CANADIAN NATIONAL HONEY BEE HEALTH SURVEY

Photo Credit: Christy Curran

2017 REPORT

British Columbia, Alberta, Manitoba, Ontario, New Brunswick, Nova Scotia, Prince Edward Island, Newfoundland

Canada

GPRC
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Technology Access Centre

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2017 Canadian National Honey Bee Health Survey

The Canadian National Honey Bee Health Survey is a four year, nation-wide initiative established to index honey bee health; the Survey began in 2014 and completed its first phase of sampling in 2017. This project was industry driven by the Alberta Beekeepers Commission and the Manitoba Beekeeper's Association, on behalf of the Grande Prairie Regional College's National Bee Diagnostic Centre – Technology Access Centre (NBDC).

The purpose of this project, the first of its kind in Canada, was to document the prevalence, intensity and distribution of pests and pathogens in Canadian apiaries. This information will help ensure that Canada, as a country, has robust data to establish a bee health database- similar to other leading beekeeping countries in the world.

To accomplish this, bee samples were collected across Canada- with a target sample size of 0.5% of registered hives. The Survey was designed to systematically expand across the country, starting in Alberta and Manitoba the first year.

Year One (2014) the Survey began in Alberta and Manitoba, resulting in samples from 163 apiaries.

Year Two (2015) the Survey expanded to 2 additional provinces, British Columbia and Ontario, resulting in samples from 212 apiaries.

Year Three (2016) the Survey moved into Eastern Canada, including Quebec, New Brunswick, Nova Scotia, Prince Edward Island and Newfoundland. Samples from the Yukon Territories were also received, resulting in samples from 314 apiaries.

Year Four (2017) the Survey included samples from all Provinces except Saskatchewan and Quebec, resulting in a total of 255 apiary samples.

The information generated by the Canadian National Honey Bee Health Survey will aid in developing relevant colony health management practices and provide the best opportunity to identify exotic organisms before they establish themselves; maintenance of healthy bee populations will allow for a sustainable apiculture industry in Canada.

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Glossary

AAFC	Agriculture & Agri-Food Canada
AB	Alberta
ABPV	Acute Bee Paralysis Virus
AFB	American Foulbrood
AHB	Africanized Honey Bees
ATTTA	Atlantic Tech Transfer Team for Apiculture
BC	British Columbia
BQCV	Black Queen Cell Virus
CBPV	Chronic Bee Paralysis Virus
cDNA	Complimentary DNA
CFIA	Canadian Food Inspection Agency
CFU	Colony Forming Unit
DNA	Deoxyribonucleic Acid
DWV	Deformed Wing Virus
EFB	European Foulbrood
EHB	European Honey Bees
GPRC	Grande Prairie Regional College
IAPV	Israeli Acute Paralysis Virus
KBV	Kashmir Bee Virus
LC-MS	liquid chromatograph-tandem quadrupole mass spectrometers
LSV	Lake Sinai Virus
MB	Manitoba
mtDNA	Mitochondrial DNA
NB	New Brunswick
NBDC	National Bee Diagnostic Centre

Glossary con't

NL	Newfoundland
NS	Nova Scotia
ON	Ontario
OTC	Oxytetracycline
PEI	Prince Edward Island
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment-Length Polymorphism
RNA	Ribonucleic Acid
SBPV	Slow Bee Paralysis Virus
SBV	Sacbrood Virus
SNP	Single Nucleotide Polymorphism
TAC	Technology Access Centre

Survey Methodology

YEAR 4: During the Summer of 2017, 255 apiary samples were collected, representing 2,524 colonies. All diagnostic tests were performed at the NBDC in Beaverlodge, Alberta with the exception of chemical residues.

Apiary Sampling: Samples were collected between July and September 2017- before fall treatments for *Varroa* and *Nosema* were applied. Sample technicians in each province were employees or contractors of the NBDC-TAC that received skill specific training for the Survey. In addition to individual contractors, AB Agriculture and Forestry, Agriculture & Agri-Food Canada (AAFC), BC Ministry of Agriculture, and the Atlantic Tech Transfer Team for Apiculture (ATTTA) each provided personnel support for sampling.

4 types of composite samples were collected from 10 randomly-chosen colonies at each apiary:

I. **LIVE BEE SAMPLE:** bees were collected in a battery box and shipped live for disease and pest analysis including *Nosema* spore count and species identification, American Foulbrood (AFB) culture and antibiotic response testing, European Foulbrood (EFB) detection, tracheal mite detection, *Apis cerana* detection, analysis of 9 honey bee viruses and the hybridization with African races of honey bees.

II. **ALCOHOL WASH SAMPLE:** bees were collected and submerged in 70% ethanol to determine *Varroa* mite levels.

III. **BROOD FRAME DEBRIS SAMPLE:** material was collected from the "knock test" of a brood frame to monitor for *Tropilaelaps* mites.

IV. **BEE BREAD SAMPLE:** bee bread was collected from 10 cells at each colony for analysis of 5 chemical residues (neonicotinoids) at the Alberta Agriculture & Forestry Agri-Foods Laboratories Branch in Edmonton, AB.

Sample Distribution: see **Map Section** for detailed figures outlining sample regions.

British Columbia	**27 Total Samples**	**Manitoba**	**42 Total Samples**
Fraser Valley	7 Samples	Central	8 Samples
Kootenay	4 Samples	Eastern Interlake	10 Samples
Northwest	3 Samples	Northwest	12 Samples
Okanagan	3 Samples	Southern	12 Samples
Peace	3 Samples	**Ontario**	**25 Total Samples**
Thompson/Cariboo	4 Samples	Northern	4 Samples
Vancouver	3 Samples	Southern	21 Samples
Alberta	**128 Total Samples**	**New Brunswick**	**8 Total Samples**
Central	13 Samples	North	4 Samples
Northeast	13 Samples	South	4 Samples
Northwest	31 Samples	**Nova Scotia**	**16 Total Samples**
Peace	33 Samples	Central	12 Samples
South	38 Samples	Western	4 Samples
		Prince Ed. Island	**7 Total Samples**
		Newfoundland	**2 Total Samples**

Visual Inspection: The 3 central brood frames were examined for brood and adult clinical disease symptoms or other colony conditions in each of the 10 colonies sampled per apiary. Results were scored for the presence or absence of a symptom or condition.

Nosema Counting/Identification: Sixty bees were macerated and analyzed for *Nosema* spp. infections. Samples were examined using a haemocytometer under light microscopy (400x) to calculate a *Nosema* spore count. Additionally, DNA was extracted from the same maceration and a PCR protocol performed to identify *Nosema* species (*N. apis*, *N. ceranae,* or both).

Varroa Counting: Bees (~1,000) were collected in 70% ethanol and agitated with a laboratory bench-top shaker to dislodge mites for the *Varroa* mite analysis. Dislodged mites were counted to provide an infestation level of the apiary, expressed as the number of mites per 100 adult bees (%).

AFB Bacterial Culture: One hundred and twenty adult bees were tested for the presence or absence of *Paenibacillus larvae*, the bacterium that causes AFB. Each sample was cultivated in triplicate on diagnostic media plates that supported the growth of the bacterium. If present, the number of bacterial colonies that grew was scored as the number of colony forming units (CFU). Samples that tested positive for *Paenibacillus larvae* were further analyzed for resistance or sensitivity towards the antibiotics Oxytetracycline (OTC) and Tylosin, which are registered for the control of AFB in Canada.

AFB Risk: Apiaries were categorized into 4 nominal groups for their propensity to develop clinical symptoms of AFB. Risk categories were designated based on the average number of bacterial colony forming units (CFU) that were cultivated on diagnostic media plates: *Not Detected, Possible Risk* (1-99 CFU), *Moderate Risk* (100-999 CFU) *and High Risk* (>1,000 CFU).

