CANADIAN NATIONAL HONEY BEE HEALTH SURVEY

Photo Credit: Christy Curran

2016 REPORT

British Columbia, Alberta, Manitoba, Ontario, Quebec, New Brunswick, Nova Scotia, Prince Edward Island, Newfoundland & Labrador, and Yukon Territory

Canada

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2016 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 will complete its first phase 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 (GPRC NBDC-TAC).

The purpose of this project, the first of its kind in Canada, is 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 are collected from across Canada- with a goal of sampling 0.5% of registered hives.

The Survey was designed to systematically expand across the country, starting in Alberta and Manitoba the first year, and to conclude its fourth year fully national in scope with over 350 samples from all Provinces.

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 & Labrador. Samples from the Yukon Territories were also received, resulting in samples from 314 apiaries.

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

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Glossary

AAFC	Agriculture & Agri-Food Canada
AB	Alberta
ABPV	Acute Bee Paralysis Virus
AFB	American Foulbrood
AHB	Africanized Honey Bees
BC	British Columbia
BQCV	Black Queen Cell Virus
CBPV	Chronic Bee Paralysis Virus
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
MB	Manitoba
mtDNA	Mitochondrial DNA
NB	New Brunswick
NBDC	National Bee Diagnostic Centre
NL	Newfoundland & Labrador
NS	Nova Scotia
ON	Ontario
OTC	Oxytetracycline
QC	Quebec

Glossary con't

PEI	Prince Edward Island
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment-Length Polymorphism
RNA	Ribonucleic Acid
SBV	Sacbrood Virus
SNP	Single Nucleotide Polymorphism
TAC	Technology Access Centre
YT	Yukon Territory

Survey Methodology

YEAR 3: During the Summer of 2016, 314 apiary samples were collected, representing 3,097 colonies. All diagnostic tests were performed at the GPRC NBDC-TAC in Beaverlodge, Alberta.

Apiary Sampling: Samples were collected between July and mid-September 2016 - before fall treatments for *Varroa* and *Nosema* were applied. Sample technicians in each province were employees or contractors of the GPRC 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, Centre de Recherche en Sciences Animales de Deschambault (CRSAD) and the Atlantic Tech Transfer Team for Apiculture (ATTTA) each provided personnel support for sampling.

3 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 and analysis of 7 honey bee viruses.
New for 2016, samples were tested for 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.

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

British Columbia	**30 Total Samples**
Fraser Valley	9 Samples
Kootenay	3 Samples
Northwest	3 Samples
Okanagan	5 Samples
Peace	3 Samples
Thompson/Cariboo	4 Samples
Vancouver	3 Samples
Alberta	**138 Total Samples**
Central	14 Samples
Northeast	14 Samples
Northwest	34 Samples
Peace	35 Samples
South	41 Samples
Manitoba	**39 Total Samples**
Central	10 Samples
Eastern Interlake	10 Samples
Northwest	9 Samples
Southern	10 Samples

Ontario	**30 Total Samples**
Central	3 Samples
Southeast	5 Samples
Southwest	22 Samples
Quebec	**35 Total Samples**
Capital	18 Samples
Northeast	6 Samples
Northwest	3 Samples
Southwest	8 Samples
New Brunswick	**11 Total Samples**
North	5 Samples
South	6 Samples
Nova Scotia	**14 Total Samples**
Central	9 Samples
Western	5 Samples
Prince Ed. Island	**8 Total Samples**
East	4 Samples
West	4 Samples
Newfoundland	**5 Total Samples**
Provincial	5 Samples
Yukon Territory	**4 Total Samples**
Territory	4 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 (Oxytet) 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 (PCR) 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 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.

A blind subset of this DNA was also sent to the University of Guelph's Honey Bee Research Centre to verify results. This analysis indicates a positive or negative detection of mtDNA with African origin.

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

African Ancestry Testing Con't:

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.

Viral Detection: RNA was extracted from 60 bees, converted into cDNA and analyzed for 7 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), and Sacbrood Virus (SBV). Apiaries were scored as "Positive" for any detection level of the virus or "Negative" for the absence of the virus.

Provincial/Territory 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 3 Regions: Central, Southwest and Southeast.

QUEBEC

Figure 5. Provincial map of Quebec, includes 4 Regions: Northeast, Northwest, Southwest and Capital.

NEW BRUNSWICK

Figure 6. Provincial map of New Brunswick, includes 2 Regions: North and South.

NOVA SCOTIA

Figure 7. Provincial map of Nova Scotia, includes 2 Regions: Western and Central.

PRINCE EDWARD ISLAND

Figure 8. Provincial map of Prince Edward Island, includes 2 Regions: West and East.

