Acta Veterinaria Brasilica March 11 (2017) 85-89

Acta Veterinaria Brasilica

Journal homepage: http://periodicos.ufersa.edu.br/revistas/index.php/acta

Short Communication

Rates of single and multiple virus infection in the honey bee, *Apis mellifera*

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A R T I C L E I N F O

Article history
Received 14 September 2016
Received in revised form 14 December 2016
Accepted 20 December 2016

Keywords:
Colony collapse disorder
Pollination
Beekeeping
Bee health

A B S T R A C T

Colony collapse disorder has increased interest in the role of viruses in honey bee health. In this study, we determine if management practices affect the rates of infection for six common viruses by comparing the rates of infection between migratory, queen breeder, and hobbyist beekeepers. The study found that migratory beekeepers had higher rates of infection for Israeli acute paralysis virus and Kashmir bee virus. Migratory beekeepers also had higher rates of infection by more than one virus within a single colony. These results highlight the importance of management practices, perhaps including housing bees at high density, for the prevention of viral infection.

INTRODUCTION

Viral infection, along with other pathogens and factors, is thought to be involved in colony collapse disorder (CCD), a syndrome that has led to large losses of bees in recent years (BERTHOUD et al., 2010; CAVIGLI et al., 2016; EVANS; SCHWARZ, 2011; GENERSCH, 2010; HIGES et al., 2008; JOHNSON et al., 2009; MULLIN et al., 2010; VAN ENGELSDORP et al., 2008, 2009).

Honey bees are infected by at least 18 viruses (reviewed in CHEN; SIEDE, 2007; RUNCKEL et al., 2011), six of which have widespread distributions (GENERSCH; AUBERT, 2010). Although viruses have not consistently been a major threat to bee health in the past, this changed with the arrival of *Varroa destructor*, a parasitic mite that is an efficient vector of viruses (BOWEN-WALKER et al., 1999; CHEN et al., 2004a, b; EMSEN et al., 2015; MARTIN et al., 2012). Viral loads, possibly elevated by mites, could be particularly acute in commercial beekeeping operations as these involve housing bees at high densities.

This study explores the presence and prevalence of virus infections in honey bees across California, which has the highest densities of colonies in the US. It examines rates of infection for six viruses—deformed wing virus (DWV), black queen cell virus (BQCV), sacbrood virus (SBV), Kashmir bee virus (KBV), acute bee paralysis virus (ABPV), and Israeli acute paralysis virus (IAPV)—in three distinct honey bee operations: migratory beekeepers, queen breeders, and hobbyists. It was predicted that the strongest levels of viral infection would be found among migratory beekeepers, as their bees are under the most stress and are exposed to the highest concentrations of bees coming from all over the country for almond pollination.

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http://dx.doi.org/10.21708/avb.2017.11.1.6479

MATERIALS AND METHODS

Nine migratory beekeepers, ten hobbyist beekeepers, and three queen breeders from across the state of California participated in the study. Migratory beekeepers were defined as having at least several hundred colonies and making a significant portion of their income from either pollination or honey production. Queen breeders were defined as having large numbers of colonies for the production of queens and package bees. Hobbyists had less than 10 colonies, usually in a large backyard or on a small farm. From each migratory beekeeper and queen breeder, we chose 10 colonies from which to sample. For hobbyist beekeepers, who typically only keep a handful of colonies, 3–5 colonies were sampled. For the migratory beekeepers and queen breeders, colonies were split between two apiaries (5 colonies each). Colonies were chosen at random within apiaries. In total, 155 colonies were sampled, of which 88 were from migratory beekeepers, 30 from queen breeders, and 37 from hobbyists. Initially, foragers from the entrance of each hive were sampled. Bees were collected using an insect vacuum and were quickly transferred to 50 mL centrifuge tubes. Then, bees from the brood nest were sampled using the same method as for foragers. Both groups of bees were immediately put on dry ice for transportation to the laboratory, where they were stored at -80 °C until testing. After collection, each hive was evaluated by making a visual inspection of the following traits: amount of adhering bees on the combs (population size), number of brood combs and nectar combs (colony size), and brood pattern. Visual inspections, which are commonly conducted, are useful for identifying obviously weak and dying colonies.

