HONEY BEE PESTS AND PATHOGENS IN ONTARIO APIARIES, 2015

Apiary Monitoring

Summary Report

Ontario Ministry of Agriculture, Food and Rural Affairs

Food Safety and Environment Division, Animal Health and Welfare Branch

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1.0 ABSTRACT

Monitoring honey bee pests and pathogens in Ontario apiaries is a key objective of Ontario's Pollinator Health Action Plan. To achieve this, the Ontario government began a 6 year monitoring project in 2015 to create an inventory of honey bee pests and pathogens found in Ontario apiaries and assess the prevalence and load of these pathogens. Apiary monitoring will continue until 2020. The data presented in this report will serve as a basis for comparison with the data acquired in subsequent years of the project.

The present study detected seven honey bee viruses and two species of *Nosema*. The prevalence of mites, brood diseases and bacterial pathogens was low. Other pests of interest such as *Tropilaelaps* mites and phorid flies (colloquially known as "zombie flies") were not detected in any of the samples tested.

2.0 INTRODUCTION

Honey bees (*Apis mellifera*) are an important part of agriculture. Managed honey bees not only produce honey but they are responsible for pollinating 80 % of all agricultural crops requiring insect pollination and are the most economically valuable pollinators world-wide. In Canada, the pollination of agricultural crops by honey bees is valued at approximately one to two billion dollars a year and in Ontario, honey bee pollination services are valued at approximately four million dollars annually. Managed honey bees pollinate a broad range of Ontario crops including apples, apricots, asparagus, blueberries, squash, and canola. The value of the honey produced in Canada in 2015 was an additional $210 million, to which Ontario contributed $31 million (StatsCan, 2015).

Despite the important contribution that honey bees add to both the economy and the environment, reports from around the world are suggesting global declines in populations of both managed honey bees and wild pollinators. It has been proposed that a number of interacting stressors are contributing to declining populations of bees, including disease and pests, exposure to pesticides, reduced habitat and climate change (Pindar et al. 2017).

In recent years, the Ontario beekeeping industry has become concerned about the observed mortality of honey bee colonies, both during the active beekeeping season (from April to October) and the overwintering period (December to May). The beekeeping industry considers 15 % overwinter mortality to be sustainable. Since 2007, overwintering losses in Ontario have ranged from a low of 12% in 2012 to an all-time high of 58 % in 2014. More recently, Ontario beekeepers reported overwinter mortality of 38% in 2015 and 18% in 2016.

In addition to recent reports of increased overwinter honey bee mortality, Ontario beekeepers began reporting honey bee mortality incidents during the active beekeeper season (spring to fall) in 2012. These honey bee incidents were reported by beekeepers to Health Canada's Pest Management Regulatory Agency (PMRA) and are defined as atypical effects observed in honey bee colonies suspected by the beekeeper to be related to pesticide exposure. The adverse effects typically observed by the beekeepers include mortality or sub-lethal effects such as twitching, an observed decrease in the number of foragers, etc. Following the increased reporting of honey bee incidents in 2012 during the early spring planting period, PMRA initiated a program to investigate the potential causes of the increased number of incident reports. The PMRA determined that pesticide-containing dust generated during planting of neonicotinoid-treated corn and soybean seed contributed to the mortalities. Bee mortality incidents continued to be reported in 2013 and the number of reports remained high. Mandatory measures to reduce exposure to pesticide-containing dust during planting of neonicotinoid treated seed were put in place prior to the 2014 planting season. Following the implementation of these measures, the number of incidents reported during the planting periods in 2014, 2015 and 2016 decreased significantly as compared to 2013 (Health Canada 2017).

The strategy for improving honey bee health in Ontario requires a comprehensive approach. This strategy must address honey bee stressors, the continued implementation of integrated pest management practices by beekeepers and crop growers, increases in pollinator-friendly forage areas and additional research on honey bee health.

To help better understand some of the stressors impacting pollinator health in Ontario, the government committed to collecting data from government monitoring and surveillance programs to use as a benchmark for managed honey bees, wild pollinators and pesticide residues in the environment as part of the Pollinator Health Action Plan. In support of this action, the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) initiated an apiary monitoring program in 2015 to better understand the prevalence and load of honey bee pests and pathogens across the province. Apiary monitoring activities involved ministry apiary inspectors observing colonies and collecting samples at four times throughout the active beekeeping season (spring to fall).

3.0 METHODS

3.1 Apiary Site and Colony Selection

Thirty-two registered bee yards across Ontario were selected for study and beekeeper involvement was voluntary. Yards were selected from a list of registered beekeepers that operated a minimum of 50 colonies in total. Eligible yards were required to have a minimum of 15 colonies in the selected yard in the spring of 2015. Beekeeping operations that provide mobile pollination services were excluded from the monitoring project. Only stationary yards were selected for study as the colonies needed to be accessible for apiary inspections from spring to fall. Using these criteria, bee yards were randomly

selected across five defined beekeeping regions in Ontario (Fig. 1). These regions are defined by geography, climate and weather patterns. Yards were selected in each region proportional to the number of beekeepers in the region. The majority of registered beekeepers maintain colonies in the central and south beekeeping regions, which is why these regions have a greater number of apiaries enrolled in the monitoring project (Table 1).

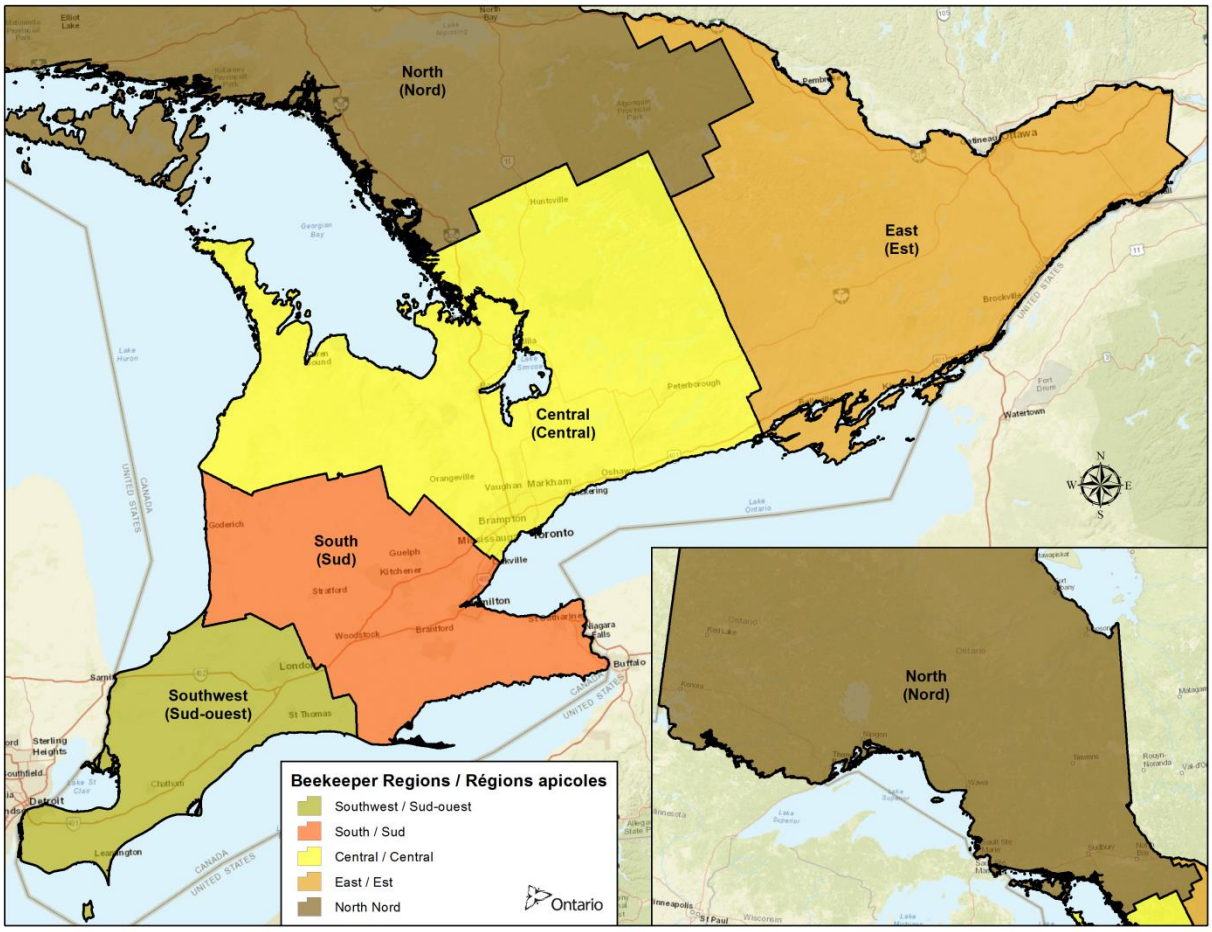

Figure 1. Beekeeping regions in Ontario.

Table 1. Number of bee yards per Ontario beekeeping region selected for the 2015 monitoring project.

Beekeeping Region	Number of Bee Yards Selected for Monitoring
Central	9
East	4
North	2
South	10
Southwest	7

All colonies in each of the 32 monitoring yards were numbered sequentially. Ten colony numbers were randomly selected from each of the 32 monitoring yards for inclusion in the monitoring project. All 10 selected colonies were evaluated by ministry apiary inspectors and were classified as either “populous” (of sufficient population to likely survive the season) or “compromised” (low population and unlikely to survive the season). Only populous colonies were included in the monitoring project; if necessary, random selection continued until 10 apparently healthy colonies were obtained.

The beekeepers continued typical management practices for these colonies and were able to extract honey and treat for pest and disease when necessary. However, the monitoring project required the beekeepers to keep the colonies at the selected yard for the season, colonies could not be requeened (replacement of honey bee queen) unless they became queenless (lacking a honey bee queen) and the colonies could not be split more than once at the start of the active beekeeping season.

