Short communication

# SURVEILLANCE OF VIRUSES IN *VARROA DESTRUCTOR* SAMPLES COLLECTED FROM HONEY BEE COLONIES IN ONTARIO, CANADA, BETWEEN 2015 AND 2019

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**Abstract**

***Varroa destructor* parasitism is associated with extreme honey bee (*Apis mellifera*) colony losses in the northern hemisphere. *Varroa destructor* causes severe damage, including a decrease in bee longevity and immunosuppression, and acts as a vector for viruses, such as *Deformed wing virus* (DWV-A). The surveillance of viral pathogens in *V. destructor* samples is essential to assess risks of emerging virulent viral variants (such as VDV-1) and evaluate their impact on honey bee health. Thus, the objective of this study was to identify viral pathogens in *V. destructor* and honey bee samples collected in Ontario, Canada, from 2015 to 2019 with the use of metagenomics and real time PCR (qPCR). DWV-A and VDV-1 had the highest abundance of viral transcripts (7.5 log2 and 5.72 log2, respectively). Acute bee paralysis virus (ABPV) and *Bee macula virus* were also identified. Viral identification and quantification in *V. destructor* samples using metagenomics will facilitate the surveillance of viral pathogens. This surveillance technique will assist diagnostic laboratories in delivering timely and accurate diagnoses and risk assessments, which in turn will help honey bee producers to take adequate measures to mitigate the damage caused by *V. destructor* and associated viruses.**

**keywords: *Apis mellifera*, honey bees, metagenomics, *Varroa destructor*, viruses, RNA sequencing,**

## INTRODUCTION

*Varroa destructor* is the main cause of honey bee colony mortality in the northern hemisphere (Guzman-Novoa et al., 2010; Le Conte et al., 2010). It acts as a mechanical and biological vector for viruses, including *Deformed wing virus* (DWV) and *Israeli acute bee paralysis virus* (IAPV) (Di Prisco et al., 2011; Gisder & Genersch, 2020). Viral infections have been linked to honey bee colony losses (McMenamin & Genersch, 2015); some cause severe damage to individual bees, like deformities in developing bees infected with DWV-A (de Miranda & Generesch, 2010) and learning impairment in adult bees (Iqbal & Mueller, 2007).

The surveillance of viruses in *V. destructor* samples is essential to assess risks of emerging virulent variants and evaluate their impact on honey bee health. The Ontario Ministry of

Agriculture, Food, and Rural Affairs (OMAFRA) conducted a monitoring project from 2015 to 2019, that included more than 1,000 honey bee colonies located in five different regions of the province; the colonies were monitored for the recording of their strength and *V. destructor* levels in the spring, summer and fall (OMAFRA, 2016). Overwinter colony mortality was also assessed, and samples of live bees were collected to identify viruses with real time PCR (qPCR), including DWV, *Black queen cell virus* (BQCV), IAPV, *Acute bee paralysis virus* (ABPV), *Chronic bee paralysis virus* (CBPV), *Kashmir bee virus* (KBV) and *Sacbrood virus* (SBV). The aim of this study was to determine the presence of pathogens in *V. destructor* samples with the use of a metagenomic technique. We determined the load and species of viruses in *V. destructor* mites retrieved from honey bee samples during the OMAFRA monitoring project (OMAFRA, 2016). Our study showed that metagenomic tools assist in active pathogen surveillance in *V. destructor* populations, which will help understand the effect of varroosis on bee health and prevent extreme colony mortality.

## MATERIAL AND METHODS

Ten *V. destructor* mites were randomly selected from each of five honey bee samples collected during the 2015-2019 OMAFRA monitoring project (OMAFRA, 2016) and kept at -80°C. Thus, a total of fifty mites were used for molecular analyses. The molecular analyses of viral pathogens were performed at the Animal Health Laboratory, University of Guelph (Ontario, Canada). The RNA from the fifty individual *V. destructor* mites was extracted using a SPEX Mini-G homogenizer and MagMAX™ 96 (Applied Biosystems; Mississauga, ON, Canada), eluting 100 µL of nucleic acid template per mite. cDNA was generated with the SuperScript™ III First-Strand Synthesis SuperMix (Invitrogen; Mississauga, ON, Canada), with the use of proprietary random hexamers as primers. For the RNAseq, 1 ng of cDNA was used to construct cDNA libraries with Nextera XT Library Preparation Kit (Illumina; San Diego, CA, USA). RNAseq was performed as 300 bp paired end reads with the use of Illumina Miseq (San Diego, CA, USA). The quality of the raw sequencing reads was assessed with FastQC (Wingett & Andrews, 2010). Taxonomic labels were assigned to metagenomic cDNA sequences with Kraken v2 (Wood & Salzberg, 2014) with a confidence score of 0.65. FastVirome pipeline (Tithi et al., 2018) was used to identify and quantify viral transcript abundance in the samples, with the use of a precomputed Kallisto index (Bray et al., 2016). Edge R in Degust was used to estimate transcript level abundance among known negative samples (control) and twenty-seven mite samples with the presence of at least one virus (Robinson et al., 2010; Powell, 2019; $p<0.05$).