NEWFOUNDLAND AND LABRADOR/ YUKON TERRITORY

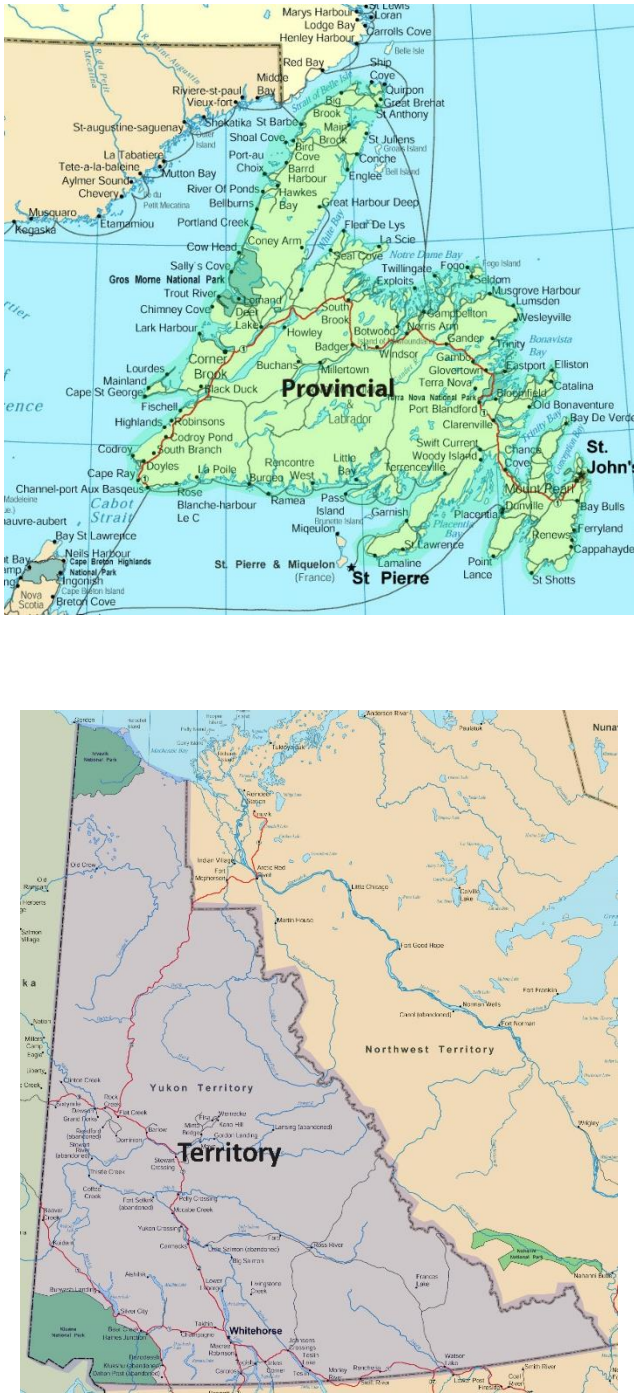

Figure 9. Provincial/territory maps of Newfoundland & Labrador and the Yukon Territory, both analyzed as a single entity- not enough samples for regional distribution.

Results

Visual Inspection (Incidence)

Disease/ Condition	BC n=300	AB n=1380	MB n=390	ON n=296*	QC n=350	NB n=102*	NS n=132*	PEI n=79*	NL n=50	YT n=18*	National Average n=3097
AFB	0	0.5	0	0	0	0	0	0	0	0	0.2%
EFB	0.7	0.1	0	0.7	2.0	2.9	0.8	1.3	0	0	0.6%
Sacbrood	1.3	0.9	0.3	0.3	0	0	0.8	0	0	0	0.6%
Chalkbrood	5.0	5.5	5.9	3.0	2.0	31.4	40.9	38.0	0	5.6	7.9%
Deformed Wing Bees	10.3	2.8	1.3	2.7	1.7	4.9	0.8	5.1	0	11.1	3.3%
Black Shiny Bees	6.7	0.3	2.6	0.3	0.6	0	0	3.8	0	0	1.3%
Small Hive Beetle (larvae or adults)	0	0	0	0	0	0	0	0	0	0	0.0%
Wax Moth (larvae or adults)	1.0	0	0.5	0.7	0	0	0	0	0	0	0.2%
Queen Cells Present	5.0	9.8	6.7	2.7	0.6	0	1.5	3.8	6.0	0	6.3%
Drone Laying Queen	0.7	1.3	1.5	0.3	0	0	0	0	2.0	0	0.9%

Table 1. Visual inspection results for each province/territory 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 (Regional & Provincial Incidence)

British Columbia	**27% Incidence**	**Quebec**	**51% Incidence**
Fraser Valley	4/9	Capital	8/18
Kootenay	0/3	Northeast	2/6
Northwest	0/3	Northwest	3/3
Okanagan	1/5	Southwest	5/8
Peace	1/3	**New Brunswick**	**81% Incidence**
Thompson/Cariboo	2/4	North	4/5
Vancouver	0/3	South	5/6
Alberta	**66% Incidence**	**Nova Scotia**	**57% Incidence**
Central	10/14	Central	6/9
Northeast	6/14	Western	2/5
Northwest	15/34	**Prince Ed. Island**	**75% Incidence**
Peace	22/35	East	3/4
South	38/41	West	3/4
Manitoba	**51% Incidence**	**Newfoundland**	**80% Incidence**
Central	6/10	Provincial	4/5
Eastern Interlake	6/10	**Yukon Territories**	**25% Incidence**
Northwest	3/9	Territory	1/4
Southern	5/10	**National Level**	56% Incidence
Ontario	**37% Incidence**	**National Total**	176/314
Central	3/3		
Southeast	1/5		
Southwest	7/22		

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

Nosema (Counting)

