



**NHBS**  
National Honey Bee  
Disease Survey



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## **2017 – 2018 APHIS National Honey Bee Disease Survey Summary Report**

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## Executive Summary

The 2017-2018 USDA Animal Plant and Health Inspection Service (APHIS) sponsored National Survey of Honey Bee Pests and Diseases was conducted in collaboration with the University of Maryland (UMD), the USDA Agricultural Research Service (ARS) and the cooperation of 40 U.S. states, Guam and Puerto Rico.

The National Survey began as a pilot survey of 3 states in 2009 to address the emerging concern about the diminishing health of honey bee colonies. After a successful pilot, the survey expanded the following year to include 13 states in a Limited National Survey. In subsequent years, funding for the National Survey increased, and the survey expanded to 34 states in 2011, 32 states in 2012 and 2013, 28 states in 2014, 37 states in 2015, and 40 states in 2016. This expansion has allowed us to augment and extend the national database of honey bee disease and pathogen information.

The primary focus of the APHIS National Survey is to verify the absence of potentially harmful exotic threats to honey bee (*Apis mellifera*) populations such as the parasitic mite, *Tropilaelaps* spp., and Slow Bee Paralysis Virus (SBPV), as well as exotic honey bee species such as the Asian honey bee (*Apis cerana*).

The secondary objective of the APHIS National Survey is to determine the incidence of known and established honey bee diseases and pests in the U.S., i.e. *Varroa destructor*, *Nosema* spp. and a series of viruses. Disease and pest information collected from the APHIS National Survey has been used to create a baseline level of reference, and to facilitate interpretation of ongoing and future epidemiological studies.

All of the data collected from the survey, including historic data from research institutions such as USDA ARS and other ongoing field sampling and management surveys, are incorporated into a single database, the nationwide Bee Informed Partnership (BIP) database. BIP is a non-profit 501(c)(3) and was originally funded as a 5 year USDA National Institute of Food and Agriculture (NIFA) grant. Results from the APHIS National Survey are available to the public on the BIP website (programmatic details here: <https://beeinformed.org/aphis/>, diagnostic data provided here: [https://bip2.beeinformed.org/state\\_reports/](https://bip2.beeinformed.org/state_reports/) and viral data provided here: [https://bip2.beeinformed.org/state\\_reports/viruses/](https://bip2.beeinformed.org/state_reports/viruses/)).

## Background

A National Survey of Honey Bee Pests and Diseases has been funded annually since 2009. This National Survey is being conducted to document which bee diseases, parasites, or pests of honey bees are present and/or likely absent in the U.S. Specifically, this survey has verified the absence of the parasitic mite *Tropilaelaps* spp. and other exotic threats to honey bee populations (e.g., *Apis cerana* and slow bee paralysis virus).

*Tropilaelaps* spp. is a parasitic mite native to Asia which, like *Varroa*, feeds on honey bee brood and vectors viruses (Chantawannakul et al., 2018). Its parasitic feeding vectors viruses, weakens or kills parasitized brood, and can cause infected colonies to abscond, which spreads the mites to new areas. Because of its faster reproduction cycle, *Tropilaelaps* dominates in regions where it coexists with *Varroa* (Guzman et al., 2017). Currently, there are no known *Tropilaelaps* species in the U.S.

*Apis cerana*, is a honey bee species found in southern and southeastern Asia that resembles the western honey bee (*A. mellifera*) in that they both build nests in cavities. *A. cerana* was the original host of *Varroa destructor* and *Nosema ceranae* (Fries, 1993; Rosenkranz et al., 2010). These parasites jumped species to the European honey bee (*A. mellifera*) when the European honey bee was introduced into southern Asia. *A. cerana* is well adapted to tropical climates, builds smaller colonies, and is known to swarm many times during the year. In tropical areas (e.g., Solomon Islands) *A. cerana* has been shown to outcompete *A. mellifera* in nectar and pollen gathering and exhibits a propensity for robbing European honey bee stores. Due to smaller colony size and lower honey production, *A. cerana* is not as well suited to migratory beekeeping for pollination as compared to *A. mellifera*. While *A. cerana* may pose a threat to the US beekeeping industry on its own, it also is the host of other mites that are extremely problematic when managing *A. mellifera* colonies in Asia – the *Tropilaelaps* spp. mites.

Slow bee paralysis virus (SBPV) is transmitted by *Varroa destructor*. The virus is present throughout Europe, though at a low (<2%) prevalence (de Miranda et al., 2010). When associated with high *Varroa* loads, the virus can result in increased bee and colony mortality (Carreck et al., 2010). After surveying for SBPV in the United States throughout the years 2013 to 2016, it was

determined that it is not present in the U.S. and resources were shifted to focus on the distribution of other viruses such as *Varroa destructor* Virus (VDV).