Each colony was tested for six viruses using reverse transcription polymerase chain reaction (RT-PCR). Five bees from each colony were ground to a fine powder in liquid nitrogen and 75 mg of powder were then used for extraction. Total ribonucleic acid (RNA) was extracted using Trizol (Thermo Fisher Scientific Inc). cDNA was generated using Promega's GoScript Reverse Transcription System, following the manufacturer's instructions (Promega Corporation, Madison, WI – USA). RT-PCR was then carried out using a thermocycler (Eppendorf Mastercycler Pro S, Eppendorf AG, USA), programs and primers, as described in Singh et al. (2011). A positive control was used for each PCR run, together with two negative controls (water and no enzyme). PCR products were run on 1% agarose gels and stained in 0.5 ug/mL ethidium bromide before being observed under ultraviolet (UV) light. For final verification, PCR products were purified using Qiagen's QIAquick Gel Extraction Kit (Qiagen Inc, Valencia, CA) and sequenced in both directions using Sanger sequencing method. Nucleotide sequences were compared to published sequences in GenBank. For each sample, the corresponding virus taken as the best hit was that with coverage greater than 95% for all samples. One-way analysis of variance (ANOVA), ordinal logistic regression, and binary logistic regression were used as statistical tests to study the influence of management practices on colony health characteristics, and on the nature and frequency of virus infections.

RESULTS

There were minor differences between the colonies in terms of the different types of operations (Table 1). Colony size was found to vary between operation types (one-way ANOVA: $F_{2,147} = 4.25$, $p = 0.016$), with migratory beekeepers having more compact colonies than hobbyists. Population density was found to differ between operators (ordinal logistic regression: log-likelihood = -165.28, $G = 20.88$, $df = 2$, $p < 0001$), with hobbyists having less densely populated colonies ($Z = 3.79$, $p < 0.001$, *odds ratio* 8.52). The number of nectar combs also varied between operations (one-way ANOVA: $F_{2,128} = 3.14$, $p = 0.047$), as did the amount of pollen (ordinal logistic regression: log-likelihood = -137.04, $G = 9.77$, $df = 2$, $p = 008$), and the number of brood combs (one-way ANOVA: $F_{2,137} = 14.95$, $p < 0.0001$). Brood quality did not differ between operation types (ordinal logistic regression: log-likelihood = -165.69, $G = 5.13$, $df = 2$, $p = 0.08$).

Table 2 shows the results for virus testing by operation type. DWV, BQCV, SBV, and APBV were not found to differ between operation types (binary logistic regression: DWV: log-likelihood = -4.60, $G = 2.87$, $df = 2$, $p = 0.24$; BQCV: log-likelihood = -84.83, $G = 3.00$, $df = 2$, $p = 0.22$; SBV: log-likelihood = -106.79, $G = 0.52$, $df = 2$, $p = 0.78$; APBV: log-likelihood = -34.58, $G = 5.00$, $df = 2$, $p = 0.08$). IAPV and KBV were found to differ between operation types (binary logistic regression: IAPV: log-likelihood = -83.22, $G = 42.19$, $df = 2$, $p < 0.001$; KBV: log-likelihood = -64.95, $G = 7.07$, $df = 2$, $p = 0.03$). For IAPV, migratory beekeepers had the highest rates of infection with a roughly five-fold higher odds ratio for infection ($Z = 3.32$, $p = 0.001$, *odds ratio* 4.98). For hobbyists, rates of IAPV infection were significantly lower than for queen breeders or migratory beekeepers. For KBV, only migratory beekeepers were found to have significantly higher rates of infection ($Z = 1.98$, $p = 0.05$, *odds ratio* 7.99). With respect to rates of multiple infection, there were differences in the rate of multiple infection between operation types (ordinal logistic regression: log-likelihood = -233.40, $G = 11.94$, $df = 2$, $p = 0.003$), with migratory beekeepers alone showing significantly higher levels of infection by more than one virus per colony ($Z = 2.98$, $p = 0.003$, *odds ratio* 0.31).

Table 1 – Results of visual inspections of colony state.

Migratory Beekeepers	Story*	Pop*	Brood quality	Brood combs*	Nectar combs*	Pollen combs*
Migratory 1	2.00	2.00	1.56	3.89	7.44	1.22
Migratory 2	2.00	2.50	1.40	3.40	5.50	2.00
Migratory 3	2.00	2.80	2.33	4.90	9.60	1.00
Migratory 4	2.00	2.80	1.70	5.80	5.60	1.70
Migratory 5	2.50	2.90	1.80	4.40	9.10	2.40
Migratory 6	1.30	1.80	1.50	3.30	4.70	1.90
Migratory 7	2.00	1.44	1.78	3.33	n/a	2.00
Migratory 8	2.00	2.40	1.70	5.50	5.40	1.00
Migratory 9	2.30	1.75	1.65	3.90	3.70	2.00
Overall	2.01	2.27	1.71	4.27	6.38	1.69
Queen Breeders	Story*	Pop*	Brood quality	Brood combs*	Nectar combs*	Pollen combs*
Breeder 1	2.00	2.00	1.50	5.30	4.70	1.70
Breeder 2	2.00	n/a	1.00	7.30	7.80	2.00
Breeder 3	2.40	2.90	2.60	n/a	n/a	2.00
Overall	2.13	2.45	1.70	6.30	6.25	1.90
Hobbyists	Story*	Pop*	Brood quality	Brood combs*	Nectar combs*	Pollen combs*
Hobbyist 1	2.00	2.00	1.33	4.00	7.00	2.17
Hobbyist 2	2.20	1.80	1.20	4.20	10.20	2.60
Hobbyist 3	3.00	1.63	1.88	5.75	7.25	1.75
Hobbyist 4	n/a	n/a	n/a	n/a	n/a	n/a
Hobbyist 5	2.70	2.20	1.20	3.60	10.40	2.00
Hobbyist 6	2.33	1.33	1.17	3.67	5.33	2.00
Hobbyist 7	2.00	1.00	1.00	1.00	6.00	1.00
Hobbyist 8	1.67	2.33	2.00	4.33	11.67	2.67
Hobbyist 9	2.00	1.33	1.67	2.50	3.33	2.00
Hobbyist 10	2.00	1.33	1.67	2.50	6.67	2.00
Overall	2.21	1.66	1.46	3.51	7.54	2.02