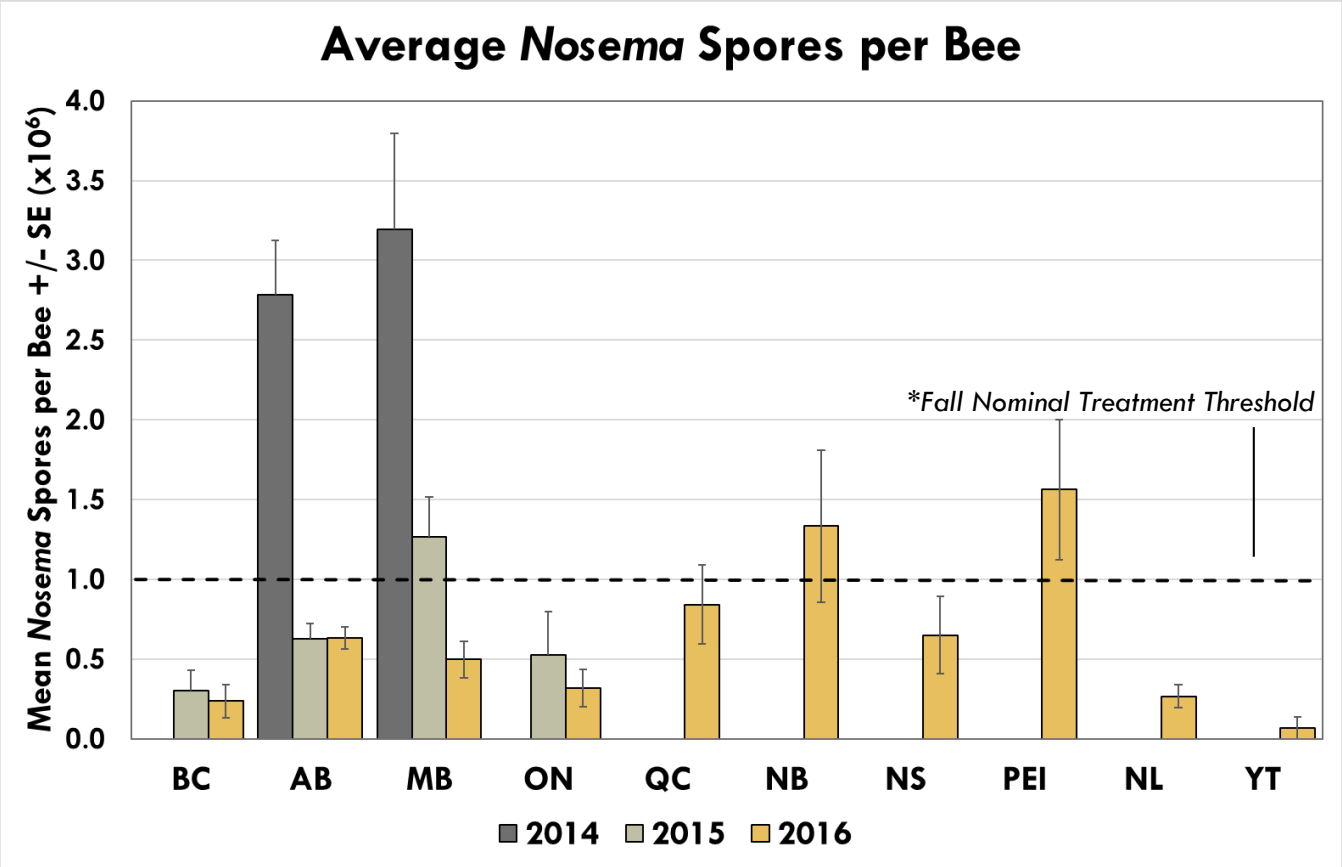

Figure 10: Average *Nosema* spore count per bee, enumerated with a haemocytometer under light microscopy (400x); reported by provincial/territory average for 2014, 2015 and 2016, when possible. The average *Nosema* spore count is represented in millions of spores per bee.

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

Nosema (Species Identification)

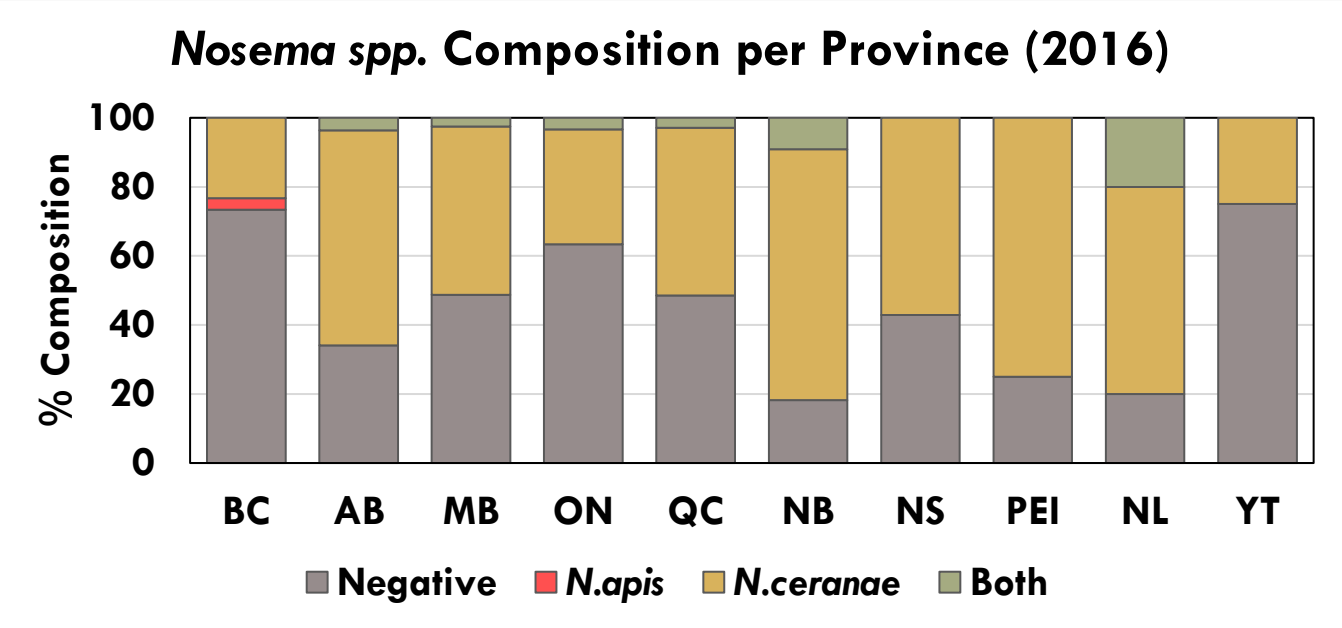

Figure 11. *Nosema* spp. composition by province/territory in 2016 detected by DNA extraction and PCR.