If exotic honey bee pests like *Tropilaelaps* spp. were to be introduced to the United States it would threaten managed honey bee colonies which are already facing unsustainably high colony loss rates (Kulhanek et al., 2017). With honey bees contributing approximately \$15 billion in U.S. crop production, ensuring the continued absence of those honey bee pests and disease is an issue of agricultural economics and national food security. The APHIS National Honey Bee Disease Survey's objective is to confirm the absence of certain exotic honey bee pests, and allows USDA to deny importation of honey bees from other nations unless the exporting nation can confirm absence of *Tropilaelaps* spp., and *Apis cerana*.

With sampling occurring throughout the majority of the U.S., this stratified semi-random survey offers one of the most systematic and comprehensive representation of the pests and disease levels in U.S. managed honey bee colonies and allowed for the establishment of baselines of disease prevalence and loads. Results from the first 6 years of this survey (survey years 2009-10 up to 2014-15) were published in that effect in Traynor *et al.*, 2016.

## **Scope of work and Methodology**

The 2017-2018 survey included sample collection in 40 states and two U.S. territories, Guam and Puerto Rico. The participating states were: Alabama, Arkansas, California, Colorado, Connecticut, Delaware, Florida, Georgia, Iowa, Idaho, Illinois, Indiana, Kansas, Kentucky, Massachusetts, Maryland, Michigan, Minnesota, Missouri, Montana, North Carolina, North Dakota, Nebraska, New Jersey, New Mexico, Nevada, New York, Ohio, Oregon, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Utah, Virginia, Vermont, Washington, Wisconsin, and West Virginia.

The objective of the survey is to establish a surveillance network that maximizes the chances of detecting the arrival of the exotic pests while being representative of the managed honey bee colonies of the U.S. The survey was open to any U.S. state and territory wishing to participate. Sampling is conducted under cooperative agreements between USDA APHIS and states. Samples

are collected by state apiary specialists and university scientists. Beekeeper participation within the states was voluntary and any identifiable information is confidential in any resulting report and publication.

## **Survey Description**

All states participating in the survey received kits to sample 24 apiaries within their state with the exception of California, which received 48 sample kits. Half of the 48 kits in California were used to sample 24 apiaries that remain in the state year-round and the remaining were used to sample 24 migratory beekeepers who travel to CA for the annual almond pollination.

In cooperating states, Apiary Specialists were provided guidelines to select apiaries in an attempt to give as close to an equal representation of the state as possible. Ideally, a state was divided into 4 quadrants with apiaries randomly chosen within each quadrant. Selected apiaries came from beekeeping operations of all types: commercial, migratory, side-liner and backyard beekeepers, with particular attention to queen breeders. When possible, ten queen producers were sampled. Of the remaining apiaries to be sampled, half were from migratory operations (apiaries that move out of the state and return prior to sampling), and half were from stationary operations (operations that only move within the state or not at all). Additional apiaries located in areas considered at higher risk of exotic pest or disease invasion, such as near deep water shipping ports, were also considered for sampling. Apiaries should have at least 8 colonies (but in most cases, at least 10 colonies, (8 of which will be sampled, with 2 extra in case inspector encounters dead outs or queen-less colonies during inspection. Dead outs and queen-less colonies should not be included in the survey sampling). Inside the apiary, samplers are instructed to select colonies at random using randomly generated lists of numbers (instructions were provided with the sampling kits sent to each participating state).

## **Samples Description**

In each of the apiaries selected, three different composite samples were collected from eight colonies: (a) adult worker bees collected in a live-bee shipping box for the analysis of viruses;

(b) adult worker bees collected in alcohol to detect and quantify *Varroa* loads, *Nosema* spores, and *A. cerana*; and (c) a sample of brood-frame debris to detect *Tropilaelaps*, called “brood frame bump” sample. In a subset of those apiaries, an additional sample of (d) brood comb wax was taken for pesticide residues analysis.

Additionally, beekeepers who managed the sampled apiaries were given a voluntary questionnaire. This short survey asks the beekeepers management information such as feeding and mite treatment practices. The data from this questionnaire has not yet been analyzed, but will be once there is an efficient sample size throughout survey years. It will be analyzed in the interest of linking management practices with disease loads.

See full instructions in the Project Plan for 2017-18, and detailed sampling protocols on the USDA APHIS website (<https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/honey-bees/survey>).

Each colony is also fully inspected to characterize their queen status and the presence of any overt disease symptoms. Information from the inspection, sample collector, beekeeper and their operation are recorded on a datasheet (see Appendix 1) and these data are entered and archived in the BIP database.

#### **(a) Live bee sample**

The live bee sample was collected from a brood frame with both capped and uncapped brood. ¼ cup of nurse bees were taken from each of the 8 colonies and collected in an aggregate sample in a live bee shipping box. Using the U.S. Postal Service (USPS), live bee shipments were mailed to University of Maryland where they were promptly frozen at -80°C. The frozen bees were tested with qRT-PCR techniques, outlined by Dr. Jay Evans at the USDA ARS Bee Research Laboratory. These molecular procedures were updated in 2013 by Dr. Eva Forsgren from the Swedish University of Agricultural Sciences (SLU) to include absolute quantification of the viral targets. As a result, the absolute quantification of viral loads (viral copies per bee) can be determined in addition to the presence or absence of a virus.