* indicates statistical significance.

DISCUSSION

The results of the visual inspections were consistent with our expectations. Our assessment of brood quality, which can be inferred by the compactness of the pattern, showed no significant differences between operation types. In general, although there was significant variation between colonies in different operation types, it was not particularly strong and was not likely to cause poor colony health due to a general lack of management.

It was found that migratory beekeepers had higher rates of infection for two viruses, IAPV and KBV, and higher rates of multiple viral infections. IAPV was one of the first viruses thought to be associated with CCD (COX-FOSTER et al., 2007). A significant association is found in this study with migratory beekeeping practices. KBV has also recently been found to be associated with CCD (VAN ENGELSDORP et al., 2009). The pathogenicity of this virus is unknown; however, its lower rates of infection overall (60% for IAPV compared to 22% for KBV) make it less likely to be a culprit for major losses than IAPV. With respect to multiple infections, this study confirms that migratory beekeepers have higher rates than stationary beekeepers. This is a significant result in that one possibility for the rise in viral loads across the US could be due to queen breeders, who are producing a higher percentage of the country's bees than ever before, and shipping sick bees throughout the country. This does not seem to be the case, however, given the relatively low rates of virus infection in queen breeding operations. Given that the bees kept by some migratory beekeepers are periodically stored in holding yards with thousands of colonies from around the country, while queen breeders and hobbyists keep their bees in relative isolation, there is a clear mechanism that could cause this pattern. Of course, this hypothesis is speculative at this point, and future work will need to look carefully at

rates of infection in migratory bees before and after the pollination season. Studies that look at the fate of newly founded (and relatively healthy) colonies before and after pollination will be particularly useful.

Table 2 – Rates of virus infection in different types of beekeeping operations. Multiple and range refer to the mean number and range of different viruses infecting a single colony.

Commercial Beekeepers	N	DWV (%)	SBV (%)	KBV* (%)	IAPV* (%)	BQCV (%)	ABPV (%)	Multiple *	Range
Migratory 1	9	100	33	11	56	89	0	2.89	1–4
Migratory 2	10	100	30	10	10	80	0	2.30	1–4
Migratory 3	10	100	0	20	20	80	0	2.20	1–3
Migratory 4	10	100	50	20	100	90	10	3.70	2–5
Migratory 5	10	100	60	10	30	90	20	3.10	2–6
Migratory 6	10	100	60	10	70	90	10	3.40	2–5
Migratory 7	9	100	56	78	89	78	11	4.11	3–5
Migratory 8	10	100	70	30	100	100	0	4.00	3–5
Migratory 9	10	100	40	10	70	20	10	2.50	1–5
Overall	88	100	44	22	60	80	7	3.13	1–6
Queen Breeders	**N**	**DWV (%)**	**SBV (%)**	**KBV* (%)**	**IAPV* (%)**	**BQCV (%)**	**ABPV (%)**	**Multiple ***	**Range**
Breeder 1	10	100	30	10	0	50	0	1.90	1–3
Breeder 2	10	100	90	0	20	90	0	3.00	2–4
Breeder 3	10	100	20	0	50	50	0	2.20	1–4
Overall	30	100	47	3	23	63	0	2.37	1–4
Hobbyists	**N**	**DWV (%)**	**SBV (%)**	**KBV* (%)**	**IAPV* (%)**	**BQCV (%)**	**ABPV (%)**	**Multiple ***	**Range**
Hobbyist 1	3	100	33	0	0	33	0	1.67	1–3
Hobbyist 2	5	80	40	0	0	60	0	1.80	1–3
Hobbyist 3	4	100	25	25	0	100	0	2.50	2–3
Hobbyist 4	5	100	60	20	0	100	20	3.00	2–4
Hobbyist 5	5	100	80	0	0	60	20	2.60	2–4
Hobbyist 6	3	100	33	33	33	67	0	2.67	2–4
Hobbyist 7	3	100	33	33	0	67	33	2.67	2–4
Hobbyist 8	3	100	100	0	33	100	33	3.67	3–5
Hobbyist 9	3	100	33	33	0	67	0	2.33	1–3
Hobbyist 10	3	100	67	0	0	100	0	2.67	2–3
Overall	37	98	51	15	7	75	11	2.56	1–5

