratio

The rate of invasion of outgoing foragers and incoming foragers were compared. No significant differences between these groups were found. Therefore, both groups are used to determine long-term trends in rates of infection in active and foraging bees.

In order to assess parasitism ratio, the collected bees were brought to the laboratory and then incubated at room temperature on 20˚C for two weeks. The samples were checked daily to record the number of emerged maggot of Zombei fly.

After about seven days of incubation, some workers dead and the fly larvae were obtained. The dead bees, fly larvae and fly pupa were counted to calculate the infection ratio of workers samples which collected in the jars from the colonies or the drop workers under light.

The Infection Ratio Equation

$$\text{Infection ratio } (\%) = \frac{\text{dead bees}}{\text{all bees}} \times 100$$

The biology of Zombei fly, *Apocephalus borealis*was observed and examined from eggs which infected worker by Phorid fly female and during larvae, pupae and adults. The samples were microscopic, photography and printed.

RESULTS AND DISCUSSIONS

Honey bees are among themost studied insects in the world due to their importance forlife. Honey bees were infected with many diseases and parasites which lead toa severe shortageof beesin thecensus. But, we have not seenlarge numbers of honey bees aggregating around light until recently.

Survey and Infection Percentage Calculation of Zombei Fly on Honeybees Collected From Different Locations in Egypt

This experiment was carried out during the period from October 2013 to May 2014. Recorded data in the table illustrated by Figure 1 explained the wide prevalence of Phorid fly in six Governorates of Egypt. Generally, data indicated that all samples are infected by the parasite, Zombei fly (Phorid fly), with different percentages. These results are in

agreement with **Core *et al* (2012)** which recorded that 77% of his sample sites yielded honey bees parasitized by *A. borealis*.

The highestinfection rate was recorded in Qalubia governorate with 37% while, the lowest infection rate was observed in Minya followed by Sharkiagovernorates with 12 and 20 %, respectively.

Table 2: Survey and Infection Percentage of *A. borealis* (Zombei Fly) on Honey Bees Collected from Different Locations in Egypt

Governorate	Location	No. of Bees Per Sample	Infection Percentage (%)	No. of Phorid Fly Larvae Per Worker	Mean of Infection (%)
Qalubia	Moshtohor	50	42	7.48	37
	Kafrelwan	50	32	5.25	
	Shbeenelknater	50	46	8.26	
	El-Khanka	50	40	5.25	
	Senhera	50	52	11.9	
	Benha	50	10	14.4	
Minya	Benimzar	50	12	5.33	12
BeniSuef	Beba	50	22	4.10	21
Alexandria	Alexandria	50	30	6.20	31
Behira	Abu qeer	50	36	4.33	32
	Kafreldwar	50	28	3.43	
	Kafreldwar	50	32	4.31	
Sharkia	Kafrsaker	50	20	3.60	20

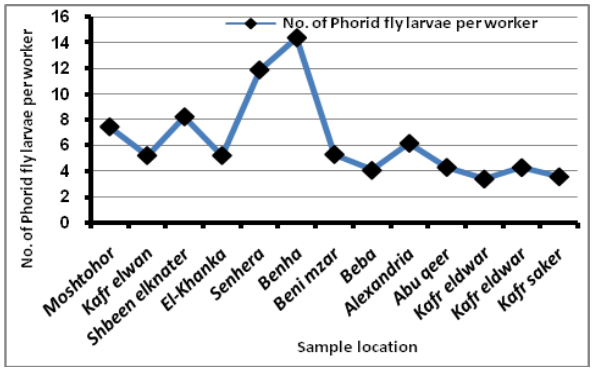

Figure 1A: Number of Phorid Fly Larvae per Worker in Different Locations in Egypt

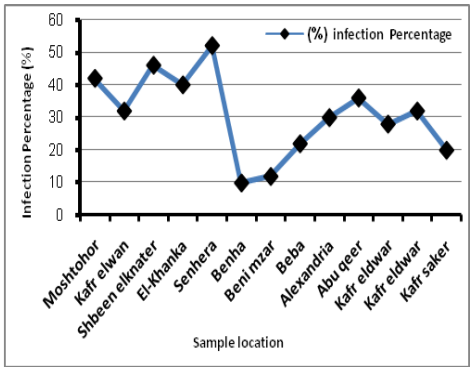

Figure 1B: Infection Percentage (%) of Phorid Fly Larvae per Worker in Different Locations in Egypt

Also, data showed that honeybee workers were infected with many Phorid fly larvae, it was ranged from 3.43 to 14.4 Phorid fly larvae/bee worker by mean of 6.45 Phorid fly larvae/bee worker.Mature phorid larvae emerged from the junction between a bee's head and thorax (Figure 2).

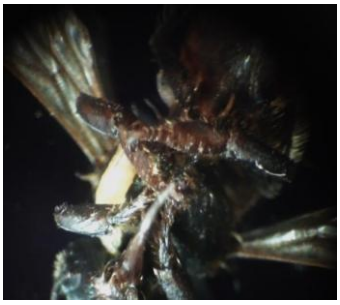

Figure 2: Final Instar Larva Exiting a Honey Bee Worker

Generally, in honey bee collected samples, we observed the incidence of all stages such as eggs, larvae, pupae and the adult of *A. borealis* (Figure 3 A, B, C & D).