3.2 Apiary Inspection and Live Bee Collection

Ministry apiary inspectors visited the selected bee yards at four times throughout the active beekeeping season to inspect the 10 selected colonies (Table 2). The first apiary inspection of the season occurred as early as possible in the spring after snow melt and was dependent on weather conditions to ensure inspections could occur without causing stress to the colonies (temperature ≥ 15°C). The second apiary inspection was planned to coincide with the conclusion of planting activities in agricultural areas. The third apiary inspection was scheduled in late summer when bee populations were near their peak and the fourth inspection was scheduled in the fall before preparing the colonies for overwintering. Due to the geographical distribution of the monitoring yards and the seasonal temperatures experienced in these areas, the timing of spring thaw and planting was variable depending on the region. This resulted in each inspection period spanning approximately 30 days where inspections in the southern region occurred earlier than in the northern region (Table 2).

Table 2. The range of dates corresponding to apiary inspections one through four in 2015.

Apiary Inspection	Date of Collections (Range)
1	April 26 – May 14, 2015
2	May 14 – June 16, 2015
3	July 15 – August 25, 2015
4	September 9 – October 15, 2015

At each apiary inspection, ministry inspectors recorded observations about the presence or absence of a honey bee queen, and checked for clinical signs of disease such as American foulbrood, European foulbrood and chalkbrood. Additionally, inspectors performed on-site diagnostics which included an estimation of the degree of infestation by *Varroa destructor* (varroa). The intensity of varroa mite infestation was determined in the field by counting the number of varroa mites found in a sample of

approximately 300 honey bees using a standard alcohol wash (Please see www.ontario.ca/beekeeping for more information on varroa mite sampling and monitoring).

One cup of live bees (approximately 100 to 300 bees) was collected from the interior of each of the 10 selected colonies per bee yard and placed in a 250 mL collection jar. Collections were made from the broodnest, where the majority of the biological activity takes place within the colony. The jars were either immediately delivered to the Animal Health Laboratory (AHL) at the University of Guelph by car or were placed in a small Styrofoam cooler on dry ice, sealed and shipped to the AHL within 24 to 72 hours of collection. Bees were euthanized using dry ice before further processing at the lab.

3.3 Bee Sample Analysis

Diagnostics were performed by the AHL. Prior to processing, all sampled bees were individually examined to ensure that they were free of varroa mites. For haplotype testing, individual varroa mites were collected from each sample jar.

Tracheal mites were detected and quantified by manual dissection using a compound microscope. Twenty-five bees from each yard were examined for evidence of tracheal mites including both the physical presence of mites in addition to tracheal scarring. The degree of infestation was categorized as high (≥6 bees positive for tracheal mites out of 25), medium (4 – 5 bees positive for tracheal mites out of 25), low (≤3 bees positive for tracheal mites out of 25) or negative (no bees found positive for tracheal mites).

Nucleic acids (RNA and DNA) were extracted using commercially available kits and RNA was reverse transcribed. Honey bee viruses and vitellogenin messenger RNA levels (a marker of honey bee health) were detected and quantified through the use of quantitative polymerase chain reaction (qPCR). The viral load was expressed as the number of viral RNA copies per bee. Pathogens were tested either at the colony (individual sample) or the yard level (composite sample).

Three of the 10 colonies per yard were randomly selected for individual colony level diagnostics using 10 bees per sample submission jar. For each colony level test, 10 bees were randomly selected from the sample submission jars and were mixed using a bead-mill homogenizer. Each of the three colonies was assessed for acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), deformed wing virus (DWV) and Israeli acute paralysis virus (IAPV).

Composite samples were prepared by selecting two honey bees from each of the 10 sample submission jars per yard to form a pool of 20 bees. The sample was mixed using a bead-mill homogenizer. The yard-level analysis was completed for black queen cell virus (BQCV), Kashmir bee virus (KBV), sacbrood virus (SBV), *Nosema* spp., phorid flies, *Spiroplasma* spp., tracheal mites, trypanosomes, vitellogenin, *Tropilaelaps* spp. and *Varroa destructor* haplotype.

3.4 Data Analysis

Viral pathogen loads, expressed as the number of viral RNA copies per bee, had a skewed distribution. Therefore, $\log_{10}$-transformation was used to reduce skewness. Samples for which pathogens were not detected or were below the limit of detection were assigned a value of one prior to $\log_{10}$-transformation.

4.0 RESULTS

Data summaries are presented below for each apiary pest or pathogen by inspection. Disease prevalence, reported as a percent, is the proportion of the sampled yards which tested positive using laboratory diagnostics during the active beekeeping season.

4.1 Viral Pathogens

Honey bees can be affected by at least 24 described viruses (de Miranda et al. 2014). Many of these viruses lack observable symptoms and physical effects that may be caused by more than one virus. For these reasons, molecular techniques are required for the detection and quantification of these viruses such as quantitative polymerase chain reaction (qPCR). Despite differing modes of transmission, sites of infection, physical effects and life stage effects, viruses may affect honey bee health and have an impact on lifespan to some degree. The degree to which viruses ultimately impact honey bee colonies under a wide range of conditions is not fully understood.

a. Acute Bee Paralysis Virus (ABPV)

ABPV is a single stranded RNA virus from the *Dicistroviridae* family (genus *Aparavirus*). It can infect honey bees at both the pupal (Bailey & Ball, 1991) and adult stages (Hunter et al. 2010). Infection may be symptomless, or can cause rapid death. ABPV is transmitted both by varroa mites and by pollen (Chen & Evans, 2006). Infection with ABPV has been shown to inhibit the honey bee immune response (Azzami et al. 2012).

The prevalence of ABPV in Canadian apiaries has been reported to be low at 0 – 20% and has not been consistently detected in all provinces (Desai et al. 2016). Research from the USA indicates that the prevalence of ABPV tends to be lower in the summer months and higher in the winter (Steinhauer et al. 2014). To date, very little is known regarding naturally occurring ABPV viral loads in bees and no

thresholds have been defined to determine a pathogenic load or otherwise detrimental to bee or colony health.

The prevalence of ABPV ranged from 23% – 65%. ABPV was detected in more apiaries with higher mean viral loads at inspection 4 compared to other inspections (Table 3).

Table 3. Descriptive statistics, prevalence and mean viral load (Log RNA copies per bee) of ABPV infection in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

Collection Period	Yards Tested	Prevalence		Log RNA copies per bee				
		Yards Positive	Percent	Mean	Standard Error	Minimum	Median	Max
Inspection 1	**30**	**7**	**23%**	**0.63**	**0.283**	**0**	**0**	**5.8**
Inspection 2	**32**	**8**	**25%**	**0.57**	**0.226**	**0**	**0**	**5.7**
Inspection 3	**32**	**10**	**31%**	**0.44**	**0.163**	**0**	**0**	**4.4**
Inspection 4	**31**	**20**	**65%**	**2.41**	**0.491**	**0**	**0.9**	**9.0**

b. Black Queen Cell Virus (BQCV)

BQCV is a widespread single stranded RNA virus from the *Dicistroviridae* family (genus *Cripavirus*). BQCV causes death in queen bee pupae, but does not appear to have detectable effects in the honey bee colony. BQCV is transmitted to larvae by nurse bees, and may also be spread by vectors such as *Varroa destructor* and *Nosema*, although this remains unproven.

The prevalence of BQCV in Canadian apiaries has been reported to be very high, generally 70 – 100% (Desai et al. 2016). The disease has been reported to be most common in spring and early summer although the prevalence remains high throughout the year. There is no data demonstrating the effects of high BQCV viral loads on bees.

The prevalence of BQCV ranged from 93 – 100% (Table 4). The prevalence and mean viral load were consistently high across all collection periods.

Table 4. Descriptive statistics, prevalence and mean viral load (Log RNA copies per bee) of BQCV infection in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

Collection Period	Yards Tested	Prevalence		Log RNA copies per bee				
		Yards Positive	Percent	Mean	Standard Error	Minimum	Median	Maximum
Inspection 1	30	28	93%	6.46	0.435	0	6.8	9.8
Inspection 2	32	31	97%	8.29	0.397	0	8.8	11.1
Inspection 3	32	32	100%	8.10	0.271	5.0	8.1	10.7
Inspection 4	31	30	97%	7.77	0.340	0	7.5	10.9

c. Chronic Bee Paralysis Virus (CBPV)

CBPV is a single stranded RNA virus that has yet to be classified into a family, although it shares many characteristics with *Nodaviridae* and *Tombusviridae*. The virus primarily targets adult bees. Infection may result in trembling, loss of hair and flight and a darkening of the body and death will typically occur within days of infection. The virus is transmitted through direct contact and possibly feces (Ribière et al. 2007).

The prevalence of CBPV in Canadian apiaries has been reported to be low, generally 0 – 30% and has not been consistently detected in all provinces (Desai et al. 2016). The virus has been reported to peak in the summer months with honey bee colony populations. Some studies show that apparently healthy bees (not showing clinical signs of infection) may harbour the virus with no effects and deceased bees have been found to have exceptionally high viral loads (Ribière et al. 2010).

The prevalence of CBPV ranged from 3% – 29% (Table 5). The prevalence and viral load increased in inspections 3 and 4.