In the 2017-2018 survey, live bee samples were analyzed for the following viruses:

1. Acute bee paralysis virus (ABPV)
2. Chronic bee paralysis virus (CBPV)
3. Deformed wing virus (DWV)
4. Israeli acute bee paralysis virus (IAPV)
5. Kashmir bee virus (KBV)
6. Lake Sinai virus-2 (LSV-2)
7. *Varroa destructor* virus (also known as deformed wing virus-B) (VDV)

#### **(b) Alcohol sample**

The alcohol preserved sample was collected from the same brood frame as the live bee sample. An additional ¼ cup of nurse bees were taken from each of the 8 colonies that were sampled in the apiary. These bees were collected into a bottle of 70% ethanol solution for preservation and sent to the University of Maryland to determine the incidence of *Varroa destructor*, *Nosema* spp. spores, and *Apis cerana*.

In the 2017-18 survey, the alcohol preserved bee samples were analyzed for the following:

1. *Nosema* spp. spore loads (in millions of spores per bee)
2. *Varroa destructor* loads (in mites per 100 bees)
3. *Apis cerana* presence or absence

#### **(c) Brood frame bump sample**

The brood bump sample was taken from debris dislodged by ‘bumping’ sampled brood frames over a collection pan. The brood frame debris was collected in a filter cloth and placed in a bottle filled with 70% ethanol solution for preservation. The brood bump sample is focused on monitoring for *Tropilaelaps* spp., but also any mites, beetles or other hive debris are observed for interest by the University of Maryland.

The brood bump samples were analyzed for:

1. *Tropilaelaps* spp. presence or absence

#### **(d) Wax pesticide residue sample**

Wax was also collected in a subsample of 10 apiaries from each state. A minimum of 3 grams of wax was collected from the same 8 colonies, preferably in the same brood area, at the same time as the other three samples described above. These samples were shipped to University of Maryland where they were catalogued and homogenized with liquid nitrogen by UMD personnel and sent to the USDA AMS Lab in Gastonia, NC for pesticide analysis.

In the 2017-18 survey, the wax samples were analyzed for:

1. 185 to 198 different pesticides measured in parts per billion (ppb) which included varroacides (beekeeper applied treatments for *Varroa* control), insecticides, herbicides, and fungicides (list of analytes is determined by the USDA AMS lab and is provided in Appendix 2)

### **Communication of Results**

All participating beekeepers, as well as Apiary Specialists, State Survey Coordinators, State Plant Regulatory Officials, and APHIS State Plant Health Directors, received a report for each sample taken. The report provides detailed results for *Varroa* load, *Nosema* load, and presence of viruses. The reports also noted the presence or absence of *Apis cerana* and *Tropilaelaps* spp. Reports also detail the national prevalence for viruses as well as specific beekeeper percentile rankings of *Varroa* load, *Nosema* spore load, and viral copy load. Reports are sent within 4-8 months of receipt of the samples.

### **Milestones and Project Timelines**

The survey design has evolved over time to reflect the recommendations of scientific experts and to fit the objectives of the program based on current information. These protocols or targets are likely to continue to change as new threats to honey bees are identified. In particular, the protocols updated have concerned the following:



- In 2011, Tracheal mites, *Acarapis woodi*, were removed from the list of pests analyzed, as there were no detections in 2009 or 2010.
- A pilot pollen pesticide survey was conducted in 2011, in which 11 states collected 3 samples of bee bread for pesticides analysis (conducted by the USDA Agricultural Marketing Service (AMS) in Gastonia, NC). In 2012, all participating states sent in 10 bee bread samples for pesticide detection and quantification. The pollen pesticide survey was suspended in 2014 due to reduced funding, but continued again in 2015. The decision to switch from bee bread to brood wax sampling was made in 2017. Current studies indicate that wax may provide a more comprehensive measure of the total number of pesticide residues within a colony. Wax is also shown to be a better predictor of colony and queen mortality (Traynor et al., 2016a).
- Speciation identification between *Nosema apis* and *Nosema ceranae* was discontinued in 2013 after finding no detections of *Nosema apis* from 2009-2010, detections of 1.3% in 2011, and 0.7% in 2012.
- Black queen cell virus (BQCV) was replaced with Lake Sinai virus-2 (LSV-2) in 2013, as the ubiquity of BQCV became known and the concern about LSV-2 became elevated.
- Absolute quantification of viral targets via Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) was adopted in favor of previous viral analysis methods in 2013, enabling direct comparison to standardized European protocols.
- All viral primers, excluding Kashmir bee virus (KBV), were updated in 2013 for increased sensitivity and specificity.

## Results

At the start of this survey year, a total of 1032 sampling kits were sent out (40 states at 24 kits per state, plus 48 for Guam and Puerto Rico and an extra 24 for California). At the conclusion of the survey year, 1008 live bee boxes were returned (97.7% return rate), 947 alcohol samples (91.8% return rate) and 947 brood frame bump samples (91.8% return rate). Of the 390 pesticide residue sample kits sent, 370 were returned and analyzed (94.9% return rate).

