Viruses in Honeybee Colonies

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ABSTRACT

Honey bee (*Apis mellifera*) health is a growing concern throughout the world as bee populations continue to dwindle. Due to their significant role in ecology and agriculture, it is of vital importance to preserve and maintain honey bee colonies. Viruses are serious threat affecting honey bee health today and are often associated with colony deaths. To determine if viruses contributed to colony losses at Purdue's apiary, we sampled 50-100 bees from 16 colonies that had dwindling populations and 22 healthy colonies. Colonies were also categorized based on which survived the winter. Bees were pooled by colony and ground together. We took a tissue sample from each colony sample and extracted RNA, which was converted to cDNA. We ran a PCR for six different pathogens (Deformed Wing Virus, Acute Bee Paralysis Virus, Kashmir Bee Virus, Blackened Queen Cell Virus, Israeli Acute Paralysis Virus, *Nosema ceranae*) to determine their presence. None of the colonies tested positive for KBV or IAPV, but all of the colonies tested positive for Deformed Wing Virus. A greater proportion of sick colonies were positive for *N. ceranae* (56%) than control colonies (32%). Quantitative real-time PCR was performed for ABPV and DWV determine pathogen concentration in the samples. Sick colonies and colonies that died during the fall and winter had significantly higher concentrations of ABPV than healthy colonies. Chi-squared tests also showed that colonies with high amounts of ABPV also tended to have high amounts of DWV, but neither virus showed an association with *N. ceranae* or BQCV. These results suggest *N. ceranae*, ABPV, and DWV may be involved with hive losses. Viruses may be interacting with each other and Varroa mites to cause these colony deaths.

INTRODUCTION

The concern over continual decline in honey bee (*Apis mellifera*) colonies has caused a worldwide interest in honey bee health. Honey bees are vital pollinators for billions of dollars worth of crops, and they produce important hive products such as honey, pollen, beeswax, and royal jelly. Their significant role in ecology and agriculture makes honey bees essential to preserve. There are a variety of parasites and pathogens that threaten bee populations, so it is necessary to research and control these diseases to maintain honey bee health.

A honey bee suffering from Deformed Wing Virus.

There are many pathogens that can cause hive death. Threats such as Varroa mites (*Varroa destructor*), American foulbrood, and *Nosema ceranae* have been attributed to colony deaths for years. Recent surveys in the US, Canada and Europe have found that Varroa is the single most important factor associated with colony losses but these mites are known to transmit viruses and weaken the bees immune response to viruses. Only recently has significant research been focused on honey bee viruses. As viruses require molecular methods to detect them, beekeepers don't know what viruses are in their colonies at all. Many honey bee pathogens have been under study as possible causes the affliction known as Colony Collapse Disorder (CCD), but no single factor has emerged as the cause. In recent a study on CCD, researchers looked at multiple pathogens in colonies apparently suffering from CCD[1]. While they also determined CCD not to have a single cause, they found CCD colonies to have more pathogens and heavier pathogen loads; when they looked at viruses specifically, their results showed that significantly more CCD colonies were infected with more than 3 viruses. Our study focused primarily on viruses as a possible factor of colony decline.

METHODS

We collected 50-100 bees on dry ice from 16 colonies with dwindling populations and 22 healthy colonies. The bees from each colony were pooled together and ground in liquid nitrogen. We took a tissue sample and extracted RNA, which was then converted to cDNA. For each pathogen of interest, we used a specific pair of primers and did a polymerase chain reaction (PCR) to determine the presence of the pathogen. We looked at six viruses: Acute Bee Paralysis Virus (ABPV), Deformed Wing Virus (DWV), Blackened Queen Cell Virus (BQCV), Israeli Acute Bee Paralysis Virus (IABPV), Kashmir Bee Virus (KBV), and Chronic Bee Paralysis Virus (CBPV).

Bee samples being ground in liquid nitrogen.

Setting up a Polymerase Chain Reaction.

We also included *Nosema ceranae* since it was only found to be present in the US in the past few years. For those pathogens that were present in the samples, we performed qRT-PCR to test for pathogen concentration in the colonies. BQCV and *N. ceranae* were not quantified due to insufficient primers.