Table 5. Descriptive statistics, prevalence and mean viral load (Log RNA copies per bee) of CBPV infection in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

Collection Period	Yards Tested	Prevalence		Log RNA copies per bee				
		Yards Positive	Percent	Mean	Standard Error	Minimum	Median	Maximum
Inspection 1	30	1	3%	0.10	0.105	0	0	3.1
Inspection 2	32	2	6%	0.18	0.128	0	0	3.0
Inspection 3	32	7	22%	0.74	0.293	0	0	6.3
Inspection 4	31	9	29%	0.96	0.385	0	0	9.6

d. Deformed Wing Virus (DWV)

DWV, a single stranded RNA virus from the *Iflaviridae* family (genus *Iflavirus*), is considered to be the most economically important honey bee virus and has been extensively studied due to the fact that this virus is transmitted and amplified by the parasitic mite, *Varroa destructor*. The virus affects the developing honey bee larvae, resulting in deformed, non-functional wings, abnormal abdomens and damaged appendages. Affected larvae die soon after emergence. Asymptomatic infected adults will generally have lower viral titres (Tentcheva et al. 2006). The virus is primarily spread by *V. destructor* mites and through feeding and timely mite control is the most effective method of controlling this virus in a colony.

In Canada, DWV is often the most prevalent viral infection and has been reported to be found in 90 – 100% of apiaries (Desai et al. 2016). Viral load has been reported to typically peak in the fall and winter months (Prisco et al. 2011; Desai & Currie 2016). Although there are no thresholds established for the pathogenicity of DWV, the viral titre has been shown to be inversely correlated with bee health and life span at both the individual and colony level (Desai & Currie 2016).

The prevalence of DWV was 57 and 66% at inspection 1 and 2, respectively and increased in the summer and fall to 81 and 97% at inspection 3 and 4, respectively (Table 6). Both prevalence and viral load increased through the summer and fall months.

Table 6. Descriptive statistics, prevalence and mean viral load (Log RNA copies per bee) of DWV infection in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

Collection Period	Yards Tested	Prevalence		Log RNA copies per bee				
		Yards Positive	Percent	Mean	Standard Error	Minimum	Median	Maximum
Inspection 1	30	17	57%	1.17	0.315	0	0.8	7.5
Inspection 2	32	21	66%	1.78	0.353	0	0.8	7.2
Inspection 3	32	26	81%	3.30	0.475	0	3.3	10.4
Inspection 4	31	30	97%	5.53	0.463	0	5.3	10.0

e. Israeli Acute Paralysis Virus (IAPV)

Similar to ABPV, IAPV is a single stranded RNA virus from the *Dicistroviridae* family (genus *Aparavirus*). The virus has been found in all life stages of honey bees, from eggs to adults (Chen et al. 2014). Infection may be asymptomatic, or can result in shivering wings and progressive paralysis (Maori et al. 2007). In all cases, IAPV infection results in the down-regulation of the honey bee immune response (Chen et al. 2014), leaving the bees in an immunocompromised state and vulnerable to other pathogens. The virus is spread both by varroa mites (Di Prisco et al. 2011) and food sources (Chen et al. 2014).

In Canada, IAPV has been reported to be found in 40 – 70% of apiaries (Desai et al. 2016). The prevalence of this virus has been reported to be lower in the spring and summer, and peak in the fall and winter months. No thresholds currently exist to determine the implications of IAPV viral loads on honey bees.

The prevalence of IAPV ranged from 23 – 69% (Table 7). Mean viral load was highest at inspection 2 and 3 (Table 7).

Table 7. Descriptive statistics, prevalence and mean viral load (Log RNA copies per bee) of IAPV infection in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

Collection Period	Yards Tested	Prevalence		Log RNA copies per bee				
		Yards Positive	Percent	Mean	Standard Error	Minimum	Median	Maximum
Inspection 1	30	7	23%	0.99	0.404	0	0	8.0
Inspection 2	32	22	69%	3.08	0.562	0	2.5	11.4
Inspection 3	32	18	56%	2.67	0.587	0	1.1	9.6
Inspection 4	31	12	39%	1.23	0.389	0	0	8.4

f. Kashmir Bee Virus (KBV)

KBV is a single stranded RNA virus from the *Dicistroviridae* family (genus *Cripavirus*), the same family and genus as BQCV. In adults, viral infection generally leads to death within a few days. In larvae, the infection may remain asymptomatic (Berenyi et al. 2006). The virus is spread by varroa mites, food sources and also from queen to egg (Shen et al. 2005).

The prevalence of KBV in Canadian apiaries has been reported to be low to moderate, generally 10 – 40% and has been detected by Desai et al. (2016) in all provinces except Alberta. Adequate data is currently not available to determine the seasonal prevalence of the virus, although because it is spread by the varroa mite, it may follow patterns similar to IAPV and DWV. No thresholds currently exist to determine the implications of KBV viral loads on honey bees.

The prevalence of KBV was low in the spring and fall (7% and 6% at inspection 1 and 4) and increased in late spring and summer to 31 and 34% at inspection 2 and 3, respectively (Table 8). Prevalence and viral load peaked in the late spring to early summer before declining into the fall months.

Table 8. Descriptive statistics, prevalence and mean viral load (Log RNA copies per bee) of KBV infection in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

Collection Period	Yards Tested	Prevalence		Log RNA copies per bee				
		Yards Positive	Percent	Mean	Standard Error	Minimum	Median	Maximum
Inspection 1	30	2	7%	0.57	0.401	0	0	9.9
Inspection 2	32	10	31%	2.07	0.588	0	0	11.2
Inspection 3	32	11	34%	2.34	0.601	0	0	10.0
Inspection 4	31	2	6%	0.55	0.389	0	0	10.3

g. Sacbrood Virus (SBV)

SBV is a single stranded RNA virus belonging to the family *Iflaviridae* (genus *Iflavirus*). In larvae, the virus can cause mortality when in the brood cell. In the field, affected larvae can be readily identified by the presence of a fluid filled sac that can be removed from the brood cell, often intact. Clinical signs of SBV in field observations are typically no more than a few infected honey bee larvae in a colony (Paul Kozak, personal communication). In adults, the virus is typically asymptomatic. Nurse bees infected with SBV may exhibit behavioural changes, including early and preferential pollen foraging and can transmit the virus to young through feeding.

The prevalence of SBV in Canadian apiaries has been reported to be low (16%) across all provinces except Manitoba where the prevalence was 44% (Desai et al. 2016). The prevalence of this virus has been reported to be lower in the spring. No thresholds currently exist to determine the implications of SBV viral loads on honey bees.

The prevalence of SBV was 50% in the spring, increased to 84% at inspection 2 and 3, and decreased to 29% in the fall months (Table 9).

Table 9. Descriptive statistics, prevalence and mean viral load (Log RNA copies per bee) of SBV infection in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

Collection Period	Yards Tested	Prevalence		Log RNA copies per bee				
		Yards Positive	Percent	Mean	Standard Error	Minimum	Median	Maximum
Inspection 1	30	15	50%	2.74	0.544	0	2.0	8.9
Inspection 2	32	27	84%	5.70	0.533	0	5.9	10.2
Inspection 3	32	27	84%	6.56	0.592	0	7.2	10.6
Inspection 4	31	9	29%	2.49	0.745	0	0	10.8

4.2 Brood Diseases

a. American Foulbrood

American foulbrood (AFB) is caused by a spore-forming bacterium, *Paenibacillus larvae*. AFB is an economically destructive and virulent disease where the spores can remain viable for at least 70 years, resistant to both boiling and dehydration (Shimanuki and Knox 1988; Grady et al. 2016). Honey bee larvae are most susceptible to AFB infection within the first 36 hours after hatching, and only a few spores (≤ 35) are needed to initiate infection (Bucher 1958). Clinical signs of disease include larvae that are slightly yellow to brown in colour which settle to the bottom of the brood cell as the larvae dies and decompose, producing billions of infective spores. Spores are spread within the colony when nurse bees clean out infected cells and spread the spores as they are feeding young larvae with contaminated mouthparts.

Apiary inspectors noted clinical signs of disease at one yard only at each of inspections 1, 2 and 3. The prevalence of AFB was low and peaked at 1.6% of colonies inspected at inspection 3 (Fig. 2). No AFB positive colonies were detected at inspection 4.

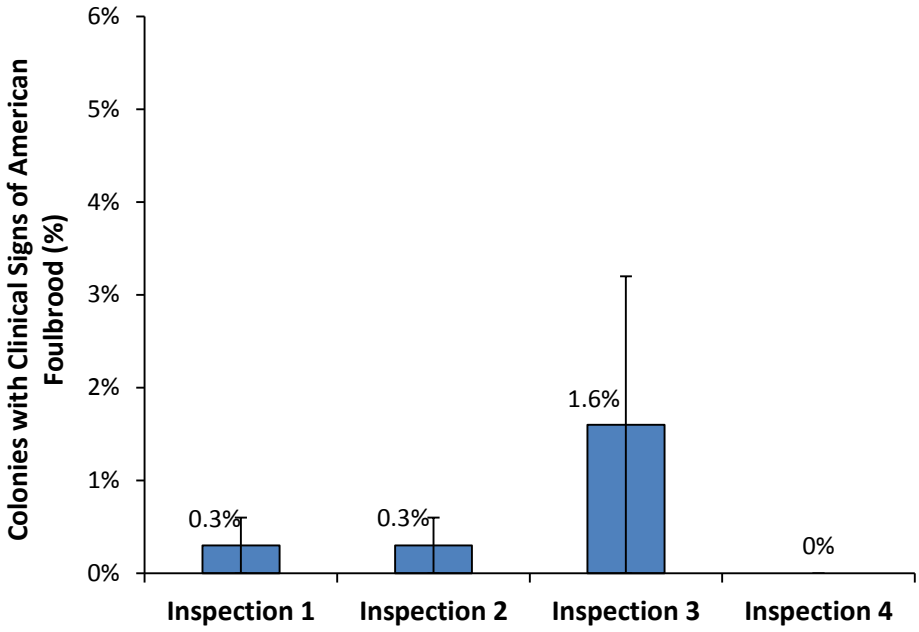

Figure 2. Mean percent of colonies ± 1 standard error with clinical signs of American Foulbrood in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

b. Chalkbrood

Chalkbrood is caused by the fungus *Ascosphaera apis*. Clinical signs of disease include dead and dried larvae covered in a hard white or black fungus with the tip of the larvae protruding from the cell (also known as “chalkbrood mummies”).