## Exotics Surveillance

All brood frame bump samples were analyzed for *Tropilaelaps* upon arrival, and no suspect mites were found. All alcohol samples were analyzed for the presence of *A. cerana* upon arrival, and no suspect bees were found.

The APHIS National Survey has confirmed the absence, as of 2018, of *Tropilaelaps* spp., and *Apis cerana*.

## Established Pests and Disease Monitoring

All trends discussed below are numerical only and have not been tested for potential confounding of sampling bias over time.

### *Nosema* spp. Spore Load and Prevalence

Of the 947 alcohol samples that were analyzed for *Nosema* spp. spore load, 419 (44.2%) tested positive (Figure 1). The average *Nosema* spore load was 0.51 million spores per bee  $\pm$  0.04 (s.e.) for samples that tested positive (Figure 2). Of all samples that were processed for *Nosema* spp. spores, 11.2% (47) exceeded the threshold thought to cause damage (more than 1 million spores per bee). Average *Nosema* spp. spore load varies throughout the year (Figures 3 and 4), with the highest loads occurring in the winter and early spring periods followed by a sharp decline in summer months when most of the samples were collected.

### *Varroa* Load and Prevalence

Of the 947 alcohol samples that were analyzed for *Varroa*, 848 (89.5%) were positive for mites (Figure 5). While the economic threshold for *Varroa* is seasonally and regionally specific, an average load of over 3 mites per 100 bees is the general threshold thought to cause damage to a colony of honey bees (Honey Bee Health Coalition, 2017). This threshold was exceeded in 31.7% (300) of all samples analyzed. The average *Varroa* load was found to be 3.37 mites per 100 bees  $\pm$  0.15 (s.e.) for samples that tested positive (Figure 6). Figures 7 and 8 illustrate the dynamic

nature and seasonality of mite populations across all years of the APHIS National Honey Bee Survey. Generally, *Varroa* populations increase exponentially in the late summer and peak in the fall.

## **Viral Load and Prevalence**

Of the 1,008 live bee boxes that were received, 996 (98.8%) of all samples were analyzed for viruses. The other 12 live bee samples were not analyzed as shipping, storage or other factors affected sample quality to such an extent that RNA quality was too poor to process. Figure 9 illustrates the prevalence of all viral targets that were tested from survey years 2010 to 2017 (ABPV, BQCV, CBPV, DWV, IAPV, KBV, LSV-2 and VDV). Figures 10 to 17 illustrate the seasonal variation in viral prevalence in the samples from past survey years compared to the 2017-18 survey year.

For the first time in the history of the survey, the most prevalent virus detected **was not** deformed wing virus (DWV), rather its close relative *Varroa destructor* virus (VDV), found in 80.7% (804) of all samples. This is an increase from the previous year of the survey (2016-2017) in which the average for VDV was 60.8%. Deformed wing virus prevalence came in a close second, occurring in 78.0% (787) of all samples. *Varroa destructor* is known to be a vector of DWV and VDV, transferring the virus from one bee to another (Bowen-Walker et al., 1999).

The least prevalent virus in the 2017-2018 survey was Kashmir bee virus (KBV) detected in 5.4% of all samples tested. Although KBV does not appear to be problematic for the U.S. honey bee population, the rising prevalence of Israeli acute paralysis virus (IAPV) may be concerning. When the survey first began in 2010, the incidence of IAPV was quite low, occurring in only 9% of all samples tested. However in recent years (2017-2018 survey year), prevalence of IAPV has risen to 20.5% (Figure 9). Another virus of growing concern is Lake Sinai virus (LSV-2). Lake Sinai virus was first detected in 2011 near Lake Sinai in South Dakota and was added to the APHIS National Survey list of viruses tested for in the 2013-2014 survey year. Prevalence of LSV-2 displays a strong seasonality across all years of the survey (Figure 16). Incidence of the virus is higher in the spring, peaking in April at 63.2% in the 2017-2018 survey year. These levels gradually decreased into the fall, and were at their lowest in October at 20.2%. A positive

correlation between the prevalence of LSV-2 and *Nosema* spores has also been observed (Traynor et al., 2016b).

Acute bee paralysis virus (ABPV) seasonality can also be seen across all survey years (Figure 10). Incidence of ABPV was at its highest in the winter months, decreasing throughout the spring and was at its lowest in the summer months. Average prevalence of ABPV has varied since the beginning of the APHIS National Survey, in the 2017-18 survey year it was detected in 17% of samples (Figure 9).

### **Pesticide Detections in Comb Wax**

During the survey year 2017-2018, 38 states (Alabama, Arkansas, California, Colorado, Connecticut, Delaware, Florida, Georgia, Iowa, Idaho, Illinois, Indiana, Kansas, Kentucky, , Massachusetts, Maryland, Michigan, Minnesota, Montana, Nebraska, New Jersey, New Mexico, New York, Ohio, Oregon, Pennsylvania, Puerto Rico, South Carolina, South Dakota, Tennessee, Texas, Utah, Virginia, Vermont, Washington, Wisconsin and West Virginia) submitted composite wax samples (370 total samples). These samples were tested by USDA AMS in Gastonia, NC through their Apiculture Pesticide Residue Screen, which includes testing for 185 to 198 different compounds.