EFB Detection: DNA was extracted from samples and a PCR protocol was applied to detect the presence or absence of European Foulbrood (*Melissococcus plutonius)*.

African Ancestry Testing:

I.PCR-RFLP Assay: DNA was extracted from 60 bees and a PCR-based restriction fragment-length polymorphism (RFLP) assay that targets three mitochondrial DNA (mtDNA) genes and employs four restriction enzymes for the discrimination of four honey bee subspecies (Eastern European, Western European, Apis mellifera lamarckii and sub-Saharan African) was performed.

As a follow-up, 30 bees were analyzed from each positive composite sample to identify individual bees positive for African genetics.

**mtDNA is maternally inherited; therefore, analysis by this method will not detect progeny of European queens mated with Africanized drones.*

II. SNP Analysis: Further examination of positive samples determined by the PCR-RFLP Assay was performed using a Single Nucleotide Polymorphism (SNP) analysis. A second subset of DNA from the individual bees positive for African ancestry was sent to the Genome Quebec sequencing facility at McGill University for a SNP assay to identify Africanized honey bees via proportion of their African ancestry. This method quantifies the ancestry of honey bees using both maternal and paternal inherited nuclear markers to distinguish between Africanized honey bees (AHB) and European honey bees (EHB).

Tracheal Mites: PCR was used to detect the presence or absence of tracheal mites (*Acarapis woodi*) from extracted DNA. Samples positively identified with *Acarapis woodi* were further investigated; 20 bees from the apiary sample were dissected for tracheal mite identification, examined under a light microscope.

Tropilaelaps Detection: Debris was collected by knocking an unsealed brood frame into a metal collection pan. The debris was screened for the presence of *Tropilaelaps* spp. mites under a dissecting microscope. *Tropilaelaps* are a parasitic mite found in Asia, not native in North America. Surveillance for *Tropilaelaps* is valuable as they are a potential invasive pest.

Apis cerana Detection: A series of morphological identification techniques were used to determine the absence of Asian honey bees (*Apis cerana*), including body size, striping pattern and colour, forewing colour, marginal cell and wing length. Furthermore, the mtDNA of individual samples was analyzed to determine the absence of Asian honey bees in the sample set.

Viral Detection: RNA was extracted from 60 bees, converted into cDNA and analyzed for 9 viruses by PCR: Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV), Israeli Acute Bee Paralysis Virus (IAPV), Kashmir Bee Virus (KBV), Lake Sinai Virus (LSV strains 1-4), Sacbrood Virus (SBV) and Slow Bee Paralysis Virus (SBPV). Apiaries were scored as "Positive" for any detection level of the virus or "Negative" for the absence of the virus.

Chemical Residue Testing: bee bread was taken from 10 cells at each colony inspected, when possible. 2 grams of this composite sample was sent to the Alberta Agriculture & Forestry Agri-Foods Laboratories Branch in Edmonton, AB for analysis of 5 neonicotinoids by multiple liquid chromatograph-tandem quadrupole mass spectrometers (LC-MS/MS).

Provincial Maps

BRITISH COLUMBIA

Figure 1. Provincial map of British Columbia, includes 7 Regions: Fraser Valley, Kootenay, Northwest, Okanagan, Peace, Thompson/Cariboo, and Vancouver Coast.

ALBERTA

Figure 2. Provincial map of Alberta, includes 5 Regions: Central, Northeast, Northwest, Peace and South.

MANITOBA

Figure 3. Provincial map of Manitoba, includes 4 Regions: Central, Eastern Interlake, Northwest and Southern.

ONTARIO

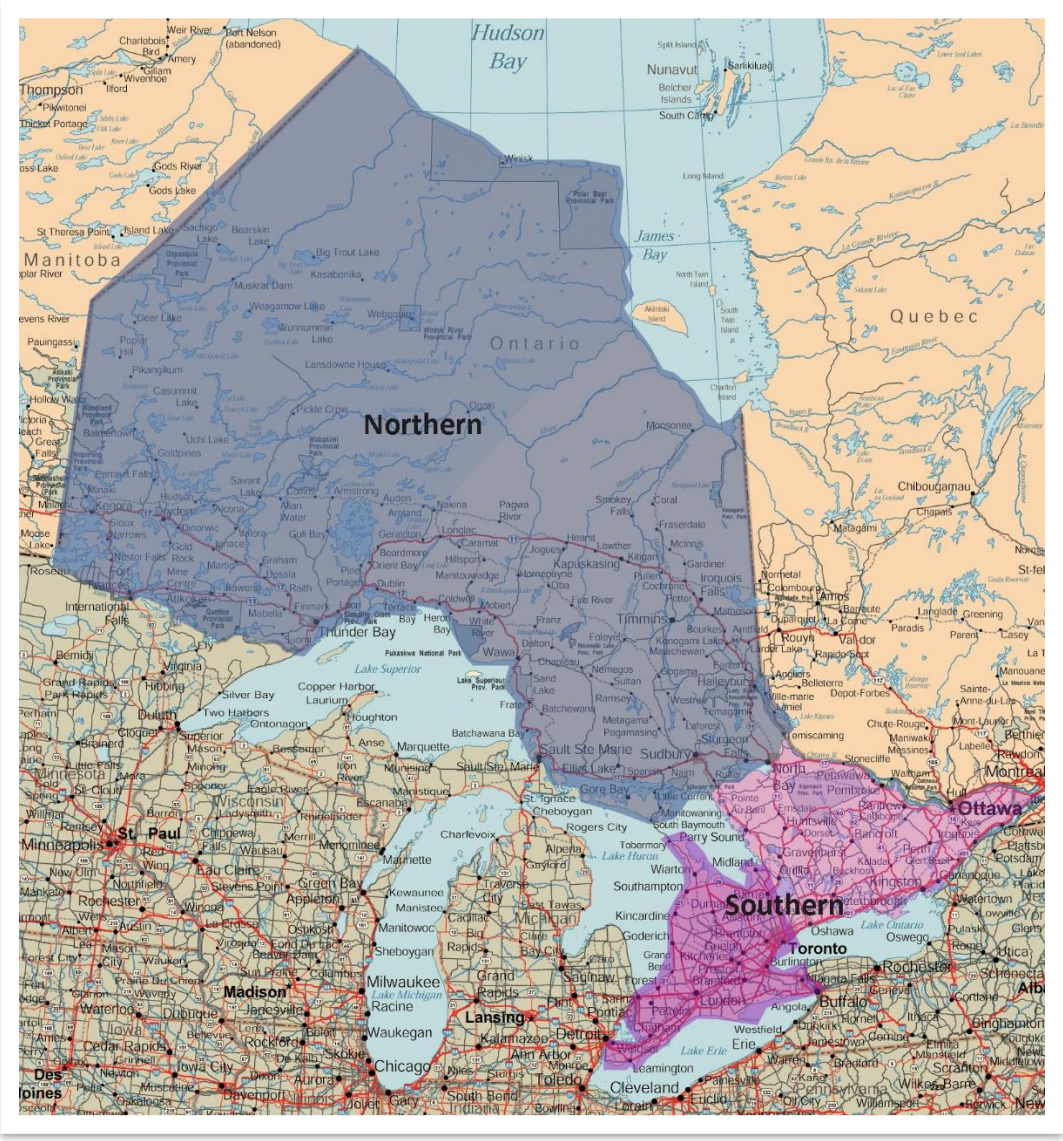

Figure 4. Provincial map of Ontario, includes 2 Regions: Northern and Southern.

NEW BRUNSWICK

Figure 5. Provincial map of New Brunswick, includes 2 Regions: North and South.

NOVA SCOTIA

Figure 6. Provincial map of Nova Scotia, includes 2 Regions: Western and Central.