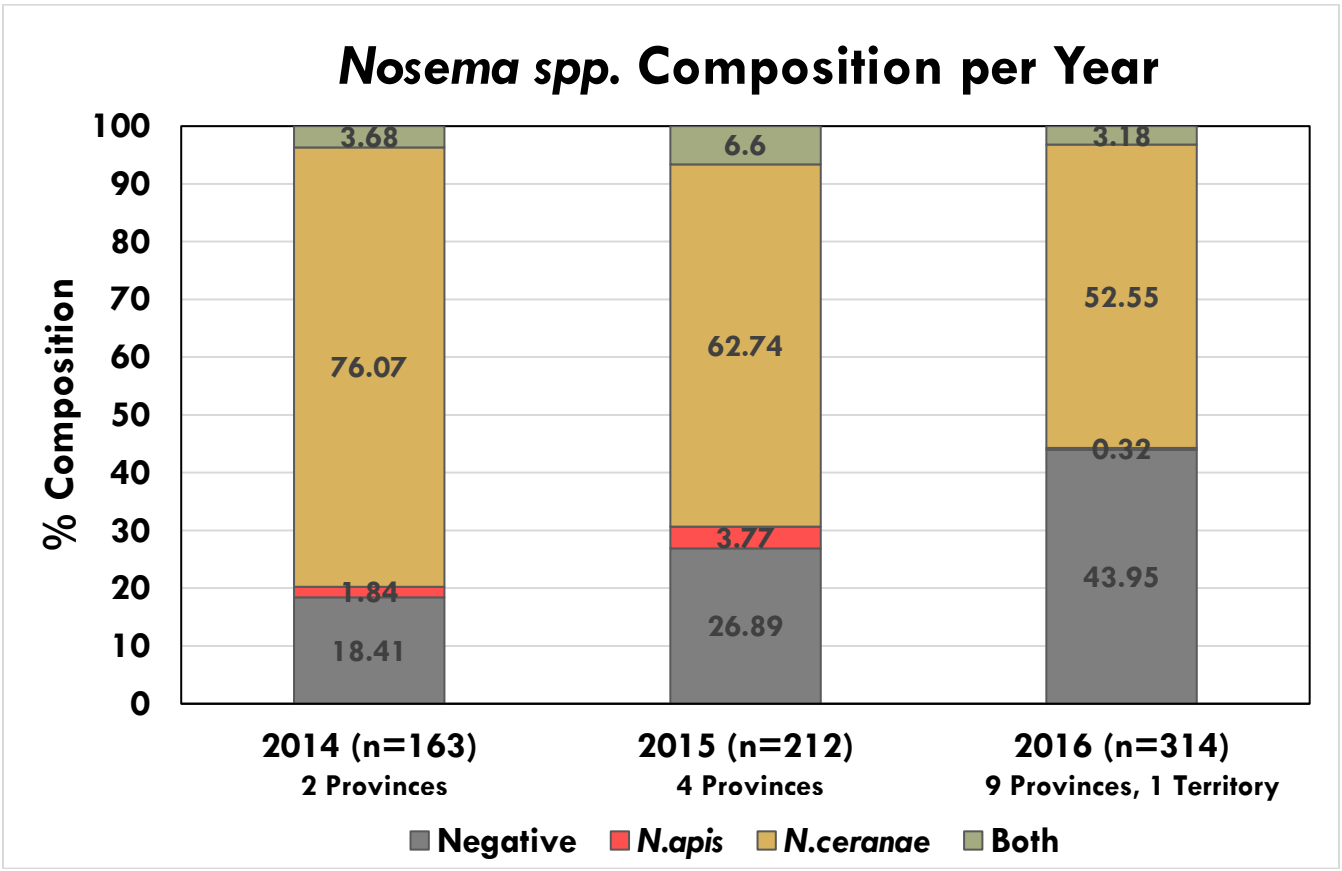

Figure 12. *Nosema* spp. composition by year detected by DNA extraction and PCR.

Varroa (Regional & Provincial Incidence)

British Columbia	**93% Incidence**	**Quebec**	**91% Incidence**
Fraser Valley	9/9	Capital	16/18
Kootenay	3/3	Northeast	6/6
Northwest	3/3	Northwest	2/3
Okanagan	4/5	Southwest	8/8
Peace	2/3	**New Brunswick**	**45% Incidence**
Thompson/Cariboo	4/4	North	2/5
Vancouver	3/3	South	3/6
Alberta	**85% Incidence**	**Nova Scotia**	**71% Incidence**
Central	12/14	Central	6/9
Northeast	10/14	Western	4/5
Northwest	29/34	**Prince Ed. Island**	**88% Incidence**
Peace	32/35	East	3/4
South	34/41	West	4/4
Manitoba	**97% Incidence**	**Newfoundland**	**0% Incidence**
Central	10/10	Provincial	0/5
Eastern Interlake	9/10	**Yukon Territories**	**50% Incidence**
Northwest	9/9	Territory	2/4
Southern	10/10	**National Level**	**84% Incidence**
Ontario	**87% Incidence**	**National Total**	265/314
Central	1/3		
Southeast	5/5		
Southwest	20/22		

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

Varroa (Counting)