The decision to switch from bee bread to brood wax sampling was made in 2017. Current studies indicate that wax may provide a more comprehensive measure of the total number of pesticide residues within a colony. Wax is also shown to be a better predictor of colony and queen mortality (Traynor et al., 2016a). In comparison with past years, this means that the prevalence of residues in wax was considerably higher than the prevalence of residues in bee bread. Of the 312 bee bread samples analyzed for the survey year 2016-17, 45 (14%) had no detected residues. In contrast, all wax samples analyzed for the survey year 2017-18 were found with at least 1 identified residue (Figure 18). On average, each sample had 14.4 different compounds detected, with as many as 38 compounds detected in a single sample.

The most prevalent type of pesticide residues found in bees' wax are miticides, likely applied by beekeepers to control infestations of *Varroa destructor*. They were found in 100% of the samples processed for the survey year 2017-18 (n=370). These miticides, also known as

varroacides, include Fluvalinate (detected in 93.8% of samples), Coumaphos (detected in 90.3% of samples), Thymol (detected in 84.9% of samples), and the Amitraz metabolite 2,4 Dimethylphenyl formamide (DMPF)(detected in 68.6% of samples) (Table 2).

Insecticides residues were found in 93% of the samples (n=344). The most prevalent insecticide residue detected was Piperonyl butoxide, found in 57.8% of samples, followed by Propargite (found in 44.6% of samples), and Chlorpyrifos (found in 33.8% of samples.)

Fungicides residues were found in 90.5% of the samples (n= 335). These fungicides include Azoxystrobin (detected in 51.4% of samples), Carbendazim (detected in 45.7% of samples) and Pyraclostrobin (detected in 38.6% of samples).

Finally, herbicides were found in 78.6% of the samples (n=291). The most frequent of them was Diuron (found in 42.9% of samples), followed by Metolachlor (found in 40% of samples) and Atrazine (found in 31.9% of samples).

Of the 203 residues tested during the course of the study, 58% (118) were found in our samples. The prevalence (%) within this survey year, the average quantity detected (ppb), and the range of detection (ppb) are provided for each pesticide tested in Table 2. If a pesticide was detected only once, a single value is given for the range and is marked with an asterisk. The relative frequency of each type of pesticide residues detections (varroacide, insecticide, fungicide or herbicide) is presented in Figure 19.

## Conclusions

In terms of exotics surveillance, the APHIS National Honey bee Disease Survey has confirmed the absence, as of 2018, of *Tropilaelaps* spp., and *Apis cerana*. The absence of these exotic pests and pathogens in the 2017-2018 Survey suggest that the current policies to prevent their introduction into the United States have been successful.

Concerning the monitoring of established pests and diseases, the survey allows us to determine baselines of seasonal variability in prevalence and loads.

*Nosema* spp. spore prevalence has been historically consistent since the origin of the APHIS National Survey. On average, *Nosema* has been detected in 50% of all samples taken. Although prevalence has remained about the same, the average load of *Nosema* spores appears to be decreasing over time. The average *Nosema* spore load for this survey (2016-2017) was 0.54 million spores per bee, which is slightly lower than the previous 5 years of the survey where the average *Nosema* spore load was 0.66 million spores per bee. This trend will continue to be monitored in subsequent years of the National Survey to determine if this decrease in *Nosema* disease load is significant.

The prevalence of *Varroa destructor* in APHIS National Survey samples has remained relatively the same since 2010, and has been detected in 90% of samples each year on average. In a similar trend as *Nosema*, *Varroa* load has decreased over time despite little to no change in prevalence. Average *Varroa* load was at its highest during the 2012-2013 survey year averaging at 5.5 mites per 100 bees and has gradually decreased until this year's survey with an average of 3.3 mites per 100 bees. An explanation could be that nationwide outreach and extension efforts towards beekeepers about monitoring and treatment of *Varroa* has been successful. An alternative explanation is that the viruses that *Varroa destructor* transmits have become more virulent, resulting in higher colony loss and therefore a drop in mite populations.

The APHIS National Honey bee Disease Survey gives us the opportunity to determine the levels of exposures of honey bees to pesticides residues in a representative sample of apiaries across the U.S. After 6 years of monitoring bee bread (stored pollen), the survey switches its focus on wax, following indications that it could be a better predictor of colony health than bee bread residues (Traynor et al., 2016a).