Apiary inspectors noted clinical signs of disease at every collection period. The prevalence of chalkbrood peaked at 10.1% in the colonies evaluated at inspection 2 (Fig. 3).

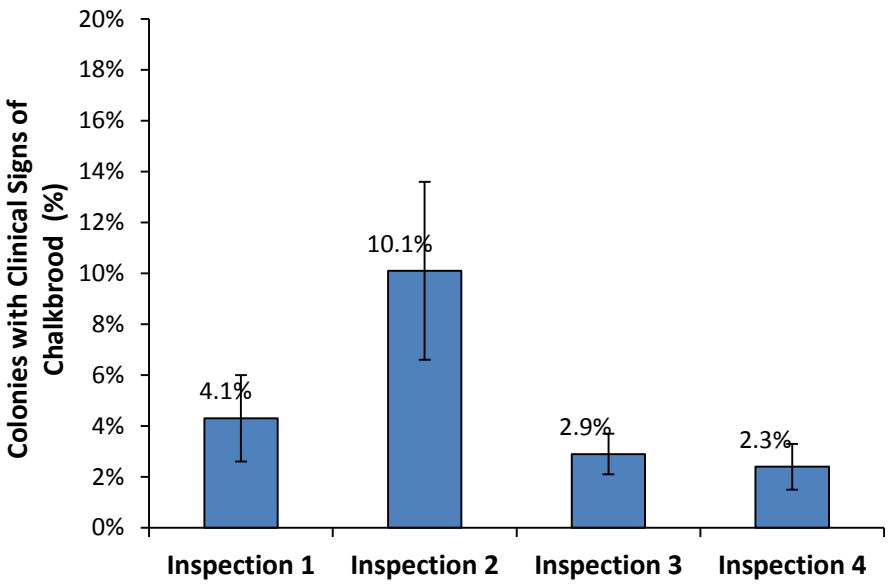

Figure 3. Mean percent of colonies ± 1 standard error with clinical signs of Chalkbrood in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

c. European Foulbrood

European foulbrood (EFB) is a honey bee disease caused by the bacteria, *Melissococcus plutonius*. Larvae become infected within the first 48 hours after hatching and EFB is often documented at higher frequencies during early summer (Grangier et al. 2015). Clinical signs of infection include dead larvae that appear yellowish or brown in color and are found curled in a C-shape at the bottom of the cell and is often accompanied by a sour odor which is distinct from AFB.

In the present study, EFB was not detected in any of the colonies inspected.

4.3 *Nosema* spp. (*N. apis* and *N. cerenae*)

Nosema are single-celled parasites of the honey bee that are classified as fungi and infect and damage the mid-gut tissue. There are two species of *Nosema* of interest, *Nosema apis* and *Nosema ceranae*. *N. ceranae* has been present in Canada since at least 1994; however, it is possible that *N. ceranae* may have been present in Canada for longer (Williams et al. 2008).

While *Nosema* is widespread, previous surveys in Ontario and Alberta have shown that *N. ceranae* was the dominant species, ranging in prevalence from 41 – 91%. Comparatively, the prevalence of *N. apis* is reported to be 4 – 34%. Occasionally, concurrent infections by both *Nosema* spp. in a single colony have

been reported, however this is less frequent than single species infections (Emsen et al. 2015). The impact of this pathogen on honey bee populations is not fully understood.

Both species of *Nosema* were detected at all inspections; however, *N. ceranae* was more frequently detected than *N. apis*. The prevalence and mean load of *N. ceranae* was higher than *N. apis* at all inspections (Fig.4).

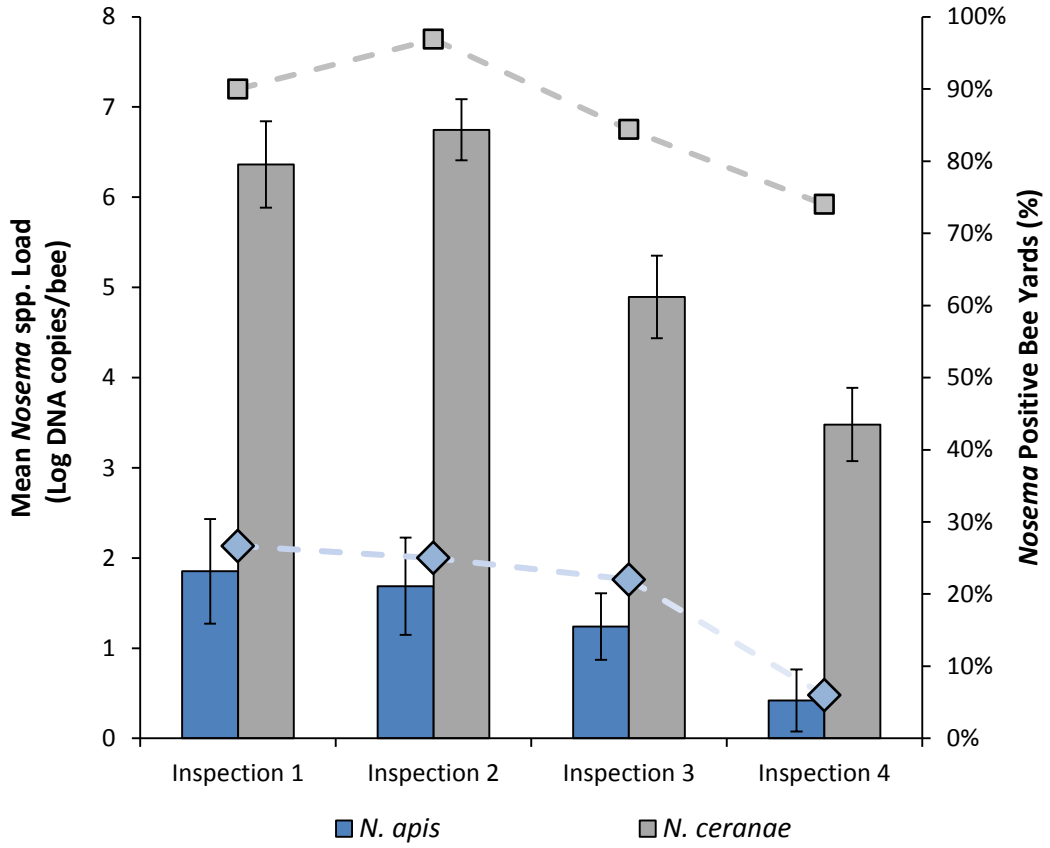

Figure 4. *Nosema* spp. in Ontario honey bee apiaries by collection period in 2015. Bars indicate the mean load (log DNA copies per bee) of *N. apis* (in blue) and *N. ceranae* (in grey) ± 1 standard error. Dotted lines indicate the percent of *Nosema* positive of bee yards at each inspection. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

4.4 Mites

a. Tracheal Mite (*Acarapis woodi*)

Acarapis woodi is an internal parasite of honey bees that live and reproduce in the tracheae of the bees. Tracheal mites have been detected on most continents, including Europe, Asia, parts of Africa, and North and South America. These mites are very small and can only be observed with a microscope. Tracheal mite infestation lack unique physical symptoms and can only be diagnosed by the mites and eggs, and evidence of scarring detected in the dissected trachea.

The prevalence of tracheal mites ranged from 3 to 13% (Fig. 5). Tracheal mites were not found in 91% of the bee yard evaluations and when mites were detected, the infestation was low (≤3 bees positive for tracheal mites out of 25).

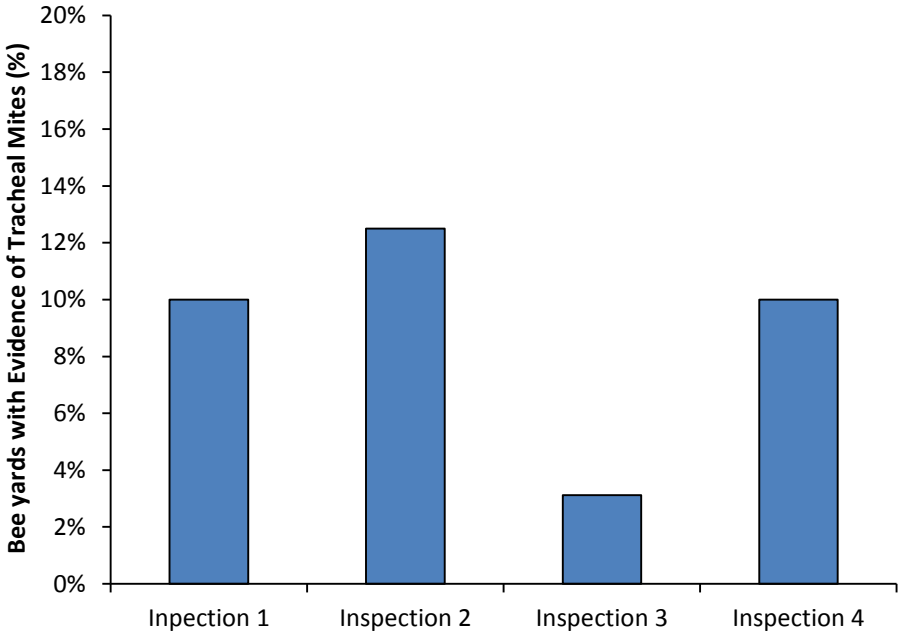

Figure 5. Percent of bee yards with evidence of tracheal mite (*Acarapis woodi*) infestation in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

b. *Varroa destructor* Infestation

Varroa destructor is an external parasite of honey bees and elevated levels of infestation may result in symptoms of stress in the colony during the active season (Dainat et al., 2012; Dainat et al., 2013). *V. destructor* mites attach to the body of the bee and weaken the bee by sucking hemolymph. In this process, RNA viruses such as the deformed wing virus (DWV) spread to bees. A significant mite infestation may lead to the death of a honey bee colony, usually in the late autumn through early spring. *V. destructor* infestation has been documented as the primary contributor to winter mortality in Ontario (Guzman et al., 2010). For the Ontario beekeeping industry, recommended treatment thresholds (2% of bees in the spring; 3% of bees in the fall) have been described by Guzmán-Novoa (2010).