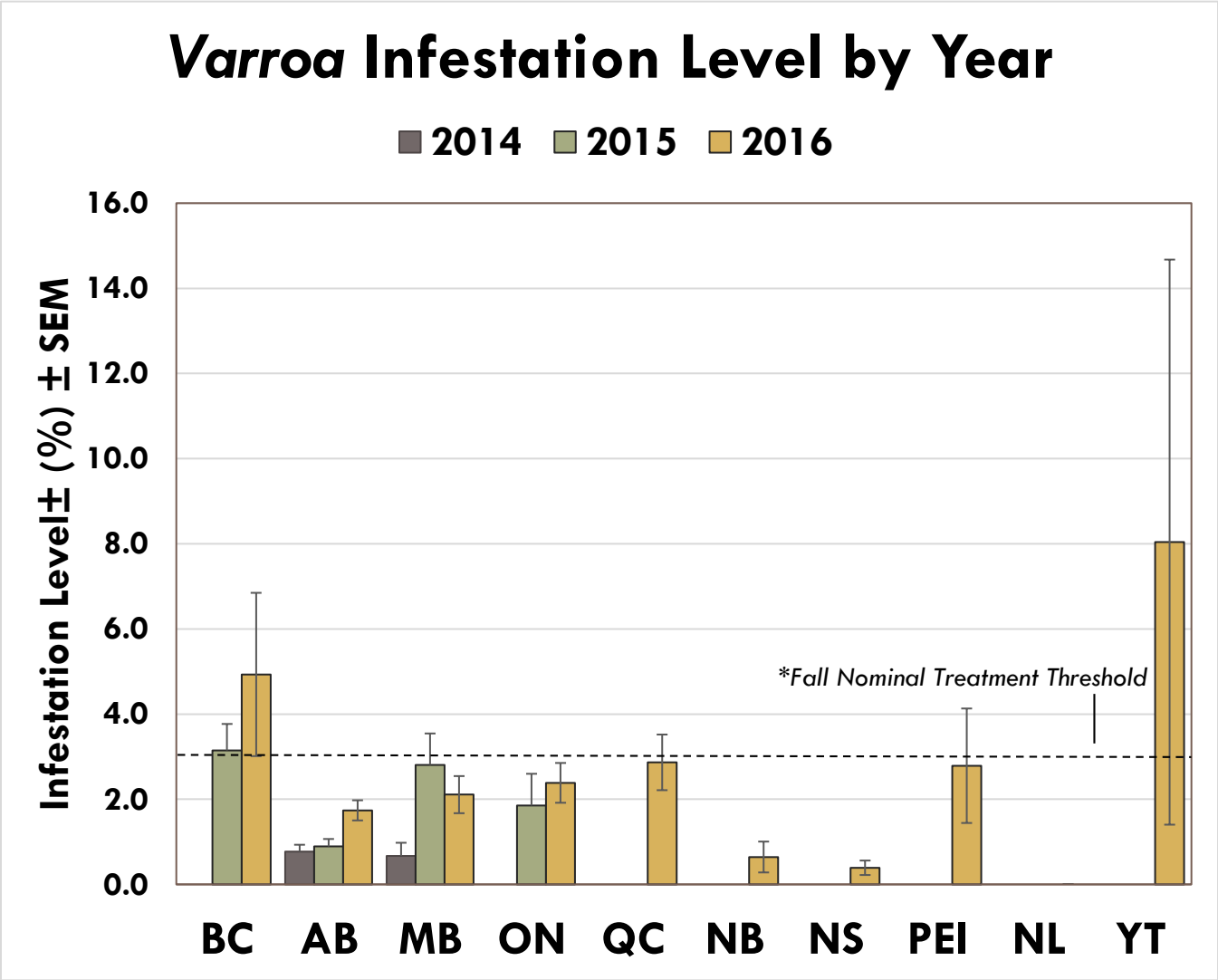

Figure 13. Average *Varroa* infestation level per province/territory, expressed as the number of mites per 100 adult bees (%).

*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	**10% Incidence**	**Quebec**	**3% Incidence**
Fraser Valley	1/9	Capital	0/18
Kootenay	0/3	Northeast	0/6
Northwest	0/3	Northwest	0/3
Okanagan	0/5	Southwest	1/8
Peace	0/3	**New Brunswick**	**9% Incidence**
Thompson/Cariboo	0/4	North	0/5
Vancouver	2/3	South	1/6
Alberta	**22% Incidence**	**Nova Scotia**	**21% Incidence**
Central	2/14	Central	3/9
Northeast	1/14	Western	0/5
Northwest	8/34	**Prince Ed. Island**	**38% Incidence**
Peace	12/35	East	2/4
South	7/41	West	1/4
Manitoba	**3% Incidence**	**Newfoundland**	**0% Incidence**
Central	0/10	Provincial	0/5
Eastern Interlake	1/10	**Yukon Territories**	**0% Incidence**
Northwest	0/9	Territory	0/4
Southern	0/10	**National Level**	**13% Incidence**
Ontario	**0% Incidence**	National Total	42/314
Central	0/3		
Southeast	0/5		
Southwest	0/22		

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

AFB (Bacterial Culture-Adult Bees)

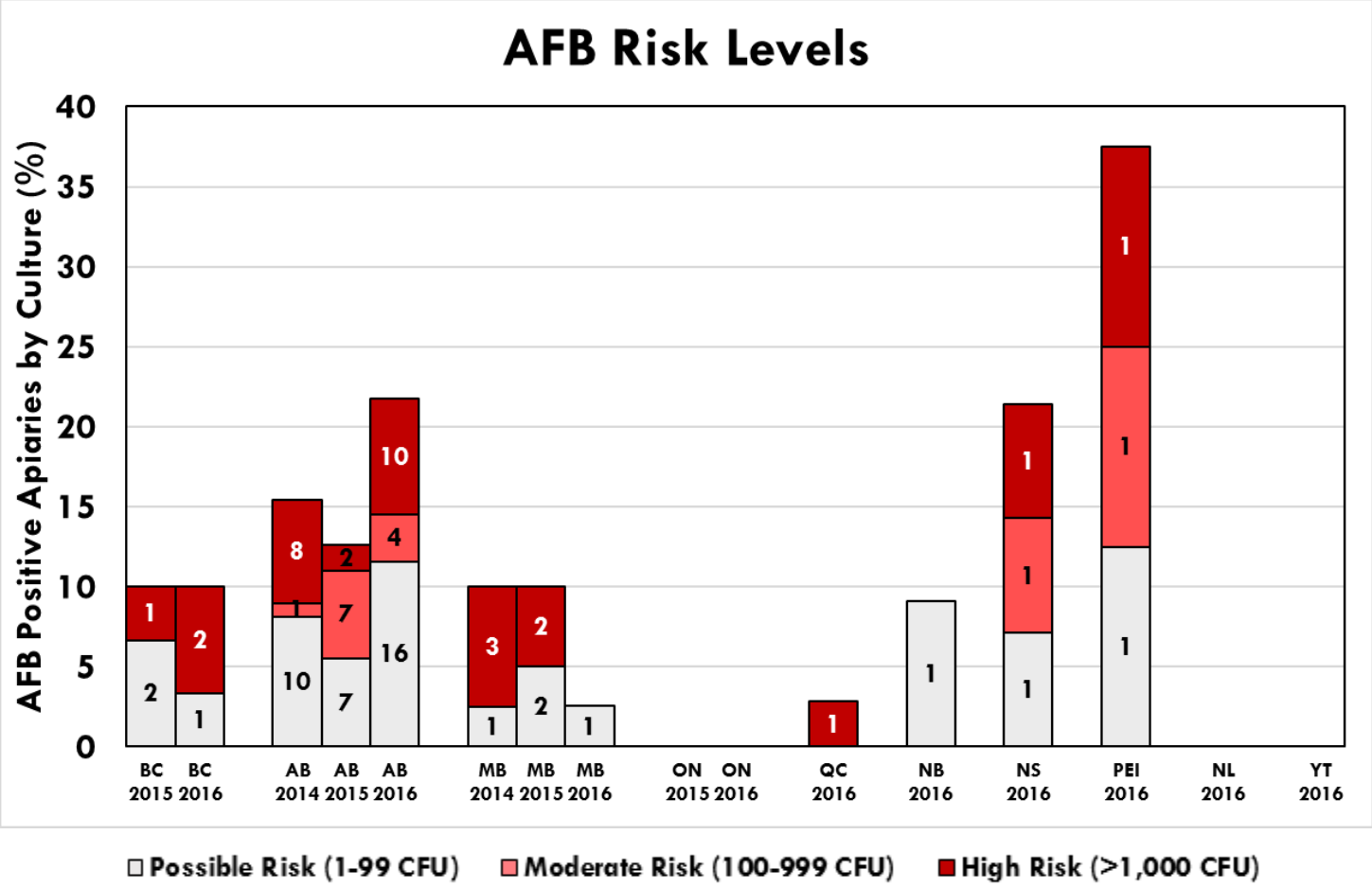

Figure 14. Based on previous research*, AFB positive apiary samples were categorized into 3 groups for their propensity to develop clinical symptoms of the disease. Risk levels were designated based on the average number of CFUs that grew on the diagnostic media plates: Possible Risk (1-99 CFU), Moderate Risk (100-999 CFU) and High Risk (>1,000 CFU). The proportion of apiaries affected in each province/territory is shown by the bar height, the number of apiary samples this represents is noted inside each bar segment. When possible, results for multiple years are provided.