The results were evaluated based on 1) colonies that appeared sick or healthy at the beginning of the study and 2) colonies that had died or survived over the winter. Pathogens were also compared with each other to determine if they tended to coexist in the colonies. All statistical analyses were conducted in SPSS 17.0.

RESULTS and DISCUSSION

None of the colonies tested positive for KBV, IABPV, or CBV. All of the colonies tested positive for DWV. BQCV presence didn't have a prevalence in sick or dead colonies, but did seem to be associated with colonies that had high levels of ABPV ($p=0.10$). ABPV showed a sporadic distribution, but was more prevalent in sick colonies and colonies that eventually died ($p=<0.01$, *see Figure 1*). Although *N. ceranae* was more prevalent in sick colonies (56%) than in healthy colonies (32%), its presence was not significantly correlated with whether a colony was dwindling in the fall or died. DWV levels did not correlate with sick/dead colonies. However, when colonies were classified as high or low for the level of DWV, Chi-square tests showed a significant correlation between this virus and ABPV (*Figure 2*).

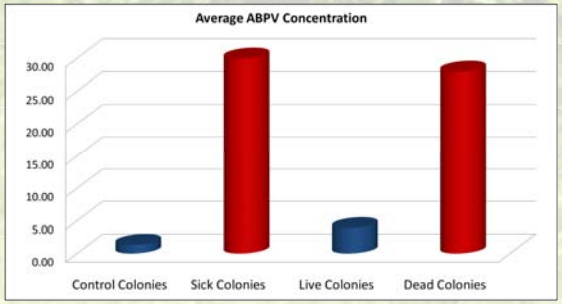

Fig. 1. Graph showing the average ABPV concentration between colonies.

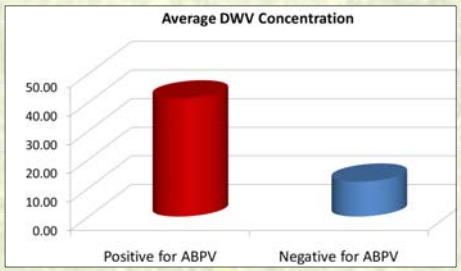

Fig. 2. Graph showing the average DWV concentration between colonies that tested positive and negative for ABPV.

Summary of Significant Test Results

Chi-squared Tests

Categorical variable 1	Categorical variable 2	Significance
High/Low ABPV	Control/Sick Colony	0.015
Positive/Negative ABPV	High/Low DWV	0.018
High/Low ABPV	High/Low DWV	0.004
Positive/Negative BQCV	High/Low ABPV	0.10

ANOVA Tests

Variable	Factor	Significance
ABPV concentration	Control/Sick colonies	0.010
ABPV concentration	Live/Dead colonies	0.018
DWV concentration	Colonies with/without ABPV	0.005

ABPV seems to be the most significant pathogen in colony deaths. *N. ceranae* showed a trend in sick colonies, but its lack of significance suggests a minor role. ABPV had strong correlation with sick/dead colonies, and DWV correlated with ABPV, even though DWV didn't seem to correlate with sick/dead colonies. This correlation between DWV and ABPV suggest an interaction between viruses. Perhaps higher DWV permits the amplification of ABPV, or one virus weakens the immune system to allow multiple infections. Relatively high levels of Varroa mites were present in the Purdue apiaries in late 2009 and these were not controlled because of a breeding program to select for mite resistance. The mites may have been an interacting factor with the viruses. Colonies that have high mites can develop "parasitic mite syndrome" which results in higher virus loads and death of the colony (*Figure 3*).

Fig. 3. Bees suffering from parasitic mite syndrome.

FURTHER RESEARCH

Repeated comparisons of viruses in dwindling colonies needs to be done before their relation to CCD can be determined. More studies comparing a combination of pathogens are needed to see whether certain pathogens react with one another to worsen colony decline.

Literature Cited

[1] vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen YP and others. *Colony Collapse Disorder: A Descriptive Study.* Plos One 2009;4(7):17.

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