V. destructor were detected at all collection periods (Figure 6) and the mean percent of bees infested was low (Fig. 6).

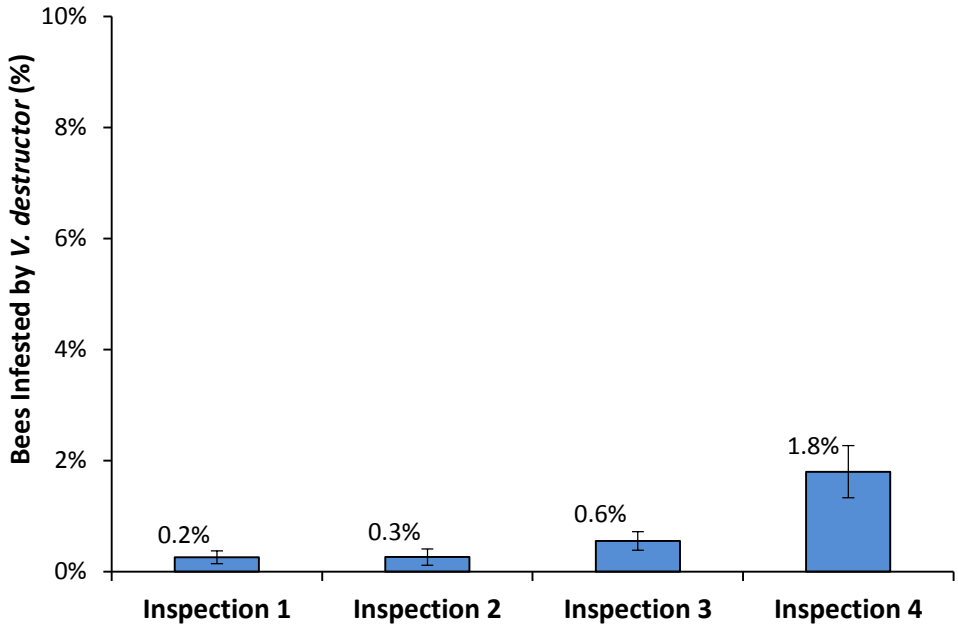

Figure 6. Mean percent of bees (± 1 standard error) infested by *V. destructor* in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

c. *Varroa destructor* Haplotype

In honey bees, two *V. destructor* haplotypes have been detected, the Korean and the Japanese haplotypes, named for the countries where they were first detected. To date, the Korean haplotype has been detected in honey bees globally whereas the Japanese haplotype has been found primarily in Japan, Thailand and the Americas (Solignac et al. 2005).

In the present study, *V. destructor* was not detected in the majority (53 – 69 %) of bee yards tested during inspections 1 through 3 (Fig. 7). At inspection 4, *V. destructor* was detected in greater than 50% of the bee yards inspected. When *V. destructor* was detected in Ontario apiaries, the Korean haplotype was dominant with only occasional Japanese or mixed haplotypes detected.

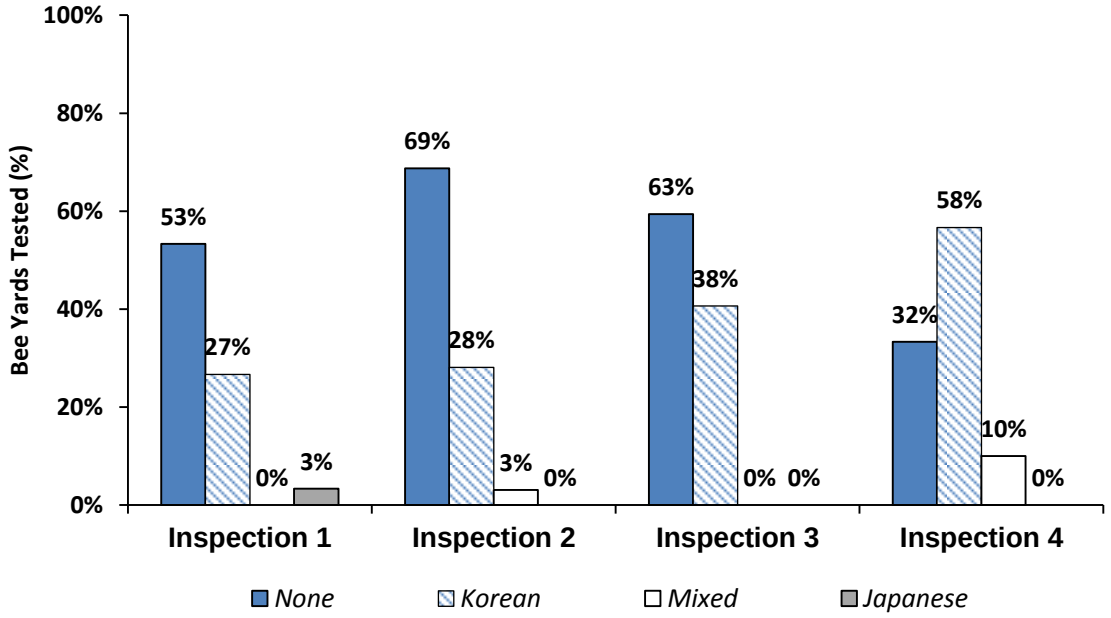

Figure 7. *V. destructor* detections and haplotype in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

d. *Tropilaelaps* spp.

The mites in the genus *Tropilaelaps* are parasites of honey bee brood that feed on bee larvae and pupae. Infestation by *Tropilaelaps* causes the death of many bee larvae (up to 50%) and subsequent colony decline. *Tropilaelaps* infestation often results in an irregular brood pattern where the dead larvae may protrude from the cells and malformed bees with distorted abdomens, stubby wings and deformed or missing legs and some of the affected bees crawl at the entrance to the colony (OIE 2008; Luo et al. 2011).

In the present study, *Tropilaelaps* spp. was not detected in any of the samples tested.

4.6 Other Honey Bee Threats

a. Phorid Fly (*Apocephalus borealis*)

Apocephalus borealis is a species of North American parasitoid phorid fly that parasitizes bumble bees and paper wasps (Core et al. 2012). Recently, *A. borealis* has been associated with honey bees in North America, including documented cases in British Columbia. Individual honey bees parasitized by *A. borealis* may be disorientated and exhibit abnormal behaviour such as hive abandonment during the night (Dutto & Ferrazzi 2014).

In the present study, *A. borealis* was not detected in any of the samples tested.

b. Small Hive Beetle

The small hive beetle (SHB; *Aethina tumida*), was first detected in Ontario in 2010. This pest is capable of damaging stressed colonies by destroying wax comb and honey bee brood in addition to spoiling honey (Hood 2004; Neumann and Elzen 2004; Neumann and Ellis 2008).

SHB was not detected in any of the yards inspected.

c. *Spiroplasma* spp. (*S. apis* and *S. melliferum*)

Spiroplasma spp. are small bacteria which infest insects and some plants. A multi-year assessment of managed honey bee colonies found that a third of colonies screened in the USA and half of the colonies screened in Brazil were infected by *Spiroplasma* spp. and *S. melliferum* was more commonly found when compared to *S. apis* (Schwarz et al. 2014). While *S. melliferum* and *S. apis* are known pathogens of honey bees (Meeus et al. 2011), the pathogenicity of these organisms in honey bee colonies has not yet been determined (Zheng and Chen 2014).

In the present study, the prevalence of *S. apis* ranged from 0% – 16%. *S. melliferum* was not detected in any of the samples tested (Table 10).

Table 10. Descriptive statistics, prevalence and mean load (Log DNA copies per bee) of *S. apis* in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

Collection Period	Yards Tested	Prevalence		Log DNA copies per bee				
		Yards Positive	Percent	Mean	Standard Error	Minimum	Median	Maximum
Inspection 1	30	0	0%	0	0	0	0	0
Inspection 2	32	5	16%	0.54	0.226	0	0	3.6
Inspection 3	32	2	6%	0.26	0.183	0	0	4.9
Inspection 4	31	5	16%	0.56	0.235	0	0	3.6

d. Trypanosomes (*Crithidia mellificae* and *Lotmaria passim*)

Trypanosomes are single celled organisms which parasitize insects. *Lotmaria passim* and *Crithidia mellificae* are trypanosomes that parasitize both honey bees and bumble bees. Parasitism by these organisms has been reported to have negative effects including learning impairment (Gegear et al. 2005) and compromised immune systems (Schwarz and Evans 2013).

The prevalence of *C. mellificae* ranged from 0% – 47% while the prevalence of *L. passim* ranged from 30% – 55%. The mean load of *C. mellificae* and *L. passim* can be found in Figure 8.