* 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)

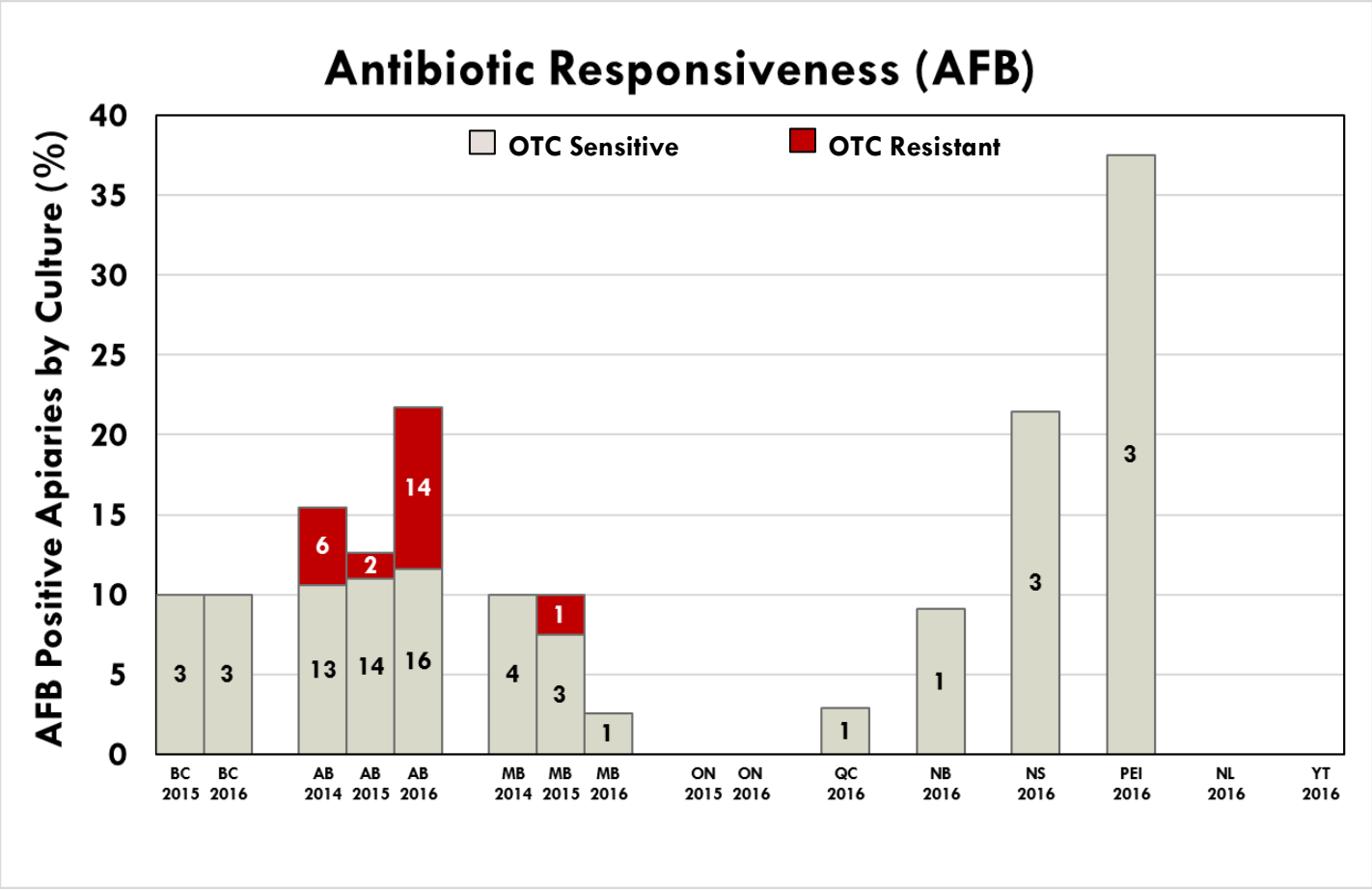

Figure 15. Samples for which AFB could be cultivated were further analyzed for resistance or sensitivity to Oxytetracycline (OTC) and Tylosin*, which are registered for the control of AFB in Canada. The graph shows the incidence of AFB positive samples with the height of each bar (similar to the AFB 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. When possible, results for multiple years are provided.

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

EFB (PCR)