## Tables and Figures

Table 1: Sample sizes and prevalence of principal targets by state

State	Sent	Alcohol sample						Tropilaelaps sample			Live bee sample		Wax sample		
		Ret.	Proc.	RR(%)	<i>Varroa</i> Prev.	<i>Nosema</i> Prev.	<i>A.cerana</i> Prev.	Proc.	RR(%)	<i>Trop.</i> Prev.	Proc.	RR(%)	Sent	Proc.	RR(%)
AL	24		23	95.8	91.7	25.0	0	23	95.8	0	24	100.0		10	
AR	24		24	100.0	100.0	0.0	0	24	100.0	0	24	100.0		10	
CA	48		26	54.2	80.8	61.5	0	26	54.2	0	26	54.2		11	
CO	24		15	62.5	73.3	13.3	0	15	62.5	0	15	62.5		6	
CT	24		24	100.0	91.7	54.2	0	24	100.0	0	24	100.0		12	
DE	24		24	100.0	87.5	83.3	0	22	91.7	0	24	100.0		10	
FL	24		24	100.0	95.8	54.2	0	24	100.0	0	22	91.7		10	
GA	24		22	91.7	100.0	50.0	0	22	91.7	0	22	91.7		10	
GU	24		24	100.0	0.0	91.7	0	10	41.7	0	14	58.3			
IA	24		24	100.0	66.7	58.3	0	24	100.0	0	24	100.0		10	
ID	24		24	100.0	79.2	58.3	0	24	100.0	0	24	100.0		10	
IL	24		24	100.0	100.0	29.2	0	24	100.0	0	24	100.0		10	
IN	24		24	100.0	100.0	25.0	0	24	100.0	0	24	100.0		10	
KS	24		24	100.0	100.0	25.0	0	24	100.0	0	24	100.0		10	
KY	24		24	100.0	83.3	33.3	0	24	100.0	0	24	100.0		10	
MA	24		24	100.0	100.0	58.3	0	16	66.7	0	24	100.0		10	
MD	24		23	95.8	95.7	52.2	0	22	91.7	0	23	95.8		9	
MI	24		24	100.0	100.0	29.2	0	24	100.0	0	24	100.0		9	
MN	24		24	100.0	91.7	58.3	0	24	100.0	0	24	100.0		10	
MO	24		24	100.0	100.0	20.8	0	24	100.0	0	23	95.8		11	



MT	24		24	100.0	83.3	41.7	0	24	100.0	0	24	100.0		10	
NC	24		23	95.8	91.3	52.2	0	23	95.8	0	23	95.8			
ND	24		0	0.0	0.0	0.0	0	0	0.0	0	24	100.0			
NE	24		24	100.0	87.5	29.2	0	24	100.0	0	24	100.0		11	
NJ	24		24	100.0	95.8	66.7	0	24	100.0	0	24	100.0		10	
NM	24		24	100.0	100.0	12.5	0	24	100.0	0	24	100.0		10	
NV	24		24	100.0	75.0	29.2	0	24	100.0	0	24	100.0			
NY	24		24	100.0	100.0	45.8	0	24	100.0	0	24	100.0		10	
OH	24		24	100.0	95.8	29.2	0	24	100.0	0	24	100.0		9	
OR	24		17	70.8	94.1	88.2	0	17	70.8	0	17	70.8		10	
PA	24		24	100.0	100.0	58.3	0	24	100.0	0	24	100.0		10	
PR	24		24	100.0	100.0	16.7	0	24	100.0	0	24	100.0		8	
SC	24		24	100.0	79.2	50.0	0	24	100.0	0	24	100.0		10	
SD	24		24	100.0	91.7	33.3	0	24	100.0	0	24	100.0		8	
TN	24		11	45.8	100.0	45.5	0	11	45.8	0	11	45.8		7	
TX	24		24	100.0	83.3	41.7	0	24	100.0	0	24	100.0		10	
UT	24		24	100.0	70.8	62.5	0	24	100.0	0	24	100.0		10	
VA	24		23	95.8	100.0	56.5	0	23	95.8	0	23	95.8		10	
VT	24		24	100.0	95.8	83.3	0	24	100.0	0	24	100.0		10	
WA	24		23	95.8	95.7	26.1	0	23	95.8	0	23	95.8		10	
WI	24		22	91.7	100.0	31.8	0	22	91.7	0	22	91.7		10	
WV	24		24	100.0	91.7	29.2	0	24	100.0	0	24	100.0		8	
Total	1032		948	91.9	87.2	43.1	0	923	89.4	0	960	93.0		369	

Legend and abbreviations:

Sent: number of sample kits sent; Ret. (returned): number of samples collected; Proc. (processed) number of samples processed; RR(%): response rate; Prev. (prevalence): percent of samples diagnosed with target.