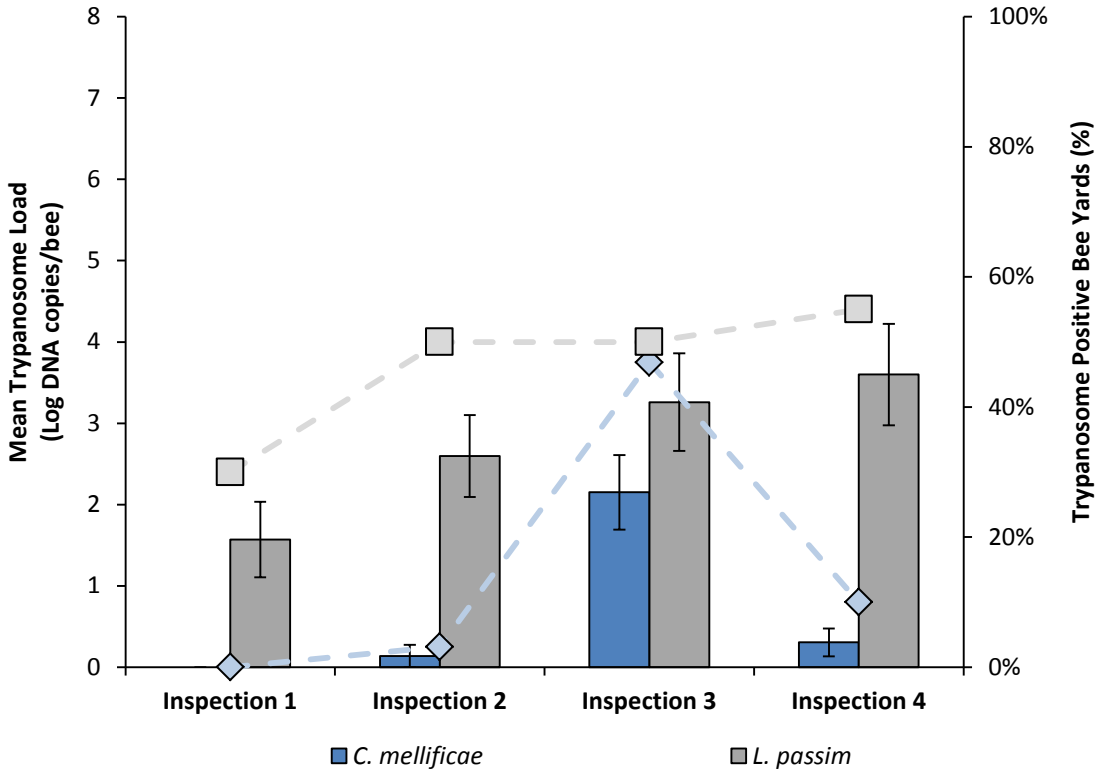

Figure 8. Trypanosomes found in Ontario honey bee apiaries by collection period in 2015. Bars indicate the mean load (log DNA copies per bee) of *C. mellificae* (in blue) and *L. passim* (in grey) ± 1 standard error. Dotted lines indicate the percent of trypanosome positive bee yards at each inspection. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

4.7 Colony Indicators

a. Honey Bee Queen

A healthy honey bee and functional colony requires a mated queen and the absence of the queen may indicate an underlying issue related to colony health.

Honey bee queens were noted in the majority of colonies inspected. Apparently queenless colonies made up fewer than 1% of colonies at inspection 1, 5.3% of colonies at inspection 2, 7.5% of colonies at inspection 3 and 5.7% of colonies at inspection 4 (Fig. 9).

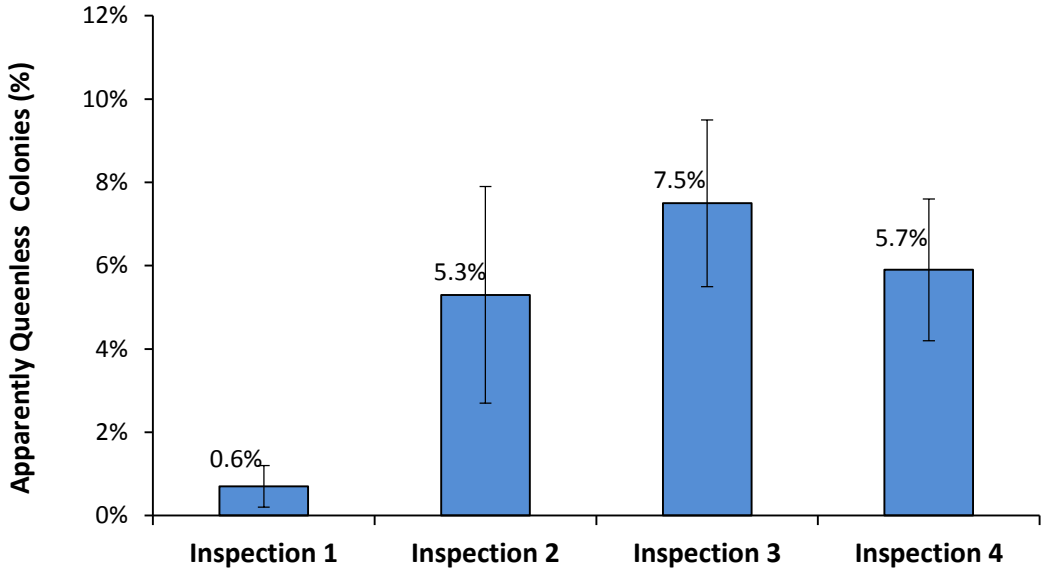

Figure 9. Mean apparently queenless colonies ± 1 standard error in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

b. Vitellogenin

Vitellogenin is lipoprotein synthesized in the fat body of honey bees. It has been documented to perform many functions and is commonly used as a molecular marker for the health of honey bees (Lin et al. 2004; Dainat et al. 2012). It is hypothesized that viral replication can impair the expression of vitellogenin in individual bees, resulting in a reduction in the expression of this gene in unhealthy or compromised honey bee colonies.

Mean vitellogenin was consistent across all collection periods, ranging from 8.75 log RNA copies/bee at inspection 1 to 9.54 log RNA copies/bee at inspection 4 (Fig. 10).

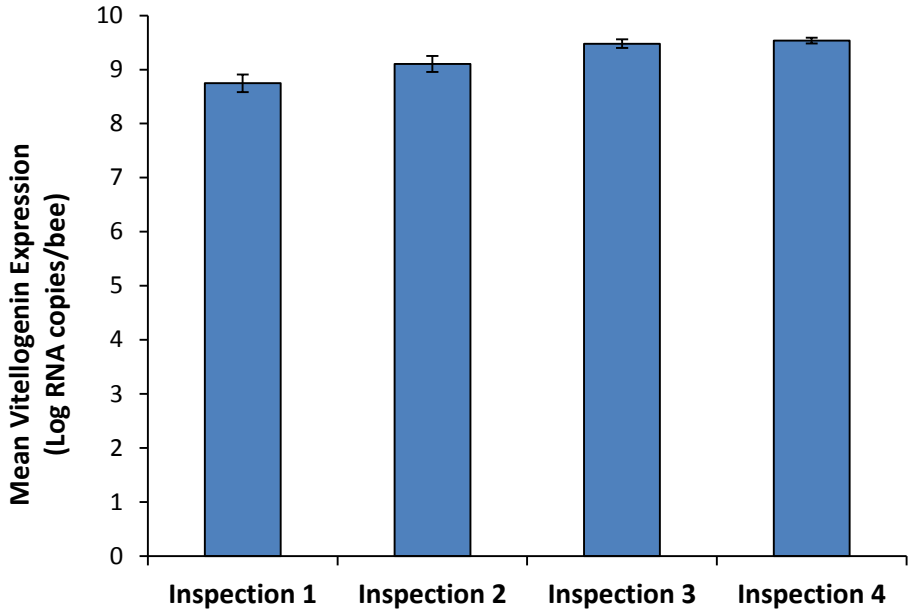

Figure 10. Mean vitellogenin expression (Log RNA copies per bee) ± 1 standard error in Ontario honey bee apiaries by collection period in 2015. Inspection 1 occurred between April 26 and May 14, inspection 2 occurred between May 14 and June 16, inspection 3 occurred between July 15 and August 25 and inspection 4 occurred between September 9 and October 15 based on weather and geographical distribution of the apiaries.

5.0 DISCUSSION

5.1 Viral Pathogens

Early studies identified common viruses in honey bee populations, and further studies have identified some modes of transmission and seasonal patterns of certain viruses. Research suggests that only a minority of honey bee populations are virus-free throughout the year (Tentcheva et al. 2004; Gauthier et al. 2007) and that honey bee viruses occur in different geographical regions (Chen & Siede, 2007). In many cases, these viruses infect honey bees without causing clinical signs of disease. In addition, several bee viruses may result in detrimental effects that are difficult to assess visually including a lesser adaptation to cold and non-beneficial changes in brood care or foraging behaviors (Aubert, 2008).

There remains uncertainty on the exact role of many honey bee viruses, either independently or in synergy with other stressors such as *V. destructor* mites. For example, many viruses are ubiquitous in populations of honey bees, have been present for decades and can be found in colonies that are healthy. Currently, there is limited research on viral loads in honey bees and peer-reviewed thresholds for honey bee viruses and their effects on colony health have not been well described. While DWV is

perhaps the most well studied viral pathogen and research has demonstrated a relationship between the presence of *V. destructor* and high viral loads, (Dainat et al. 2012; Dainat & Neumann 2013, Desai & Currie 2016) defined thresholds for viral loads are lacking.

Recent quantitative work has focused on documenting the prevalence of viruses in Canadian honey bee colony populations and evaluating viral loads (Desai et al. 2016). Viral load, not just presence or prevalence, is an important determinant in bee health. Studies on the prevalence and quantification of honey bee viruses have shown that all of the viruses investigated in the present study are present in Ontario (Emsen et al. 2015; Desai et al. 2016).

Prior work by Desai et al. (2016) characterised the occurrence and frequency of seven honey bee viruses (DWV, BQCV, SBV, IAPV, KBV, CBPV, and ABPV) in eight Canadian provinces using collections of honey bees from 2009 and 2010. The results of the present study confirm that the viruses detected by Desai et al. (2016) continue to be detected in Ontario apiaries. Additionally, the three most common viruses (BQCV, DWV, SBV) detected in this study were also the most commonly detected viruses in France, Italy and the USA further confirming the general prevalence of these particular viruses (Tentcheva et al. 2004; Porrini et al. 2016).