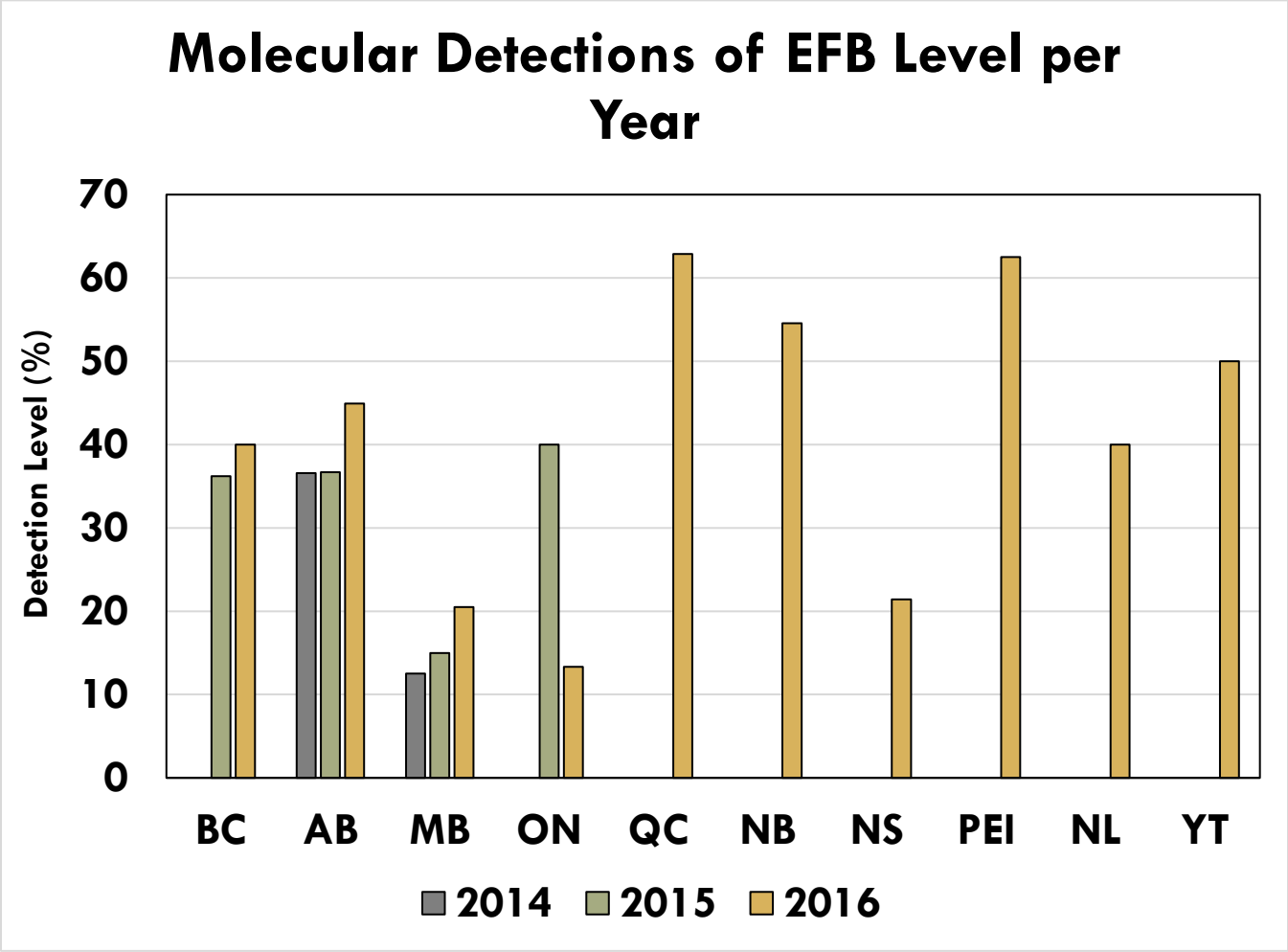

Figure 16. Molecular detection of EFB per province in 2014, 2015, and 2016, when possible, detected by PCR*.

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

Tracheal Mites (PCR & Dissection)

In the **2014 Survey,** tracheal mites were not detected in any samples from **Alberta** and **Manitoba.**

In the **2015 Survey,** tracheal mites were not detected in any samples from **British Columbia** or **Ontario.**
In **Alberta: 6/127** and in **Manitoba: 1/40 samples** tested **positive** for tracheal mites by PCR - but not confirmed by dissection.

In the **2016 Survey,** tracheal mites were not detected in any samples from **Manitoba, Ontario, Nova Scotia, Prince Edward Island, Newfoundland & Labrador** or **Yukon Territories.**
In **Alberta: 7/138, British Columbia: 3/30, New Brunswick: 1/11 and Quebec: 1/35 samples** tested **positive** for tracheal mites by PCR - but not confirmed by dissection.

Tropilaelaps (Microscopy)

Tropilaelaps specimen have not been identified in any samples from the Survey, for any year (2014-2016).

African Ancestry Testing

PCR-RFLP ASSAY

mtDNA of African origin was detected in 26 composite samples from 5 Provinces and 1 Territory.

Province/Territory	Positive Samples
British Columbia	3 of 30 Apiaries
Alberta	4 of 138 Apiaries
Manitoba	4 of 39 Apiaries
Ontario	5 of 30 Apiaries
Quebec	9 of 35 Apiaries
Yukon Territory	1 of 4 Apiaries
NATIONAL TOTAL	**26 of 314 Apiaries**

SNP ANALYSIS

SNP sequencing data for all 26 composite samples positive by PCR-RFLP Assay ranged from **0.6 -15.9% (average 5.6%) 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)