The RNA of honey bees (pool of ten whole bodies, from the same samples used to retrieve *V. destructor* mites) and *V. destructor* mites was extracted and used to calculate the number of viral genome copies (gc) with the use of a LC480 thermocycler (Roche; Mississauga, ON, Canada). Each reaction consisted of 2 µl template, 1 µl forward and reverse primers (500 nM), 10 µl LC480 SYBR mastermix (1×) and 7 µL nuclease-free $H_2O$. The target gene copy number was calculated as per Bustin et al. (2009) with specific primers for ABPV, BQCV and DWV-A (Van Engelsdorp et al., 2009); CBPV and KBV (Boncristiani et al., 2012); IAPV (this study; F5'CAGTTACAGCAGTCGTATGG and R5'TTGTGTACGTTGGGAGTATTG) and SBV (this study; F'ACCGATTTGTTTAATGGTTGGG and R5'ATTCCAGATTCTTCGTCCACTC). The DWV-A and ABPV genome copy numbers in mites and the corresponding bee samples were subjected to a Spearman's correlation test, as the data was not normal based on Shapiro Wilk test ($\alpha$ of 0.05).

## RESULTS AND CONCLUSION

Kraken was used to identify eight viruses relevant to honey bee health in thirty-five out of the fifty *V. destructor* samples, including DWV-A, VDV-1, and *Bee macula virus* (Tab. 1 and Suppl. Tab. 1). Variability was observed in the number of viruses present in the samples

Table 1.

Taxon identification number (NCBI), description, number of positive *V. destructor* samples (from a total of 50 samples tested), and average reads in the clade based on Kraken v2 outputs (confidence score of 0.65)

| Taxon identification number (NCBI) | Description | Number of positive samples | Average reads from positive samples (Kraken v2) |
|---|---|---|---|
| 198112 | DWV-A | 18 | 84.89±87.68 |
| 232800 | VDV-1 | 9 | 444.44±0.00 |
| 2056384 | VDV-2 | 13 | 6.96±0.81 |
| 2056385 | VDV-3 | 12 | 16.92±0.00 |
| 1682186 | *Bee macula virus* | 10 | 176.90±0.00 |
| 1100043 | *Apis mellifera filamentous virus* | 1 | 309 |
| 92395 | BQCV | 1 | 3 |
| 92444 | ABPV | 3 | 32.67±0.00 |

Table 2.

Viral copy numbers and number of positive honey bee samples and *V. destructor* samples (n=50) base on qPCR

| Accession number (NCBI) | Description | Number of honey bee positive samples | Number of *V. destructor* positive samples | Mean viral copy numbers (±SEM) in honey bees | Mean viral copy numbers (±SEM) in *V. destructor* |
|---|---|---|---|---|---|
| AY053371.1 | ABPV | 0 | 10 | Not detected | $2.41x10^{9}$±0.0 |
| MT482476.1 | BQCV | 14 | 1 | $4.41x10^{5}$±$4.24x10^{5}$ | $6.15x10^{6}$±0.0 |
| MN114562.1 | CBPV | 1 | 0 | $1.39x10^{1}$ | Not detected |
| MT096529.1 | DWV-A | 36 | 38 | $6.79x10^{9}$±$5.49x10^{9}$ | $1.44x10^{10}$±$5.57x10^{9}$ |
| MG599488.1 | IAPV | 1 | 1 | $5.71x10^{3}$ | $5.71x10^{3}$ |
| MT096516.1 | KBV | 1 | 1 | $1.80x10^{2}$ | $1.79x10^{2}$ |
| MT482477.1 | SBV | 7 | 7 | $1.89x10^{5}$±$1.74x10^{5}$ | $1.89x10^{5}$±0.00 |

and the number of reads of each virus, which indicated a complex distribution of viruses in the tested samples. Additionally, RNA sequences of DWV-A (NC_004830.2), VDV-1 (NC_006494.1), VDV-2 (NC_040601.1), VDV-3 (NC_040313.1), ABPV (NC_002548.1), *Apis filamentous virus* (NC_027925.1), KBV (NC_004807.1), *Bee macula virus* (NC_027631.1), and transcripts of *Apis rhabdovirus* were identified with the use Kallisto. Only DWV-A (NC_004830.2) and VDV-1 (NC_006494.1) were higher compared to samples with zero viral abundance (control) (7.5 and 5.72 log2 fold, respectively; $p<0.05$). These results in line with previous studies show that DWV-A and VDV-1 are among the most abundant viruses in honey bee populations (Ryabov et al., 2017). In addition, a positive correlation between the genome copy numbers of DWV-A in mites and the corresponding bee samples ($r=0.60$, $p=0.05$) but not for ABPV ($r=-0.059$, $p=0.95$) confirms a strong relationship between DWV-A, *V. destructor* and their host