In the present study, the prevalence of ABPV peaked at 65%, which is greater than expected based on previous work (Desai et al. 2016). Similar to the seasonal pattern described by Steinhauer et al. (2014) where the prevalence of ABPV tends to be lower in the summer months and higher in the winter, ABPV was detected in more apiaries in 2015 heading into the winter months. This follows the seasonal pattern of increased *V. destructor* throughout the active beekeeping season and may partially explain the observed seasonal pattern of ABPV as *V. destructor* mites are vectors of the disease (Genersch & Aubert, 2010).

Similar to previous work by Desai et al. (2016), the prevalence of BQCV in the present study was consistently above 90% in all collection periods. BQCV has been associated with *Nosema* spp. and other viruses, but it is unclear if *Nosema* directly transmits the virus (Bailey et al. 1981).

CBPV is one of the few viruses that cause distinct symptoms in adult bees. Similar to the findings of Desai et al. (2016), the prevalence of CBPV was low in Ontario apiaries and increased in the summer months. CBPV outbreaks have been described to follow one of two epidemiological pathways, crowded and confined conditions due to bad weather forcing bees into the colony where virus is transmitted or very high density of colonies in a forage area (Genersch & Aubert, 2010).

As expected, the prevalence and viral load of DWV in the present study increased through the summer and fall months, likely as a result of the close association of this virus with *V. destructor*. Studies have shown that in the absence of *V. destructor*, DWV is benign and low DWV loads have been detected in honey bee colonies without serious impacts to colony health (Genersch & Aubert, 2010; Dainat et al. 2012). As DWV is vectored and amplified by *V. destructor* mites, increased mite infestations will further increase DWV (Genersch & Aubert, 2010). While there are no thresholds established for the pathogenicity of DWV, research in Switzerland has provided evidence that the presence and number of worker bees in a colony with DWV symptoms is correlated with *V. destructor* infestation and these two

variables can be used to predict colony loss (Dainat and Neumann, 2013). This provides support for anecdotal reports from Ontario beekeepers who have observed that colonies with high levels of symptomatic DWV individuals are less likely to survive winter (Paul Kozak, personal communication).

Compared to other honey bee viruses, IAPV is a relatively new discovery and as such, there are few studies documenting the range and prevalence of the virus. IAPV has been documented to be prevalent in the Middle East, Australia and North America (Genersch & Aubert, 2010). In 2006, when "Colony Collapse Disorder" (CCD) was first being investigated in the USA, IAPV had been associated with early reports of high honey bee mortality in colonies examined for CCD (Cox-Foster et al. 2007). Since that time however, IAPV has also been commonly associated with normal and healthy colonies as well. No causative agent has been identified for the suite of symptoms reported as CCD and there have not been official reports of CCD in Canada. IAPV has been reported to be found in 40 – 70% of Canadian apiaries with prevalence and viral load lower in the spring and summer, followed by a peak in the fall and winter months (Desai et al. 2016). This trend was not observed in the present study, where the general trend indicated that both prevalence and viral load of IAPV peaked in the late spring early summer before declining into the fall months. It should be noted however that the colonies in the present study were not investigated over the winter months.

KBV has been documented to be prevalent in honey bee populations in North America and New Zealand but is rarely found in Europe (Genersch & Aubert, 2010). In Canadian apiaries, KBV has been detected in all provinces except Alberta (Desai et al. 2016). Although the implications of KBV viral loads on honey bees are currently unknown, the virulence of KBV appears to differ depending on the mode of transmission. For example, early studies on KBV showed rapid onset of mortality in adult bees when injected with purified KBV particles compared to no obvious effects when bees were exposed through oral transmission (Bailey et al. 1979). Adequate data is currently not available to determine the seasonal prevalence of the virus. In the present study, however, the general trend indicated that prevalence and viral load peaked in the late spring early summer before declining into the fall months.

SBV prevalence has been investigated in various countries and host organisms including *Apis mellifera* and *A. cerana* (the Asiatic honey bee). Previous work by Desai et al. (2016) has indicated that the prevalence of SBV in Canadian apiaries is low in all provinces except Manitoba where the prevalence was 44% and that this virus is reported less frequently in the spring. In the present study, however, the prevalence of SBV was found to be moderate in the spring (50%) and increase to 84% during the summer. The prevalence and seasonality observed in the present study is contrary to what was expected based on the literature (Desai et al. 2016). High prevalence of SBV in colonies has been correlated with low winter survival (Desai & Currie 2016). While SBV causes high mortality in *A. cerana* colonies, infection appears to be less detrimental to *A. mellifera* colonies (Gong et al. 2016).

Currently, there are no established thresholds for honey bee viruses. While the seasonal prevalence of the above-mentioned honey bee viruses can be reported, viral loads can only be categorized as not detected, low, medium and high based on the distribution of the current data collected in 2015 from these sample sites. Additionally, the virulence of these viral pathogens is not completely understood and the scientific literature indicates that virulence may vary seasonally, geographically and by host

organism. Further monitoring is required to understand the prevalence and viral load of these pathogens in Ontario apiaries.

5.2 Brood Diseases

Clinical signs observed in the field that distinguish AFB from other brood diseases include a characteristic foul odor and sunken, perforated cell cappings. When the infection is new, the decomposed larvae have a mucus-like consistency where they can be stretched out of the brood cell (up to 30 cm). When the infection is old, the decomposed larvae form a hard, black, brittle scale that is difficult to remove from the bottom of the cell wall. At a population level, AFB represents a major risk to honey bee colonies as it can be easily transmitted from one bee yard to another. All colonies where AFB was detected were voluntarily destroyed by the beekeeper to prevent the spread to non-infected colonies both within the bee yard and in surrounding apiaries.

Each year, the prevalence of pests and diseases are assessed by ministry apiary inspectors as part of regulatory compliance activities. Of the 8,822 colonies inspected across Ontario in 2015, AFB was found in 84 honey bee colonies or 0.95% of the colonies inspected. In the present study of 320 colonies, AFB was found in only one yard at each inspection (Inspections 1, 2 and 3) and peaked at 1.6% of colonies inspected. Samples of AFB that have been tested for resistance to known treatments have confirmed that the strains of AFB circulating in Ontario are still susceptible to registered antibiotics (oxytetracycline and tylosin) and there have been no instances of antibiotic resistant AFB in Ontario to date (Paul Kozak, personal communication).

In heavy chalkbrood infestations, colonies may have multiple “chalkbrood mummies” in the honey bee brood and may litter the bottom board or entrance of the colony. Chalkbrood in honey bees is typically understood to be a brood disease of minor importance, rarely causing damage in honey bee colonies (Morse & Flottum 2013).

Of the 8,822 colonies inspected across Ontario in 2015, chalkbrood was detected in 5.8% of colonies and EFB was found in 0.13% of colonies. In the present study, chalkbrood peaked at 10% at inspection 2 which is greater than expected from the results of province-wide regulatory inspections. EFB however, was not detected in the bee yards assessed as part of the monitoring project.

European foulbrood is not typically understood to be a brood disease of major importance (Morse and Flottum, 2013). EFB infected larvae often die before the brood cell is capped and when dried can be easily removed from the cell, unlike AFB where the larvae dies after the cell is capped and are typically difficult to remove from the cell. Although EFB is spore-forming, it is not nearly as virulent, persistent or contagious as AFB.

5.3 *Nosema* spp.

Nosema spores are picked up by honey bees from the surrounding environment. Once in the gut tissue the pathogen replicates producing large numbers of spores which are excreted in the feces of the honey bee, continuing the transmission cycle (Fries et al. 1992). The effects of *Nosema* make it difficult for bees to digest food, resulting in nutritional deficiency, starvation and diarrhea.

Recent work by Dosselli et al. (2016) concluded that *N. apis* infection influences foraging activities, which could reduce foraging ranges of colonies and impact their ability to provide pollination services. *N. ceranae* has been documented to reduce the lifespan of individual worker honey bees, be more virulent, and proliferate when combined with environmental stressors (Alaux et al. 2010; Pettis et al. 2012; Goblirsch et al. 2013; Pettis et al. 2013). In Ontario, no correlation was found between *N. ceranae* and colony mortality but a correlation between *N. ceranae* and reduced colony strength in the early spring was identified (Guzman et al. 2010; Emsen et al. 2015). As expected, this study found *N. ceranae* to be the dominant species in the Ontario apiaries studied.

To date, a threshold has been established for *N. apis*, whereby treatment is recommended when there are greater than one million spores per individual bee in the spring (Jaycox, 1980). This threshold is decades old and was established in the southern USA and therefore may not be appropriate for conditions in Ontario. No threshold currently exists for *N. ceranae*; as this species is the dominant species in Ontario apiaries, more research is needed to develop a threshold.

5.4 Mites

Parasitism of honey bees by tracheal mites can have serious negative effects on colony health such as decrease in production, reduction in life span of individual bees and increased winter mortality (Bailey 1958; Eischen et al. 1989; Otis and Scott-Dupree 1992). Physical impacts of tracheal mite infestations on the individual bee include pierced and scarred trachea, and in some cases damage to flight muscles and hypopharyngeal glands (Liu et al. 1989a; Liu et al. 1989b). Mortality in individual bees parasitized by tracheal mites is believed to occur as a result of disruption to respiration, caused by mites blocking the flow of air through the tracheae and the damage caused by the mites to the integrity of the tracheae. Additionally, parasitism by tracheal mites results in a loss of hemolymph and may assist in the introduction of microorganisms into the hemolymph (Liu et al. 1989b). When over 30% of the bees in a colony become parasitized, honey production may be reduced and the likelihood of winter survival decreases with a corresponding increase in infestation (USDA, 2016). Of the apiaries surveyed in the present study, it appears as though the prevalence of tracheal mites is low in Ontario. This is not surprising given that Ontario beekeepers are using formic acid and other methods of treatment to control *V. destructor* mites, which will also impact populations of tracheal mites (Liu and Nasr 1992). Additionally, Ontario beekeepers have been working with the Ontario Beekeepers' Association Technology Transfer Program to incorporate honey bee stocks that have been demonstrated to harbour fewer mites (Otis and Scott-Dupree 1992; Lin et al. 1996; Nasr et al. 2001).