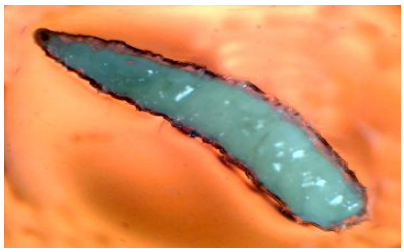

Figure 3A: Larva of Phorid Fly

Figure 3B:Pupa of Phorid Fly

Figure 3C: Emerging of Phorid Fly

Figure 3D: Adult of Phorid Fly

The anatomy of the adult fly showed that it had a lapping mouth parts and the female had a short ovipositor Figure 4 (A & B).

Figure 4A: Mouth Parts of Phorid Fly Adult

Figure 4B: Ovipositor of Phorid Fly

Data clearly showed that phorid-parasitized bees have anabnormal behavior of give up their hives at night. However, we can't eliminate the possibility that some parasitized bees also abandon their hive during normal foraging times and die at some distance from the hive.This phenomenonmayexplain thestate of collapse (CCD) that has emerged inbeesin Egyptfrommany years. This explanation is consistent with**Core *et al* (2012)** who reported that*A. Borealis* as a novel parasite of honeybees and documents hive abandonment behavior consistent with asymptom of CCD.

CONCLUSIONS

On the other hand, **Runckel*et al* (2011)** indicated that the seasonal variation could be associated with the life cycle of the fly in which rates of parasitism of honey bees fluctuate as *A. borealis* populations seasonally increase and decline.

REFERENCES

1. **Aizen MA, Harder LD. (2009).** The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr. Biol, 19:15–18.

2. **Arce, A. P. and Pedraza, R. (2012)**."Evaluation of Phorid Fly (*Apocephalus borealis*) parasitism of Feral Honey Bee (*Apismellifera*) colonies in South Orange County.".KSBR and the Department of Biological Sciences, Saddleback College.

3. **Brues, C. T. (1924).**"Notes on Some New England Phoridæ (Diptera)".*Psyche: A Journal of Entomology***31**: 41–44.

4. **Castro, J. (2012)**. "Fly Parasite Turns Honeybees into Zombies | Live Science ".

5. **Coghlan, A. (2012)**. "Parasitic fly could account for disappearing honeybees". New Scientist.

6. **Core, A; Runckel, Ch; Ivers, J; Quock, Ch; Siapno, T; DeNault, S; Brown, B; DeRisi, J, Smith, C. D, Hafernik, J. (2012)**. A New Threat to Honey Bees, the parasitic Phorid Fly *Apocephalus borealis*. PLoS ONE, 7(1):1-9

7. **Disney, R. H. L. (1994).**Scuttle Files: The Phoridae. Chapman & Hall, London, ISBN 0-412-56520-X.

8. **Doughton, Sandi (2012)**. "Start's first case of 'zombie bees' found in Kent". The Seattle Times newspaper, Seattle, Washington, US.

9. **Engelsdorp, D; Evans J.D; Saegerman C, *et al*. (2009).** Colony collapse disorder: a descriptive study. PLoS One, 4:64-81.

10. **Engelsdorp, D; Underwood, R; Caron, D, Hayes, J. (2007).** An estimate of managed colony losses in the winter of 2006–2007: A report commissioned by the apiary inspectors of America. Am. Bee J, 147:599–603.

11. **Figard, K. (2012).**"Seeking ZombeeHunters".The San Francisco Examiner newspaper, San Francisco, US, P:12.

12. **Gittleson, B. (2014).**"'Zombie' Bees Surface in the Northeast". ABC News, Australia.

13. **Johnson, R. M; Evans, J. D; Robinson, G. E. and Berenbaum, M. R. (2009).** Changes in transcript abundance relating to colony collapse disorder in honey bees (*Apismellifera*). PNAS, 106(35): 14790-14795.

14. **Otterstatter, M. C; Whidden, T. L. and Owen, R. E. (2002).**Contrasting frequencies of parasitism and host mortality among Phorid and conopid parasitoids of bumble-bees. The Royal Entom. Soci, Eco. Entom, 27: 229-237.

15. **Runckel C, Flenniken ML, Engel J, Ganem D, Andino R, *et al*. (2011)**. Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, Nosema,andCrithidia. PLoS ONE 6(6): e20656. doi:10.1371/journal.pone.0020656.

16. **Talk, T. (2012).**"Flight of the Living Dead: Dr. John Hafernik". TedTalk.

17. **Williams, G. R; Tarpy, D. R; Engelsdorp, D; Chauzat, M; Cox-Foster, D. L; Delaplane, K. S. Neumann, P; Pettis, J. S; Rogers, R. E. L. and Shutler, D. (2010).**Colony collapse disorder in context.Bioessays, 32: 845-846, WILEY Periodicals, Inc.