Table 2: Pesticides residues detections in wax samples

Pesticide	Type	N (samples)	Detections	(among which "trace")	Prevalence (%)	Average detection if positive for target (ppb)	Range if positive for target (ppb)
1-Naphthol	Insecticide	350	1	0	0.29	430.0	430 - 430
2,4 Dimethylphenyl formamide (DMPF)	Varroacide	370	254	44	68.65	2,390.2	Trace - 78,900
2,6-Dichlorobenzamide (BAM)	Herbicide	370	6	2	1.62	9.8	Trace - 13
3-Hydroxycarbofuran	Insecticide	250	0	0	0.00	NA	NA
4-Hydroxychlorothalonil	Fungicide	370	2	0	0.54	22.0	19 - 25
Acephate	Insecticide	370	1	0	0.27	51.0	51 - 51
Acetamiprid	Insecticide	370	13	8	3.51	8.6	Trace - 15
Acetochlor	Herbicide	370	71	55	19.19	298.4	Trace - 740
Acrinathrin	Insecticide	370	0	0	0.00	NA	NA
Alachlor	Herbicide	370	0	0	0.00	NA	NA
Aldicarb	Insecticide	370	0	0	0.00	NA	NA
Aldicarb sulfone	Insecticide	330	0	0	0.00	NA	NA
Aldicarb sulfoxide	Insecticide	347	0	0	0.00	NA	NA
Ametoctradin	Fungicide	370	18	5	4.86	11.5	Trace - 70
Atrazine	Herbicide	370	118	50	31.89	18.5	Trace - 207
Avermectin	Insecticide	313	0	0	0.00	NA	NA
Azinphos-methyl	Insecticide	370	6	2	1.62	1,576.0	Trace - 2,670
Azoxystrobin	Fungicide	370	190	27	51.35	37.5	Trace - 1,070
Bensulide	Herbicide	350	0	0	0.00	NA	NA
Bentazon	Herbicide	350	0	0	0.00	NA	NA
Bifenazate	Insecticide	97	5	2	5.15	17.0	Trace - 31
Bifenthrin	Insecticide	325	5	0	1.54	149.6	36 - 315
Boscalid	Fungicide	370	66	10	17.84	32.8	Trace - 232
Bromacil	Herbicide	370	5	2	1.35	13.7	Trace - 16
Bromopropylate	Insecticide	370	0	0	0.00	NA	NA
Bromuconazole	Fungicide	370	0	0	0.00	NA	NA
Buprofezin	Insecticide	370	44	1	11.89	30.0	Trace - 543
Captan	Fungicide	370	0	0	0.00	NA	NA
Carbaryl	Insecticide	370	20	10	5.41	58.7	Trace - 357
Carbendazim	Fungicide	370	169	64	45.68	112.4	Trace - 2,510
Carbofuran	Insecticide	370	2	0	0.54	4.0	4 - 4

Carfentrazone-ethyl	Herbicide	370	0	0	0.00	NA	NA
Chlorantraniliprole	Insecticide	370	34	22	9.19	34.1	Trace - 59
Chlorfenapyr	Insecticide	370	1	1	0.27	NA	NA
Chlorfenvinphos	Insecticide	370	0	0	0.00	NA	NA
Chlorothalonil	Fungicide	370	89	56	24.05	3,653.6	Trace - 57,400
Chlorpropham	Herbicide	370	1	1	0.27	NA	NA
Chlorpyrifos	Insecticide	370	125	64	33.78	27.6	Trace - 273
Chlorpyrifos methyl	Insecticide	370	0	0	0.00	NA	NA
Chlorthal-dimethyl (DCPA)	Herbicide	370	43	33	11.62	6.0	Trace - 20
Clofentezine	Insecticide	370	0	0	0.00	NA	NA
Clothianidin	Insecticide	370	0	0	0.00	NA	NA
Coumaphos	Varroacide	370	334	53	90.27	274.8	Trace - 9,310
Coumaphos oxon	Varroacide	370	196	0	52.97	27.0	1 - 1,050
Cyantraniliprole	Insecticide	370	1	0	0.27	44.0	44 - 44
Cyazofamid	Fungicide	370	0	0	0.00	NA	NA
Cyflufenamid	Fungicide	370	0	0	0.00	NA	NA
Cyflumetofen	Insecticide	370	0	0	0.00	NA	NA
Cyfluthrin	Insecticide	370	0	0	0.00	NA	NA
Cyhalothrin	Insecticide	353	0	0	0.00	NA	NA
cyhalothrin lambda	Insecticide	17	0	0	0.00	NA	NA
Cymiazole	Varroacide	370	0	0	0.00	NA	NA
Cymoxanil	Fungicide	370	0	0	0.00	NA	NA
Cypermethrin	Insecticide	370	1	1	0.27	NA	NA
Cyphenothrin	Insecticide	370	0	0	0.00	NA	NA
Cyprodinil	Fungicide	347	122	1	35.16	96.4	Trace - 3,780
Cyromazine	Insecticide	353	1	1	0.28	NA	NA
DDE, p,p'	Insecticide	310	39	37	12.58	17.0	Trace - 18
DEET	Insecticide	370	114	9	30.81	143.7	Trace - 3,850
Deltamethrin	Insecticide	370	0	0	0.00	NA	NA
Diazinon	Insecticide	370	0	0	0.00	NA	NA
Diazinon oxon	Insecticide	370	0	0	0.00	NA	NA
Dichlorvos (DDVP)	Insecticide	370	1	0	0.27	4.0	4 - 4
Dicloran	Fungicide	370	2	2	0.54	NA	NA
Dicofol	Insecticide	310	2	2	0.65	NA	NA
Difenoconazole	Fungicide	370	50	13	13.51	18.1	Trace - 129
Diiflubenzuron	Insecticide	370	57	8	15.41	171.4	Trace - 7,240
Dimethenamid	Herbicide	370	5	2	1.35	5.7	Trace - 6
Dimethoate	Insecticide	370	7	2	1.89	26.6	Trace - 101
Dimethomorph	Fungicide	370	0	0	0.00	NA	NA
Dinotefuran	Insecticide	370	0	0	0.00	NA	NA
Diphenamid	Herbicide	370	0	0	0.00	NA	NA
Diphenylamine	Fungicide	370	41	23	11.08	7.1	Trace - 16