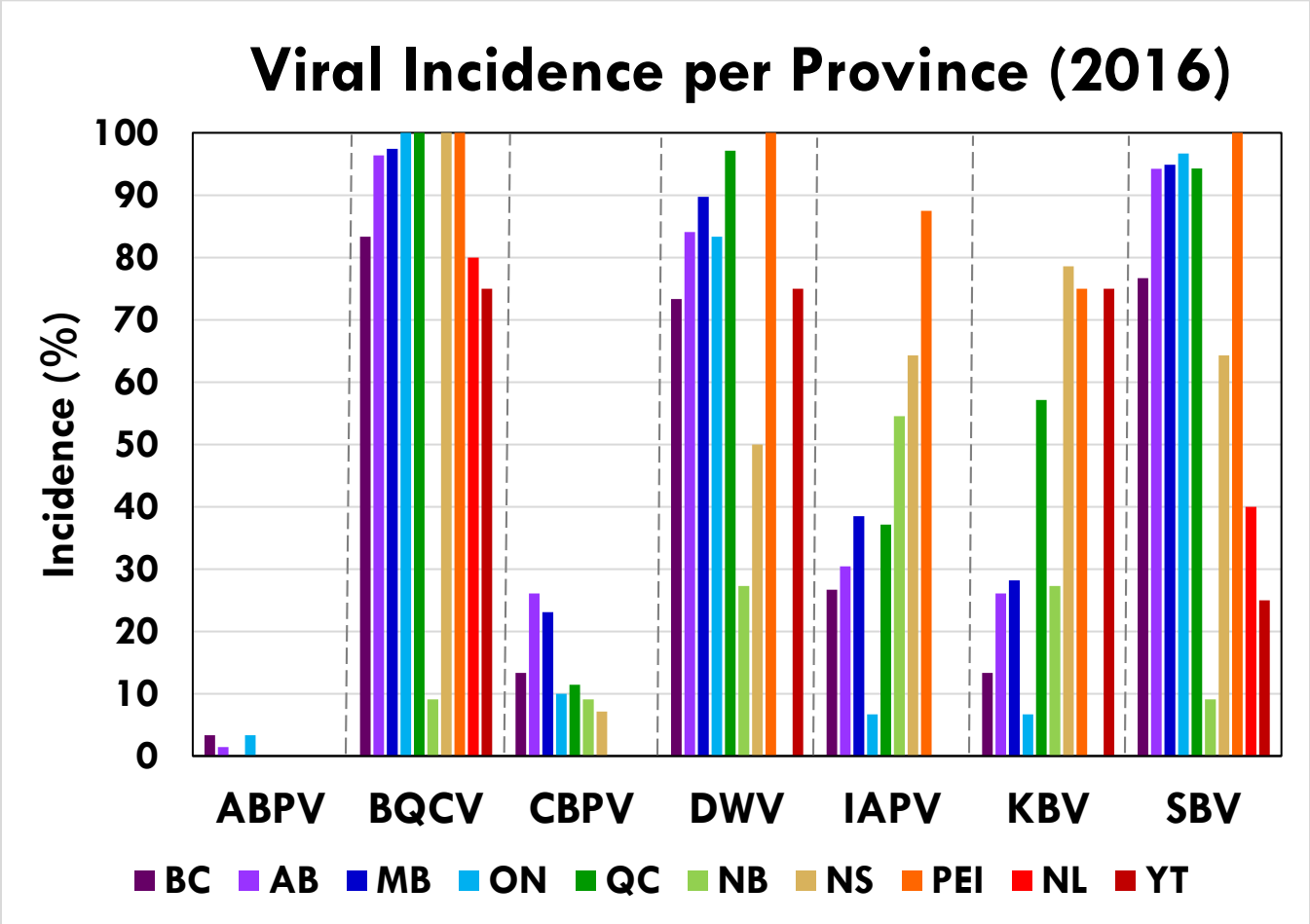

Figure 17. Viral incidence per province for 2016 detected from extracted RNA, converted into cDNA and PCR method; apiaries were scored as 'Positive' for any detection level of the virus or 'Negative' for the absence of the virus.

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

Notes

Sample Size

The protocol for Year Three (2016) projected sampling in British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Quebec, New Brunswick, Nova Scotia, and Prince Edward Island. This goal was met with the exception of Saskatchewan; Saskatchewan Beekeepers Association declined to participate in the Survey, consistent with their response in 2015.

In several provinces, complete composite apiary samples were unable to be collected due to unexpected circumstances. In New Brunswick: 8/110 colonies; Nova Scotia: 8/140 colonies; and Ontario: 4/300 colonies were missed due to weak colony conditions the day of sampling. In Prince Edward Island: 1/80 colonies was missed due to inclement weather conditions. In the Yukon Territory, only 18/40 colonies were collected from as commercial beekeeping in this area has been newly developed.

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-TAC 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.

Discussion/Summary

- Chalkbrood was documented at noticeably higher levels in the Maritime provinces (NB, NS, PEI); all three provinces had an incidence 31% and above. All other provinces fell below 6%.

- *Nosema* was detected in 28 of the 31 provincial/territory regions included in the Survey. Only three BC regions (Kootenay, Northwest and Vancouver) did not show presence of *Nosema*. The highest average level of spores provincially was reported in PEI with ~1.5 million spores/bee and the lowest level was found in the Yukon Territory with ~50,000 spores/bee. *Nosema* infection in the provinces sampled multiple years (BC, AB, MB and ON) have declined overall since the Survey started in 2014.

- *Nosema ceranae* was the most prevalent species detected in all provinces/territories in 2016. In addition, *Nosema ceranae* has been the most common species found in Canada every year of the Survey; *Nosema apis* was only identified in one sample as a single infection this year, from British Columbia. Results from 2016 record the first documented findings of *N. ceranae* in Newfoundland & Labrador.

- Newfoundland & Labrador is the only province reported to be *Varroa*-free, based on our findings in the 2016 Survey. *Varroa* was detected in all other regions sampled in 2016, with provincial infestation levels ranging from 0.4% in NS to 8.0% in the Yukon Territory. In the provinces sampled multiple years (BC, AB, MB and ON) a trend of increasing infestation levels of *Varroa* exists.

- Upon visual inspection, AFB was only identified in AB in 7 colonies from 2 individual apiaries. When cultivated in the lab from adult honey bees, AFB was detected in samples from 13 of the 31 regions. High risk (>1,000 CFU) samples by culture were identified in BC, AB, QC, NS and PEI.

- AFB positive samples from AB were the only cases that indicated resistance to the antibiotic Oxytetracycline, 14 samples in total. Samples from AB and MB are the only provinces documented to date showing resistance to OTC. All AFB positive samples were sensitive to the antibiotic Tylosin.

- EFB was detected molecularly in every province, ranging from 13.3% incidence in ON to 62.9% incidence in QC. In the provinces sampled multiple years (BC, AB, MB and ON) a trend of increasing incidence of EFB is present, with the exception of ON.

- Tracheal mites have been detected molecularly in samples from 2015 and 2016, but never confirmed by dissection to date.

- *Tropilaelaps* have not be identified in any samples collected for the Survey.

- Using the current method recognized for the detection of AHB and certification of queen exports by the CFIA, 26 apiary level samples tested positive for African Ancestry in the 2016 Survey from BC, AB, MB, ON , QC and YT. 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 26 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 0.6% to 15.9% with an overall average of 5.6%.

- The most prevalent viruses detected in the survey were Black Queen Cell Virus (BQCV), Deformed Wing Virus (DWV) and Sacbrood Virus (SBV). Conversely, Acute Bee Paralysis Virus (ABPV) was entirely absent in samples from MB, QC, NB, NS, PEI, NL and YT.

Year Four (2017) will conclude the first phase of the Canadian National Honey Bee Health Survey. As the last year, the panel of diagnostics will expand further to include chemical residue testing of bee bread, molecular detection of 2 additional viruses (Lake Sinai Virus and Slow Bee Paralysis Virus) and presence of *Apis cerana*. In addition, results from all years will be statistically analyzed in depth to create a detailed summary of honey bee health for Canada.

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