PRINCE EDWARD ISLAND

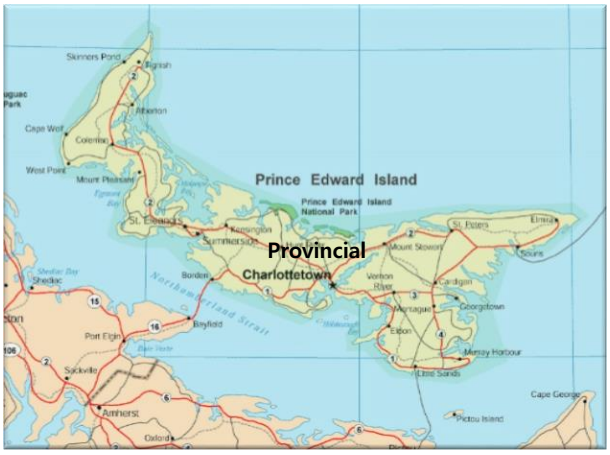

Figure 7. Provincial map Prince Edward Island, analyzed as a single entity- not enough samples for regional distribution.

NEWFOUNDLAND

Figure 8. Provincial map of Newfoundland, analyzed as a single entity- not enough samples for regional distribution.

Results

Visual Inspection (Incidence)

Disease/ Condition	BC n=268*	AB n=1280	MB n=418*	ON n=243*	NB n=76*	NS n=149*	PEI n=70	NL n=20	National Average n=2,524
AFB	0	0.2	0	0	0	0.7	0	0	**0.2%**
EFB	3.0	0.2	0	0	4.0	1.3	1.4	0	**0.6%**
Sacbrood	1.1	0.7	0.2	0	0	0	0	0	**0.5%**
Chalkbrood	2.6	4.3	2.6	7.0	26.3	39.6	21.4	0	**7.3%**
Deformed Wing Bees	1.9	1.8	0.2	2.9	18.4	4.0	0	0	**2.2%**
Black Shiny Bees	4.1	0.1	1.9	2.5	22.4	2.7	4.3	0	**2.0%**
Small Hive Beetle (larvae or adults)	0	0	0	0	0	0	0	0	**0%**
Wax Moth (larvae or adults)	0	0.1	0	0.8	1.3	0	0	0	**0.2%**
Queen Cells Present	4.1	11.7	1.9	5.4	1.3	0.7	0	0	**7.3%**
Drone Laying Queen	1.1	10.6	0.5	0	0	0	0	0	**5.6%**

Table 1. Visual inspection results for each province identifying the presence or absence of brood and adult clinical disease symptoms or other colony conditions. The three central brood frames from the ten colonies sampled per apiary were inspected.

*The number of colonies (n) does not represent 10 colonies per apiary. Some composite samples in these regions were incomplete (<10 colonies per apiary) due to discovery of weak colonies or inclement weather during sampling.

Nosema (Incidence)

British Columbia	**22% Incidence**	**Ontario**	**48% Incidence**
Fraser Valley	3/7	Northern	2/4
Kootenay	0/4	Southern	10/21
Northwest	0/3	**New Brunswick**	**88% Incidence**
Okanagan	0/3	North	4/4
Peace	1/3	South	3/4
Thompson/Cariboo	0/4	**Nova Scotia**	**75% Incidence**
Vancouver	2/3	Central	10/12
Alberta	**61% Incidence**	Western	2/4
Central	13/13	**Prince Ed. Island**	**57% Incidence**
Northeast	7/13	Provincial	4/7
Northwest	16/31	**Newfoundland**	**50% Incidence**
Peace	12/33	Provincial	1/2
South	30/38	**National Level**	58% Incidence
Manitoba	**64% Incidence**	**National Total**	147/255
Central	6/8		
Eastern Interlake	8/10		
Northwest	3/12		
Southern	10/12		

Table 2. *Nosema* incidence (number of apiaries affected per region and provincial total), identified by microscopy.

Nosema (Count)

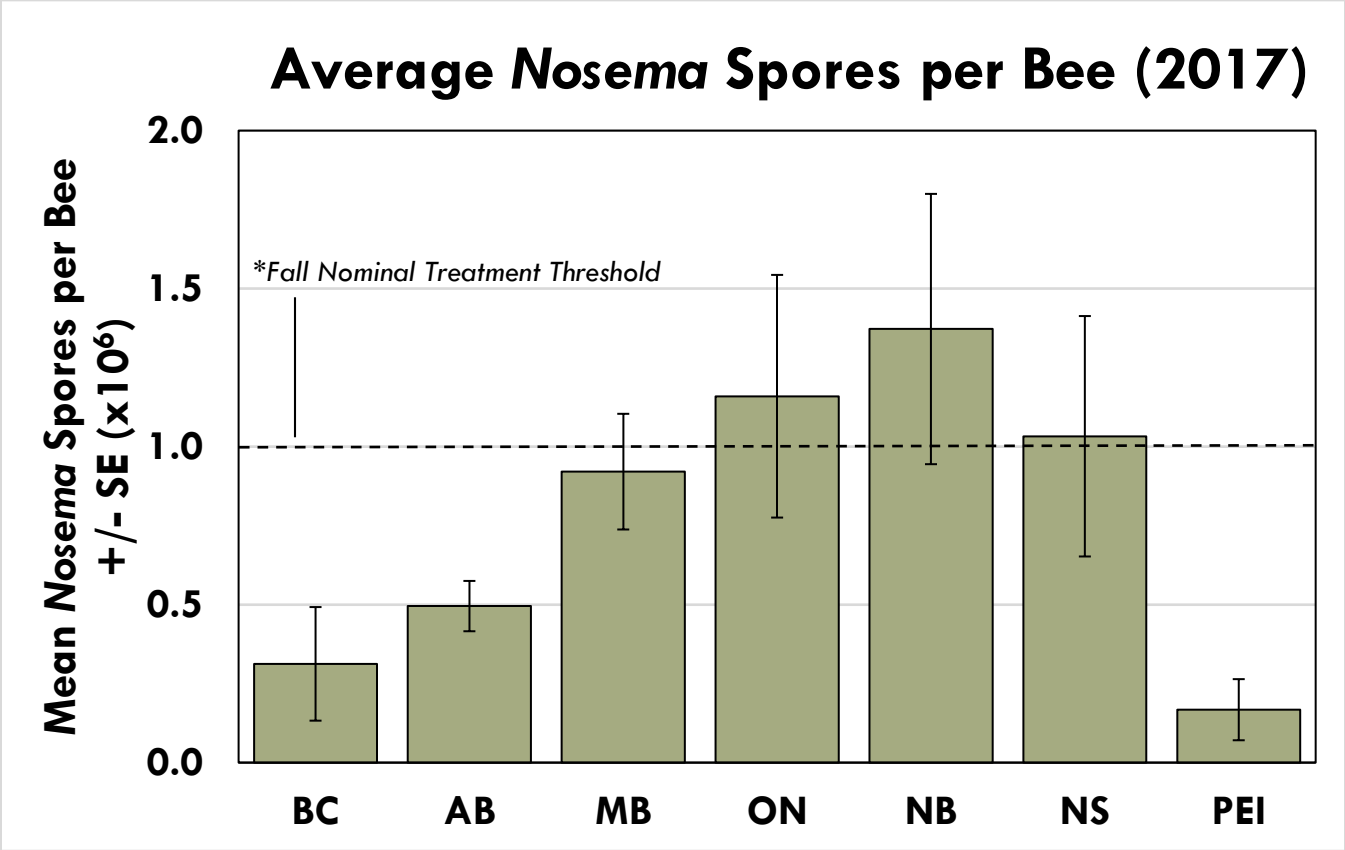

Figure 9: Average *Nosema* spore count per bee, enumerated with a haemocytometer under light microscopy (400x); reported by provincial average. The average *Nosema* spore count is represented in millions of spores per bee.