(Bowen-Walker & Gunn, 1999) . Only one virus identified in honey bee samples with qPCR was not present in mites (CBPV; Tab. 2). Additionally, the presence of *Bee macula virus* and *Apis rhabdovirus* in *V. destructor* samples indicates that further studies on their transmission and pathogenicity are needed. As previously proposed, high throughput techniques assist in the identification of emerging viruses in honey bee populations (McMenamin & Flenniken, 2018; Abou-Shaara, 2019). Our study shows that RNAseq combined with Kraken and Kallisto, can be used in disease monitoring programs to identify viral pathogens in *V. destructor* populations. This study identified virulent variants of DWV (VDV-1, VDV-2 and VDV-3; Levin et al., 2016; Natsopoulou et al., 2017), but no positive samples of *Slow bee paralysis virus* or *Lake Sinai virus* were found, nor bacterial or fungal diseases relevant to bee health were identified in the metagenomic analysis. The diagnostic tools used in this study can aid in the active surveillance of novel viral variants in *V. destructor* populations and help prevent extreme honey bee colony losses linked to viral diseases.

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**Author contributions**

Conceptualization: HYC and PK. Methodology: HYC, PB-R, JLZ, QY, LL, AK, DS. Investigation: HYC, PB-R, JLZ, QY, LL. Formal analyses: HC, PB-R, QY, LL, NM. Writing original draft: NM and EG-N. Writing, reviewing and editing: NM, EG-N, HYC, PK, PB-R, JLZ, QY, AK, DS.

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Supplementary Table 1.

Presence of viruses and viral levels (number of RNA sequences or transcripts) of 35 out of the 50 *V. destructor* mites used in the study and analyzed with Kraken (Wood & Salzberg, 2014)

| Sample ID | Year of sampling | 198112 (DWV-A) | 232800 (VDV-1/ DWV-B) | 2056384 (VDV-2) | 2056385 (VDV-3) | 1682186 (*Bee Macula -like virus*) | 1100043 (*Apis mellifera filamentous virus*) | 92395 (BQCV) | 92444 (ABPV) |
|---|---|---|---|---|---|---|---|---|---|
| 15-054309-0010 | 2015 | 1 | 0 | 6 | 0 | 7 | 0 | 0 | 0 |
| 15-054393-0010 | 2015 | 0 | 0 | 3 | 25 | 59 | 0 | 0 | 0 |
| 15-054972-0010 | 2015 | 0 | 0 | 7 | 0 | 0 | 309 | 0 | 0 |
| 15-055033-0010 | 2015 | 0 | 0 | 3 | 49 | 0 | 0 | 3 | 0 |
| 15-055622-0010 | 2015 | 22 | 0 | 12 | 57 | 534 | 0 | 0 | 0 |
| 15-055628-0010 | 2015 | 0 | 0 | 1 | 31 | 0 | 0 | 0 | 0 |
| 15-055681-0010 | 2015 | 0 | 0 | 8 | 7 | 0 | 0 | 0 | 0 |
| 15-057660-0010 | 2015 | 744 | 0 | 3 | 13 | 0 | 0 | 0 | 0 |
| 15-057733-0010 | 2015 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| 15-059145-0010 | 2015 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| 16-072563-0010 | 2016 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| 16-073231-0014 | 2016 | 212 | 0 | 0 | 0 | 546 | 0 | 0 | 0 |
| 16-076218-0011 | 2016 | 310 | 0 | 0 | 10 | 447 | 0 | 0 | 0 |
| 16-079403-0012 | 2016 | 82 | 0 | 0 | 2 | 94 | 0 | 0 | 0 |
| 16-079404-0011 | 2016 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 17-071828-0003 | 2017 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| 17-073654-0001 | 2017 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| 17-074745-0002 | 2017 | 6 | 752 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-077871-0001 | 2017 | 62 | 16 | 0 | 1 | 47 | 0 | 0 | 60 |
| 17-080092-0002 | 2017 | 16 | 19 | 2 | 0 | 2 | 0 | 0 | 0 |
| 17-080430-0001 | 2017 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 17-080445-0001 | 2017 | 1 | 604 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-083166-0001 | 2017 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-073335-0002 | 2018 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 18-075350-0001 | 2018 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-075359-0001 | 2018 | 2 | 2236 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-076045-0004 | 2018 | 21 | 0 | 0 | 0 | 23 | 0 | 0 | 37 |
| 18-077007-0001 | 2018 | 16 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| 18-079834-0001 | 2018 | 0 | 366 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-081107-0001 | 2018 | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-084972-0002 | 2018 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19-073791-0001 | 2019 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19-076087-0002 | 2019 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 |
| 19-077315-0001 | 2019 | 4 | 1 | 0 | 3 | 0 | 0 | 0 | 0 |
| 19-081189-0005 | 2019 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

DWV= *Deformed wing virus*, VDV= *Varroa destructor virus*, BQCV= *Black queen cell virus*, ABPV= *Acute bee paralysis virus*