Tracheal mites have been difficult to detect in recent surveillance programs in the USA (Rose et al. 2010), possibly related to the ongoing usage of miticides by beekeepers to control mite infestations. At present, aside from mixed infestations of tracheal mites and *V. destructor* mites, tracheal mites are considered to be a pest that is largely manageable in Ontario honey bee colonies with current treatment, breeding and management options available to beekeepers (Paul Kozak, personal communication).

V. destructor mites are considered by many to be one of the greatest threats to honey bee health based on its current distribution and virulence (Rosenkratz 2010; Guzmán-Novoa 2016). Although *V. destructor* coevolved with the Asian honey bee (*Apis cerana*) it has spread to most western honey bee (*Apis mellifera*) populations globally, excluding Australia. *V. destructor* has been in most parts of Ontario since the early 1990s and is in found in virtually all honey bee colonies in the province. Ontario beekeepers rely on different methods to control this pest, including the use of registered chemical treatments and management techniques. Academics in the field are currently working on breeding honey bees that demonstrate grooming behavior which protects against *V. destructor* (Guzmán-Novoa et al. 2012).

In Ontario, *V. destructor* has been shown to be the main culprit for death and reduced populations of overwintered honey bees (Guzmán-Novoa et al. 2010). Monitoring for *V. destructor* and effective and timely treatments are crucial for honey bee colony survival (Lee et al. 2010; Currie & Gatien 2006). Ontario beekeepers are encouraged to monitor for *V. destructor* throughout the season and treat colonies when needed. The recommended treatment thresholds described by Guzmán-Novoa et al. (2010) are 2% of bees in the spring and 3% of bees in the fall. The present study showed that the mean percent of bees infested with *V. destructor* was low and was, for the most part, below the recommended treatment thresholds.

Determining the presence, distribution and prevalence of the different *V. destructor* haplotypes may be important if future research demonstrates differences in the reproductive capacity or virulence between haplotypes. As expected, the Korean haplotype was the dominant haplotype detected in the present study. This was expected as the Korean haplotype has been detected in honey bees globally whereas the Japanese haplotype has been found primarily in Japan, Thailand and the Americas (Solignac et al. 2005).

The *Tropilaelaps* mite is thought to be restricted to Asia and to date there have been no documented cases of *Tropilaelaps* mites in North America. Unmanaged *Tropilaelaps* infestations typically lead to the death of the colony. Where the distribution of this pest overlaps with *V. destructor*, *Tropilaelaps* is considered a worse threat to honey bee health (Rath et al. 1995). In addition to the direct impacts of *Tropilaelaps* on honey bees, this pest can also vector numerous pathogens (primarily viruses) similar to *V. destructor* (Dainat et al. 2009). The results of the present study continue to indicate the absence of *Tropilaelaps* in Ontario apiaries. The severity of this pest and the risk to honey bees globally reinforces the importance of surveillance.

5.5 Other Honey Bee Threats

Adult female phorid flies will lay eggs in the insect host's abdomen. The larvae of the fly grows within the body of the host, consuming the tissues and eventually chewing its way out of the body, killing the host in the process. While *Apocephalus borealis* is native to most of North America, including Ontario, it has expanded its host range to include the non-native honey bee (Core et al. 2012). Recently, *A. borealis* has been documented in British Columbia but at present there is not adequate evidence to suggest parasitism of honey bees is anything more than incidental. Although the relationship is not well understood, studies have found that larvae and adult phorid flies often test positive for DWV and *N. ceranae* and honey bees from parasitized colonies frequently are affected by both DWV and *N. ceranae*. This suggests that *A. borealis* may be a potential vector or reservoir of these pathogens (Core et al. 2012). The results from the present study indicates that *A. borealis* is not currently found in association with Ontario apiaries. Independent sampling by the ZomBee Watch Team at San Francisco State University has yet to find honey bees parasitized by *A. borealis* in Ontario (www.zombeewatch.org).

The SHB is native to sub-Saharan Africa and first spread to the United States in 1996 and has become established throughout most of the country. Since Ontario first detected this apiary pest in 2002, the provincial government in partnership with industry have undertaken a number of actions including education, regulatory and supporting regional research to mitigate the spread and potential impact of SHB in Ontario.

SHB can also be a vector for honey bee pathogens such as DWV (Eyer et al. 2009). While SHB can directly impact honey bee colonies, the presence of the pest has not been linked to overwintering losses (Schafer et al. 2010). The impacts of SHB can typically be mitigated through best management practices (Elzen et al. 1999; Ellis 2005a; Ellis 2005b). As the known distribution of SHB is generally restricted within Ontario, it is not surprising that SHB was not detected in the current study.

Previous work by Schwarz et al. (2014) found *Spiroplasma* spp. to be prevalent in the USA and found that *S. melliferum* was more common than *S. apis*. Additionally, it appears as though the presence of one pathogen made bees more susceptible to the other. Contrary to the work by Schwarz et al. (2014), the present study found that the prevalence of *S. apis* was low in Ontario apiaries and *S. melliferum* was not detected in any of the samples tested. Originally thought to be a pathogen of concern for beekeepers exclusively in the spring, new work shows that *Spiroplasma* spp. might pose a threat year-round. Similar to Schwarz et al. (2014), the present study provides evidence for seasonal prevalence of *S. apis* with peaks in both spring and fall among the colonies tested. While there is evidence to suggest that bees pick up these pathogens when they feed on nectar from certain plants that can act as bacteria-transmission sites, it is currently unknown if *S. melliferum* and *S. apis* are factors in honey bee mortality and it is unclear how virulent *Spiroplasma* spp. bacteria are to bees.

Studies have shown that trypanosomes are found globally in honey bees. Researchers investigating the prevalence of trypanosomes in the USA found that *C. mellificae* infection was detected at every time-point and from every geographic location tested and the prevalence of this pathogen peaked in January (Runckel et al. 2011). Additionally, Runckel et al. (2011) found that *C. mellificae* infections were

associated with *N. ceranae* which is commonly found in Ontario apiaries. Under laboratory conditions, honey bees infected by *C. mellificae* had significantly different life spans compared to uninfected individuals (Higes et al. 2016) and no significant negative impacts have been observed when honey bees are coinfected with *L. passim* and *N. ceranae* (Tritschler et al. 2017).

Results from the present study showed a peak in *C. mellificae* prevalence in the summer, although it should be noted that samples were only collected during the active beekeeping season. Research by Ravoet et al. (2015) found that *L. passim* is the dominant trypanosome species in colonies surveyed in Belgium, Japan and Switzerland, which is similar to the results presented here. While trypanosomes have been documented in weak colonies, the specific role they play in honey bee health is unclear and more work is needed to understand these relationships. At this time, there is no threshold for what constitutes a damaging level of these pathogens and they are not yet determined to be of primary importance to honey bee health.

5.6 Colony Indicators

The health and productivity of a honey bee colony is directly influenced by the presence of a functional queen. Potential causes of queen issues may range from disease, inadequate mating conditions, age of the queen, environmental stimuli and beekeeper management. While honey bee colonies have been shown to have a multitude of different pathogens (Chen & Siede, 2007; Desai et al. 2016; Steinhauer et al. 2014), the queen bee may be exempt from a number of these pathogens (Delaney et al. 2011). A survey of commercially produced honey bee queens in the USA found that the overall disease and pest prevalence in queens is low (Delaney et al. 2010). Although the prevalence of disease in honey bee queens was not investigated in the present study, honey bee queens were noted in the majority (greater than 90%) of colonies inspected, suggesting that colony issues related to queen health were unlikely or not detected.

Vitellogenin acts as a reproductive protein (Nelson et al. 2007), influences queen longevity (Corona et al. 2007) and behavioral specialization (Amdam et al. 2004) including social organization of the colony (Nelson et al. 2007), in addition to having antioxidant properties (Seehuus et al. 2006). Because of these many functions, the expression of vitellogenin is often used as an indicator of health. In honey bees, vitellogenin acts as an antioxidant to promote longevity and is used by nurse bees for the production of brood food (Di Pasquale et al. 2016). Previous research has shown that vitellogenin expression is highly sensitive to changes in pollen availability (Di Pasquale et al. 2016) and is influenced by pathogen infections, such as *N. ceranae* (Goblirsch et al. 2013). In the present study, only worker bees were tested and there were no differences in vitellogenin expression across the collection dates. While it is not known what the nutritional status was of the colonies tested, the consistent expression of vitellogenin across collection periods may suggest that changes in pollen availability were not a factor.

6.0 LIMITATIONS

There are a number of limitations regarding the interpretation of the data collected from Ontario apiaries in 2015. Primarily, there are no established thresholds for many of the honey bee pathogens. The work outlined in this report aimed to survey honey bee pathogens at selected apiaries across Ontario to gain a better understanding of pathogen prevalence and load. Aside from thresholds defined for *V. destructor* and *N. apis*, cause and effect relationships cannot be drawn because there are no data to suggest that a particular pathogen load is pathogenic or otherwise detrimental to the health of individual bees or the colony as a whole. Further research is required to define thresholds for honey bee pathogens and this is outside of the scope of the current project.

The beekeeping population in Ontario is diverse, but only commercial operators (having 50 or greater colonies) of stationary bee yards were studied. While extending the scope of this study to small-scale and migratory beekeepers would be interesting, commercial beekeepers were selected as this group represents the largest proportion of honey bee colonies in Ontario.

Due to the field monitoring nature of this study rather than a controlled experiment, results should be viewed as exploratory. The apiary monitoring data collected in 2015 will serve as a basis for comparison with subsequently acquired data from future years. It must be emphasized that the disease detections outlined in the present study are reported at the apiary level and may not accurately reflect the individual colony level data within each yard.

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