Results for NL are unable to be provided for *Nosema* in order to maintain beekeeper confidentiality due to the low sample size (n=2).

*Fries I., Ekbohm G., Villumstad E. (1984). *Nosema apis*, sampling techniques and honey yield. J. Apic. Res. 23, 102-105.

Nosema (Species Identification)

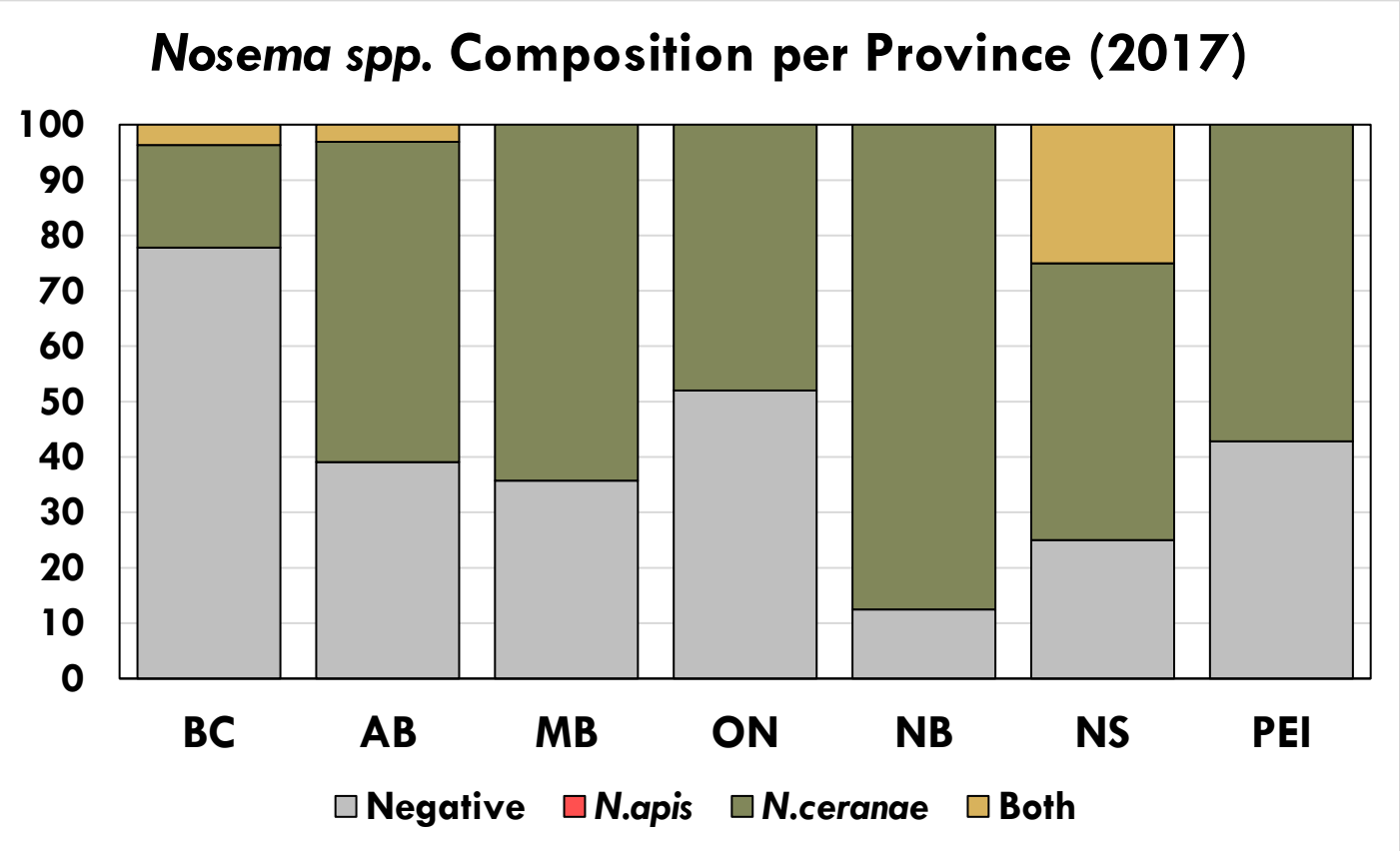

Figure 10. *Nosema* spp. composition by province in 2017 detected by DNA extraction and PCR.

Results for NL are unable to be provided for *Nosema* in order to maintain beekeeper confidentiality due to the low sample size (n=2).

Varroa (Incidence)

British Columbia	**93% Incidence**	**Ontario**	**92% Incidence**
Fraser Valley	7/7	Northern	4/4
Kootenay	4/4	Southern	19/21
Northwest	3/3	**New Brunswick**	**63% Incidence**
Okanagan	2/3	North	2/4
Peace	2/3	South	3/4
Thompson/Cariboo	4/4	**Nova Scotia**	**63% Incidence**
Vancouver	3/3	Central	6/12
Alberta	**62% Incidence**	Western	4/4
Central	7/13	**Prince Ed. Island**	**86% Incidence**
Northeast	5/13	Provincial	6/7
Northwest	23/31	**Newfoundland**	**0% Incidence**
Peace	20/33	Provincial	0/2
South	24/38	**National Level**	**71% Incidence**
Manitoba	**76% Incidence**	**National Total**	180/255
Central	6/8		
Eastern Interlake	10/10		
Northwest	8/12		
Southern	8/12		

Table 3. *Varroa* incidence (number of apiaries affected per region and provincial total), detected using laboratory alcohol washes of adult bees.

Varroa (Count)