* indicates statistical significance

REFERENCES

BERTHOUD, H., et al. Virus infections and winter losses of honey bee colonies (Apis mellifera). **Journal of Apicultural Research**. v.49, n.1, p.60-65, 2010.

BOWEN-WALKER, P. L., et al. The transmission of deformed wing virus between honeybees (Apis mellifera L.) by the ectoparasitic mite Varroa jacobsoni Oud. **Journal of Invertebrate Pathology**. v.73, n.1 , p. 101-106, 1999.

CAVIGLI, I. et al. Pathogen prevalence and abundance in honey bee colonies involved in almond pollination. **Apidologie. v.** 47, p. 251–266, 2016.

CHEN, Y. P., et al. Transmission of Kashmir bee virus by the ectoparasitic mite Varroa destructor. **Apidologie**. v.35, n.6, p. 441-448, 2004a.

CHEN, Yan Ping, SIEDE, Reinhold. Honey bee viruses. In: K. Maramorosch, et al., Eds., **Advances in Virus Research**, Vol 70, p. 33-80, 2007.

CHEN, Y. P., et al. Multiple virus infections in the honey bee and genome divergence of honey bee viruses. **Journal of Invertebrate Pathology**. v. 87, n.2, p. 84-93, 2004b.

COX-FOSTER, D. L., et al. A metagenomic survey of microbes in honey bee colony collapse disorder. **Science**. v. 318, n. 5848, p. 283-287, 2007.

EMSEN, B., et al. Lower Virus Infections in Varroa destructor Infested and Uninfested Brood and Adult Honey Bees (Apis mellifera) of a Low Mite Population Growth Colony Compared to a High Mite Population Growth Colony**. PLoS ONE 10** (2): e0118885.doi:10.1371/journal.pone.0118885, 2015.

EVANS, J. D., SCHWARZ, R. S. Bees brought to their knees: microbes affecting honey bee health. **Trends in Microbiology**. v.19, n. 12, p. 614-620, 2011.

GENERSCH, E. Honey bee pathology: current threats to honey bees and beekeeping. **Applied Microbiology and Biotechnology**. v.87, n. 1, p. 87-97, 2010.

GENERSCH, E., AUBERT, M. Emerging and re-emerging viruses of the honey bee (Apis mellifera L.). **Veterinary Research**. v.41, n.6, p. 41-54, 2010.

HIGES, M., et al. How natural infection by Nosema ceranae causes honeybee colony collapse. **Environmental Microbiology**. v.10, n. 10, p. 2695-2669, 2008.

JOHNSON, R. M., et al. Changes in transcript abundance relating to colony collapse disorder in honey bees (Apis mellifera). **Proceedings of the National Academy of Sciences of the United States of America**. v.106, n. 35, p. 14790-14795, 2009.

MARTIN, S. J., et al. Global Honey Bee Viral Landscape Altered by a Parasitic Mite. **Science**. v.336, n 6086, p. 1304-1306, 2012.

MULLIN, C. A., et al. High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health. **PloS One** 5 (3): e9754. doi: 10.1371/journal.pone.0009754, 2010.

RUNCKEL, C., et al. Temporal Analysis of the Honey Bee Microbiome Reveals Four Novel Viruses and Seasonal Prevalence of Known Viruses, Nosema, and Crithidia. **PloS One** 6 (6): e20656. doi: 10.1371/journal.pone.0020656, 2011.

SINGH, R., et al. RNA Viruses in Hymenopteran Pollinators: Evidence of Inter-Taxa Virus Transmission via Pollen and Potential Impact on Non-Apis Hymenopteran Species. **PloS One** 5 (12): e14357. doi: 10.137/journal.pone.0014357, 2010.

VAN ENGELSDORP, D., et al. A Survey of Honey Bee Colony Losses in the US, Fall 2007 to Spring 2008. **PloS One** 3 (12): e4071. doi:10.1371/journal.pone.0004071, 2008.

VAN ENGELSDORP, D., et al. Colony Collapse Disorder: A Descriptive Study. **PloS One** 4 (8): e6481. doi: 10.1371/journal.pone.0006481, 2009.