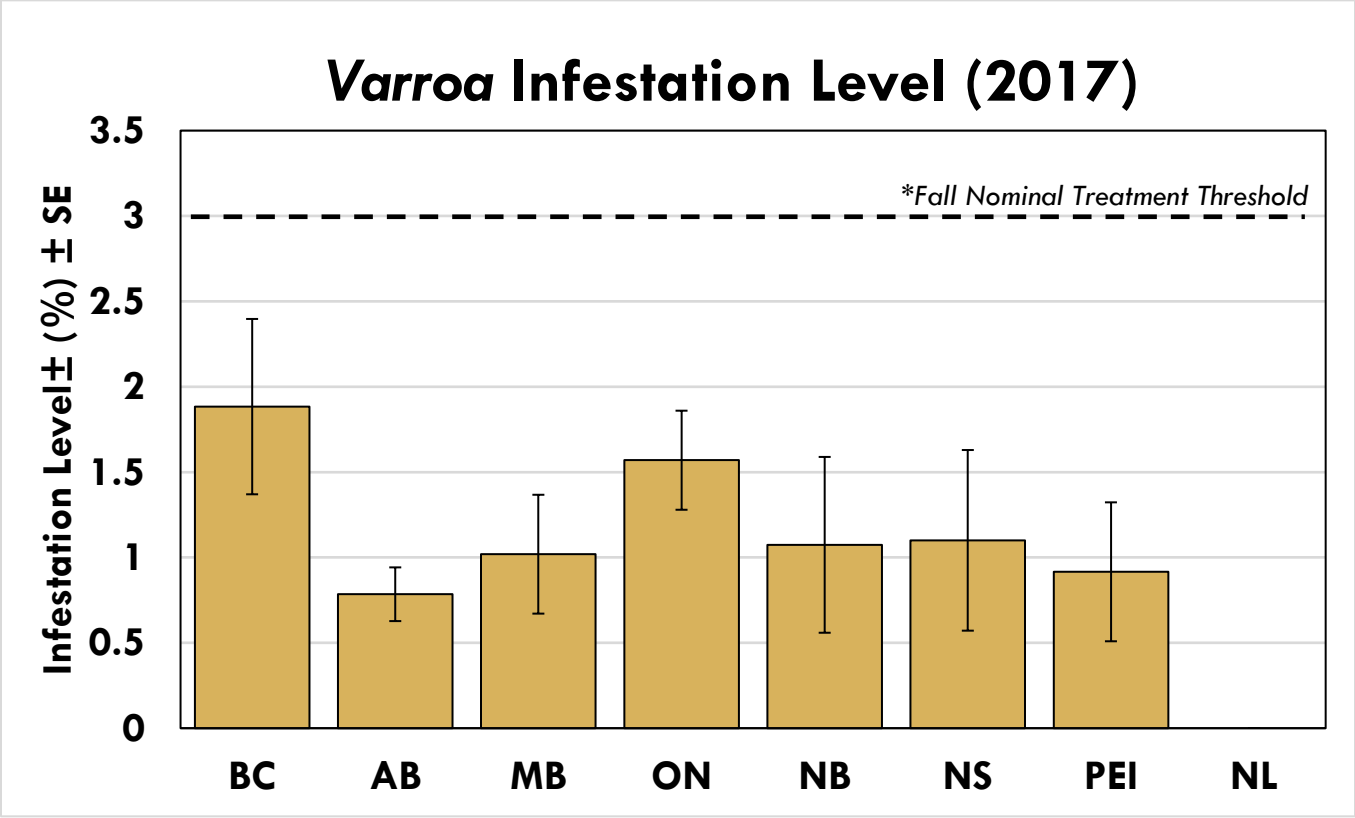

Figure 11. *Varroa* infestation level reported by provincial average, expressed as the number of mites per 100 adult bees (%).

*Currie, R.W. 2008. *Economic Threshold for Varroa on the Canadian Prairies*. University of Manitoba, Dept. of Entomology.

AFB (Bacterial Culture-Adult Bees)

British Columbia	**11% Incidence**	**Ontario**	**0% Incidence**
Fraser Valley	0/7	Northern	0/4
Kootenay	0/4	Southern	0/21
Northwest	0/3	**New Brunswick**	**13% Incidence**
Okanagan	1/3	North	0/4
Peace	0/3	South	1/4
Thompson/Cariboo	0/4	**Nova Scotia**	**13% Incidence**
Vancouver	2/3	Central	2/12
Alberta	**13% Incidence**	Western	0/4
Central	0/13	**Prince Ed. Island**	**0% Incidence**
Northeast	0/13	Provincial	0/7
Northwest	5/21	**Newfoundland**	**0% Incidence**
Peace	9/33	Provincial	0/2
South	3/38	**National Level**	**9% Incidence**
Manitoba	**2% Incidence**	National Total	24/255
Central	0/8		
Eastern Interlake	0/10		
Northwest	0/12		
Southern	1/12		

Table 4. Incidence of apiaries positive for American Foulbrood (AFB), as determined by bacterial culture of adult bee samples, per province and region, when applicable.

AFB (Bacterial Culture-Adult Bees)

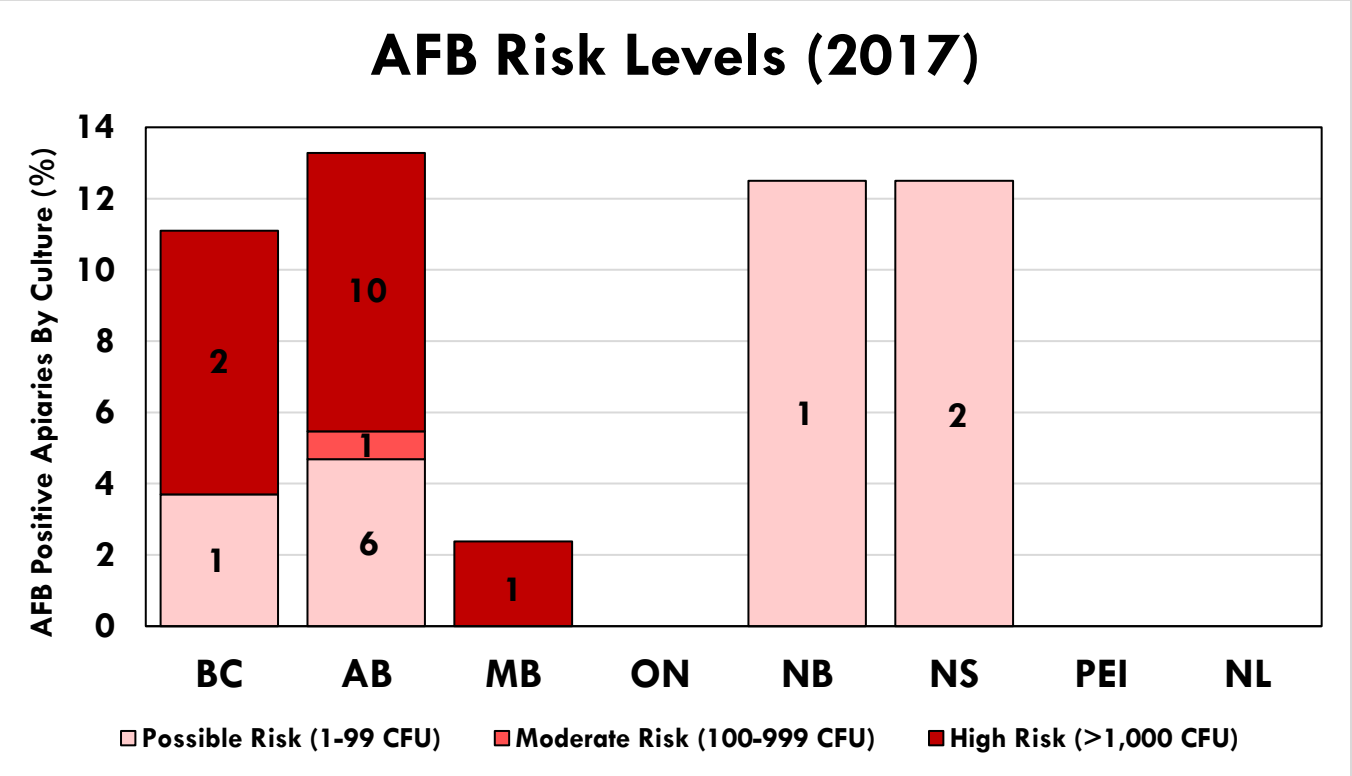

Figure 12. Based on previous research*, AFB positive apiary samples were categorized into 3 groups for their propensity to develop clinical symptoms of the disease. Risk levels were designated based on the average number of CFUs that grew on the diagnostic media plates: Possible Risk (1-99 CFU), Moderate Risk (100-999 CFU) and High Risk (>1,000 CFU). The proportion of apiaries affected in each province is shown by the bar height, the number of apiary samples this represents is noted inside each bar segment.

* Pernal S.F., Melathopoulous, A.P. (2006) Monitoring for American foulbrood spores from honey and bee samples in Canada. Apiacta 41, 99-109.

Pernal S.F., Albright R.L., Melathopoulous, A.P. (2008). Evaluation of the shaking technique for the economic management of American foulbrood disease of honey bees (Hymenoptera; Apidae). J. Econ. Entomol 101: 1095-1104.

AFB (Bacterial Culture-Adult Bees)

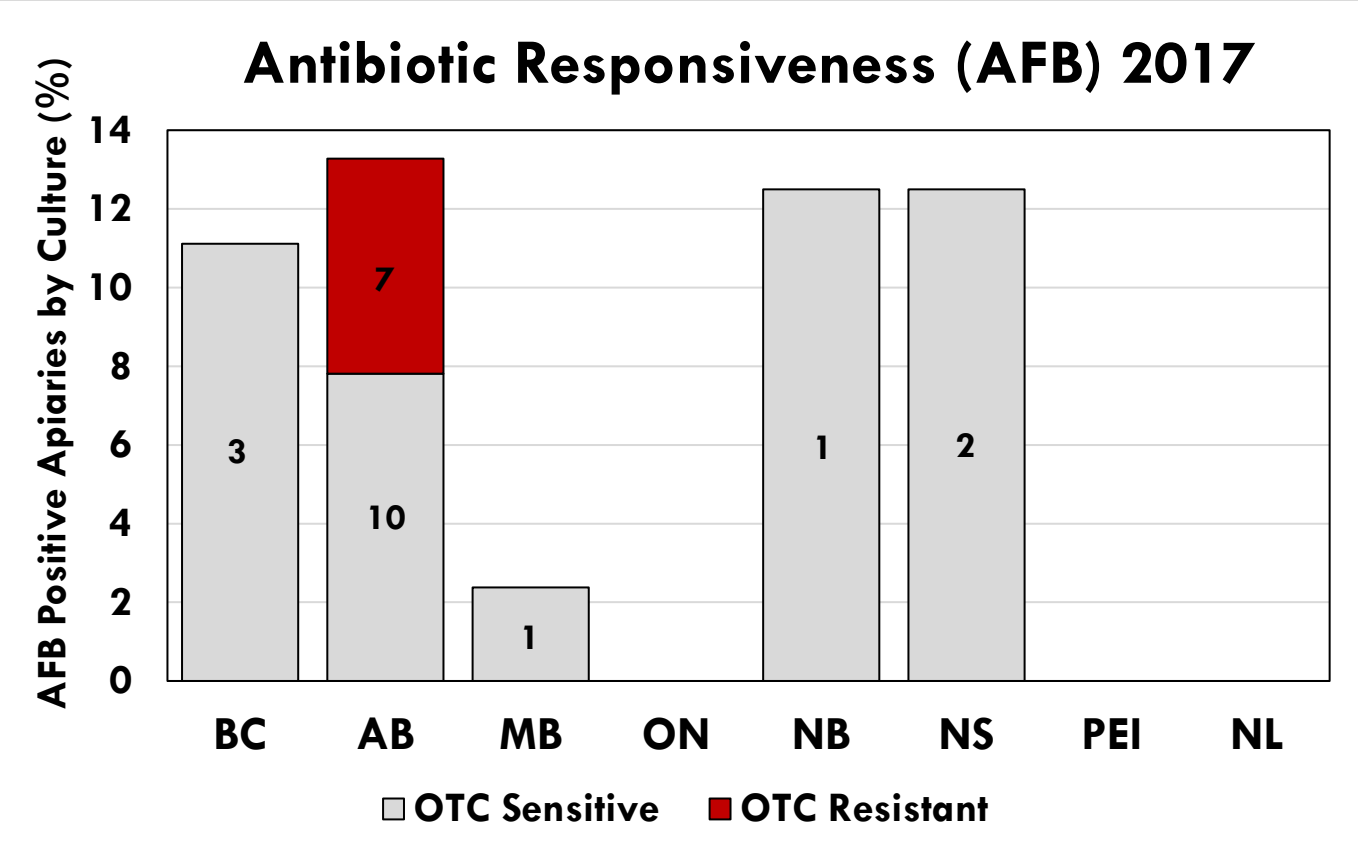

Figure 13. Samples for which AFB could be cultivated were further analyzed for resistance or sensitivity to Oxytetracycline (OTC) and Tylosin*, which are registered for the control of AFB in Canada. The graph shows the incidence of AFB positive samples with the height of each bar (similar to the AFB risk level graph on the previous page), but differentially displays the proportion that were sensitive or resistant to OTC. The number of apiary samples the proportion represents is noted inside each bar segment.

*All samples positive for AFB were sensitive to the antibiotic Tylosin.

EFB (PCR Detection)

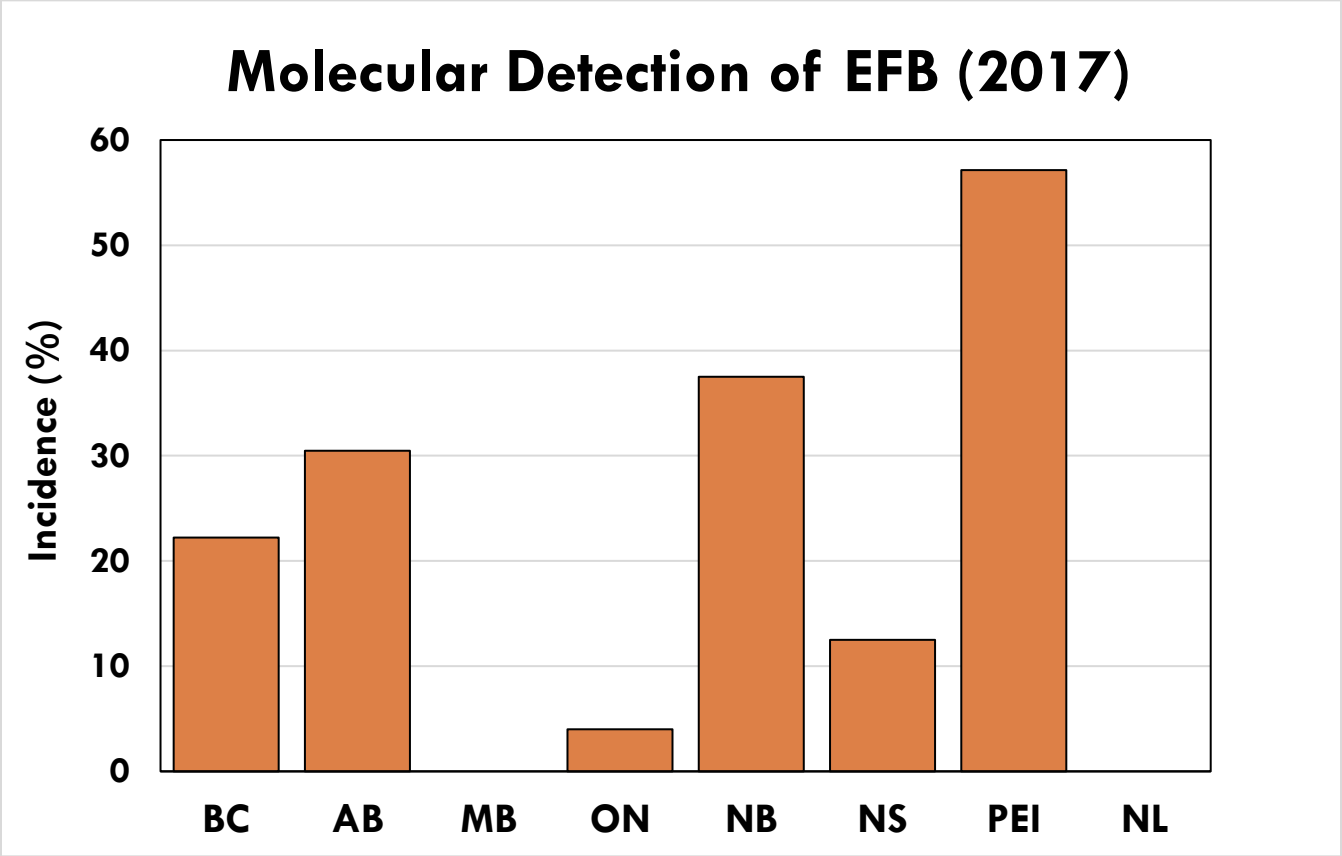

Figure 14. Incidence of EFB per province for 2017, detected molecularly by PCR*.

**Positive detection by PCR does not conclusively diagnose an active condition within the apiary.*

Tracheal Mites (PCR Detection & Dissection)

In the **2017 Survey,** tracheal mites **were not detected** in any samples from **Ontario, Nova Scotia** or **Newfoundland.**

In **Alberta: 3/128, British Columbia: 2/27, Manitoba: 1/42, New Brunswick: 1/8 and Prince Edward Island: 1/7 samples** tested **positive** for tracheal mites by PCR.

Only 1 from Alberta was confirmed by dissection.

Tropilaelaps (Microscopy)

Tropilaelaps specimen have not been identified in any samples from the Survey, for any year (2014-2017).

Apis cerana (Microscopy, PCR-RFLP Assay)

No *Apis cerana* specimen were detected in any samples from the 2017 Survey.

African Ancestry Testing

PCR-RFLP ASSAY

mtDNA of African origin was detected in 22 composite samples from 5 Provinces.

Province	Positive Samples	Incidence
British Columbia	5 of 27 Apiaries	18.5%
Alberta	6 of 128 Apiaries	4.7%
Manitoba	6 of 42 Apiaries	14.3%
Ontario	4 of 25 Apiaries	16.0%
Prince Edward Island	1 of 7 Apiaries	14.3%
NATIONAL TOTAL	**22 of 255 Apiaries**	**8.6%**

SNP ANALYSIS

A second subset of DNA from individual bees positive for African ancestry by PCR-RFLP assay was sent to the Genome Quebec facility at McGill University for Single Nucleotide Polymorphism (SNP) analysis.

SNP sequencing data for all 22 composite samples positive by PCR-RFLP Assay ranged from **0 -11.5% African ancestry**. These values fall well below the 25% threshold instituted by Dr. Zayed and collaborators above which bees are considered Africanized. These values are also consistent with the range found through other recent analyses of Canadian bee stock by Dr. Zayed's group.

Viral Incidence (PCR Detection)

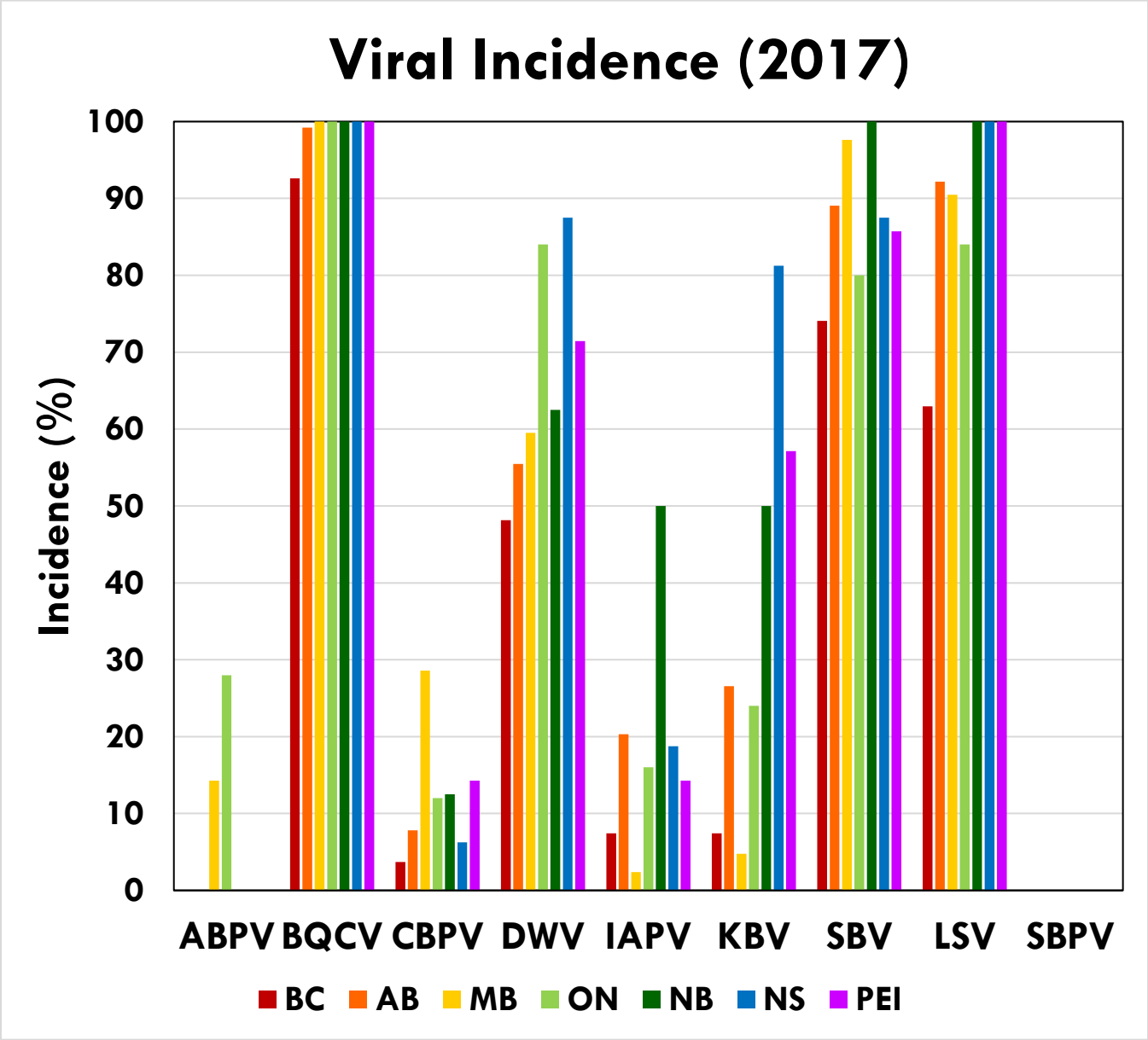

Figure 15. Viral incidence per province for 2017 detected by PCR; apiaries were scored as 'Positive' for any detection level of the virus or 'Negative' for the absence of the virus.

**Positive detection by PCR does not conclusively diagnose an active condition within the apiary.*

Results for NL are unable to be provided for Viral Detections to maintain beekeeper confidentiality due to the low sample size (n=2).

Chemical Residue Testing (LC-MS/MS)

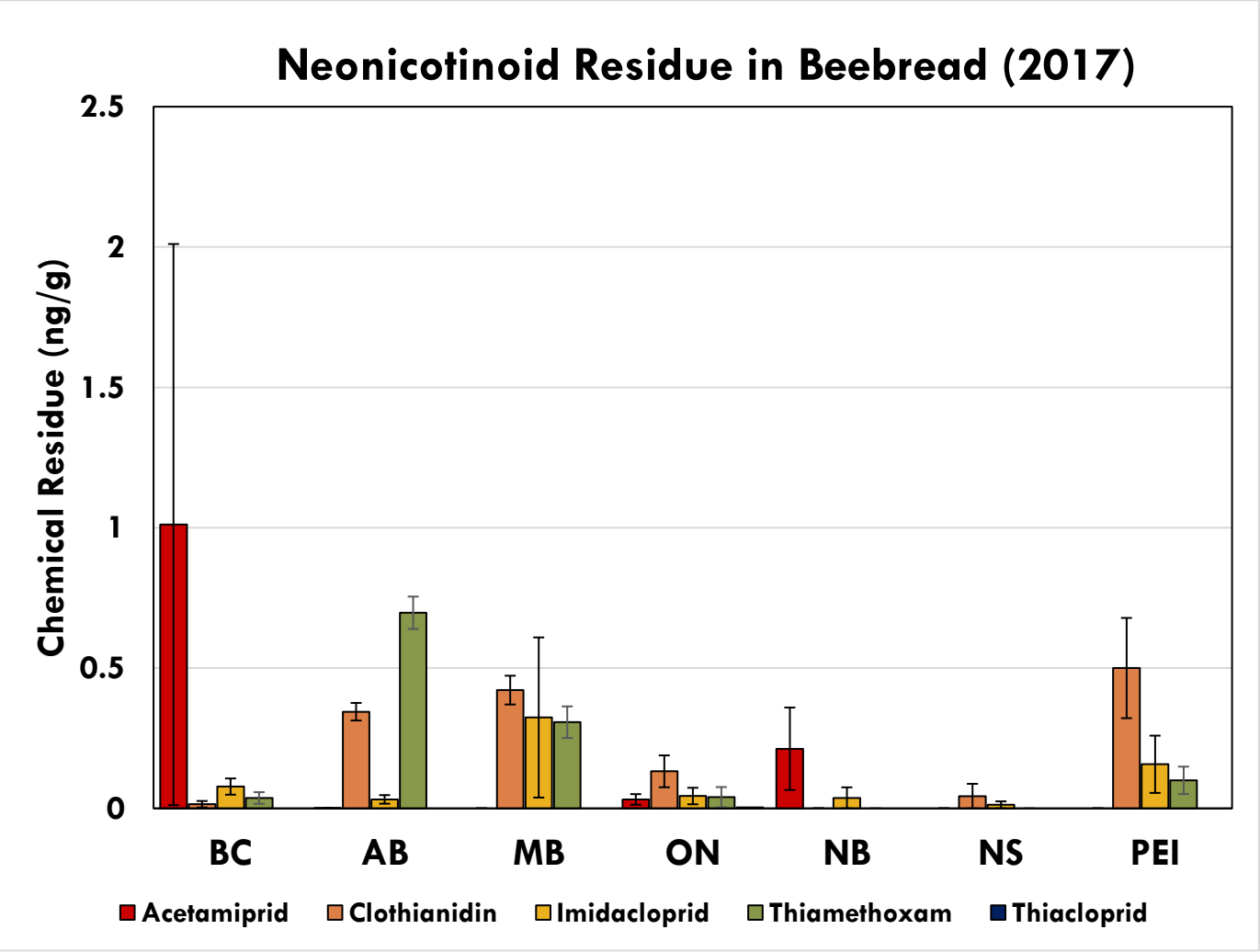

Figure 16: Average chemical residue detected by liquid chromatography coupled to tandem quadruple mass spectrometry (LC-MS/MS) per province, represented as ng/g of bee bread.

Results for NL are unable to be provided for Chemical Residues because bee bread samples were not collected.

Notes

Sample Size

The protocol for Year Four (2017) projected sampling in all Provinces. This goal was not met as samples were not collected from Saskatchewan or Quebec.

In several provinces, complete composite apiary samples were unable to be collected due to unexpected circumstances. In British Columbia: 2/270, Manitoba: 2/420, Ontario: 7/250, New Brunswick: 4/80 and Nova Scotia: 11/160 colonies were missed due to weak colony conditions the day of sampling or inclement conditions.

Only 2 samples were collected from Newfoundland. As a result, some data was not released to maintain beekeeper confidentiality; bee bread samples were also missing for this province.

Testing Limitations

PCR: The use of PCR is an effective diagnostic technique, but is also very sensitive. Therefore, a *positive detection using the technique does not conclusively diagnose an active or overt condition.* Specifically, PCR detection of EFB and the viral panel require further development, such as quantitative PCR, to more accurately associate positive detections with possible clinical symptoms in an apiary.

African Ancestry Testing: Testing for African Ancestry in honey bees is an evolving technique; the current diagnostic standard for the detection of AHB by the Canadian Food Inspection Agency (CFIA) and the US Animal and Plant Health Inspection Service (APHIS) uses a PCR-based RFLP assay. This method targets mtDNA genes, but because mtDNA is maternally inherited, this analysis fails to detect progeny of European queens mated with Africanized drones.

Even though it has inherent limitations, this method is recognized as the current diagnostic standard for the detection of AHB. It is also used as the standard technique by which queen breeding apiaries are certified as being free of the sub-Saharan type of AHB. Specifically, this test is an export certification requirement that CFIA imposes on California breeders that ship queens to Canada.

As described previously, an emerging technique using a Single Nucleotide Polymorphism (SNP) analysis has been developed out of York University (Zayed et al.) to identify AHB by using both maternal and paternal inherited nuclear markers to distinguish between AHB and EHB.

Although positive samples were identified in the Survey using the current diagnostic standard (PCR-RFLP Assay), the NBDC is not aware of any highly-defensive behavior or incidents involving bees from the affected apiaries.

As defensive behavior is primarily a paternal effect, progeny from these colonies are likely to retain typical EHB behaviors when mated with European drones. It is plausible that the African ancestry identified in these hives was introduced through importation of stock and maintained, possibly for several generations, if queens were not artificially replaced by beekeepers.

Our results clearly show the limitations of the current method used to identify AHB colonies, thus research and development of a more informative AHB detection method, such as the SNP-based techniques, is required.

Summary Points

- Chalkbrood was documented at noticeably higher levels in the Maritime provinces (NB, NS, PEI); all three provinces had an incidence 21% and above. All other provinces fell below 7%.
- *Nosema* was detected in 20 of the 24 provincial regions included in the Survey. Only four BC regions (Kootenay, Northwest, Okanagan and Thompson/Cariboo) did not show presence of *Nosema*. The highest average level of spores provincially was reported in NB with ~1.37 million spores/bee and the lowest level was found in Newfoundland.
- *Nosema ceranae* was the most prevalent species detected in all provinces in 2017. In addition, *Nosema ceranae* has been the most common species found in Canada every year of the Survey; *Nosema apis* was not identified as a single infection this year.
- Newfoundland was found to be *Varroa*-free again this year. *Varroa* was detected in all other regions sampled in 2017, with provincial infestation levels ranging from 0.8% in AB to 1.9% in BC.
- Upon visual inspection, AFB was only identified in AB in 3 colonies from 1 apiary and in 1 colony in NS. When cultivated in the lab from adult honey bees, AFB was detected in samples from 8 of the 24 regions nationwide. High risk (>1,000 CFU) samples by culture were identified in BC, AB, and MB.
- AFB positive samples from AB were the only cases that indicated resistance to the antibiotic Oxytetracycline, 7 samples in total. All AFB positive samples were sensitive to the antibiotic Tylosin.
- EFB was detected molecularly in every province except MB and NL, ranging from 4% incidence in ON to 57% incidence in PEI.
- Tracheal mites have been detected molecularly in samples from 2015-2017, but this was the first year they were confirmed by dissection in one sample from AB.
- *Tropilaelaps* have not been identified in any samples collected for the Survey.
- Exotic pest, *Apis cerana* was not identified in any samples in 2017.
- Using the current method recognized for the detection of AHB and certification of queen exports by the CFIA, 22 apiary level samples tested positive for African Ancestry from BC, AB, MB, ON, and PEI. This analysis uses mtDNA that only reflects maternal genetics. Further analysis using a new technique that accounts for both maternal and paternal genetics indicated that all 22 positive samples identified initially, show low levels of African ancestry. Genetic sequencing to identify AHB with this technique sets 25% as the threshold instituted above which bees are considered Africanized. None of the samples met this threshold; samples ranged from 0% to 11.5%.
- The most prevalent viruses detected in the Survey were Black Queen Cell Virus (BQCV) and Sacbrood Virus (SBV). Conversely, Acute Bee Paralysis Virus (ABPV) was entirely absent in samples from BC, AB, NB, NS, PEI, and NL. Lake Sinai Virus was added to the panel this year and

with the exception of BC at 63%, all other provinces had an incidence >84%. Slow Bee Paralysis Virus (SBPV) was another new addition to the panel for 2017, and was not identified in any samples.

- Analysis of neonicotinoids identified the presence of Clothianidin in 63% of samples (0.4±0.03 ppb), Thiamethoxam in 62% of samples (0.7±0.05 ppb), Imidacloprid in 12% of samples (0.8±0.39 ppb), Acetamiprid in 3% of samples (3.7±3.33 ppb) and only 1 case of Thiacloprid. On average, these levels are below those normally associated with sub-lethal effects on honey bees, though a small number of elevated levels were found in specific beekeeping operations.

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