Diuron	Herbicide	296	127	6	42.91	11.4	Trace - 203
Emamectin Benzoate	Insecticide	333	0	0	0.00	NA	NA
Endosulfan I	Insecticide	370	0	0	0.00	NA	NA
Endosulfan II	Insecticide	370	4	4	1.08	NA	NA
Endosulfan sulfate	Insecticide	370	0	0	0.00	NA	NA
Epoxiconazole	Fungicide	264	0	0	0.00	NA	NA
Esfenvalerate	Insecticide	17	0	0	0.00	NA	NA
Esfenvalerate/Fenvalerate	Insecticide	353	0	0	0.00	NA	NA
Ethion	Insecticide	370	0	0	0.00	NA	NA
Ethofumesate	Herbicide	370	0	0	0.00	NA	NA
Etofenprox	Insecticide	370	1	0	0.27	81.0	81 - 81
Etiozazole	Insecticide	370	1	0	0.27	4.0	4 - 4
Famoxadone	Fungicide	370	2	1	0.54	11.0	Trace - 11
Fenamidone	Fungicide	370	8	6	2.16	3.0	Trace - 4
Fenarimol	Fungicide	284	0	0	0.00	NA	NA
Fenazaquin	Varroacide	370	1	0	0.27	2.0	2 - 2
Fenbuconazole	Fungicide	370	55	6	14.86	42.8	Trace - 705
Fenhexamid	Fungicide	370	0	0	0.00	NA	NA
Fenoxaprop-p-ethyl	Herbicide	370	0	0	0.00	NA	NA
Fenpropathrin	Insecticide	370	0	0	0.00	NA	NA
Fenpyroximate	Varroacide	370	191	63	51.62	88.2	Trace - 4,740
Fipronil	Insecticide	370	0	0	0.00	NA	NA
Fipronil sulfide	Insecticide	370	0	0	0.00	NA	NA
Fipronil sulfone	Insecticide	370	1	1	0.27	NA	NA
Flonicamid	Insecticide	370	2	1	0.54	30.0	Trace - 30
Fludioxonil	Fungicide	370	33	20	8.92	127.2	Trace - 434
Fluometuron	Herbicide	370	1	1	0.27	NA	NA
Fluopicolide	Fungicide	370	8	2	2.16	6.5	Trace - 16
Fluopyram	Fungicide	370	87	0	23.51	33.1	1 - 503
Fluoxastrobin	Fungicide	370	3	1	0.81	6.5	Trace - 10
Flupyradifurone	Insecticide	370	11	6	2.97	97.2	Trace - 403
Fluridone	Herbicide	370	0	0	0.00	NA	NA
Flutriafol	Fungicide	370	1	0	0.27	18.0	18 - 18
Fluvalinate	Varroacide	370	347	41	93.78	1,070.4	Trace - 76,800
Fluxapyroxad	Fungicide	370	55	9	14.86	12.6	Trace - 158
Hexazinone	Herbicide	370	2	1	0.54	3.0	Trace - 3
Hexythiazox	Fungicide	370	95	62	25.68	5.6	Trace - 26
Imazalil	Fungicide	350	1	0	0.29	46.0	46 - 46
Imidacloprid	Insecticide	370	6	1	1.62	12.6	Trace - 16
Indoxacarb	Insecticide	370	11	8	2.97	18.0	Trace - 32
Iprodione	Fungicide	370	96	37	25.95	453.0	Trace - 3,370
Kresoxim-methyl	Fungicide	370	4	3	1.08	9.0	Trace - 9

Linuron	Herbicide	370	0	0	0.00	NA	NA
Malathion	Insecticide	370	9	5	2.43	262.5	Trace - 784
Mandipropamide	Fungicide	370	13	8	3.51	5.4	Trace - 10
Metalaxyl	Fungicide	370	21	10	5.68	9.6	Trace - 29
Metconazole	Fungicide	370	18	5	4.86	42.2	Trace - 159
Methamidophos	Insecticide	370	0	0	0.00	NA	NA
Methidathion	Insecticide	370	0	0	0.00	NA	NA
Methomyl	Insecticide	370	0	0	0.00	NA	NA
Methoprene	Insecticide	370	17	12	4.59	5,582.0	Trace - 6,300
Methoxyfenozide	Insecticide	370	84	0	22.70	34.7	1 - 1,020
Metolachlor	Herbicide	370	148	124	40.00	100.1	Trace - 521
Metribuzin	Herbicide	370	2	2	0.54	NA	NA
MGK-264	Insecticide	370	9	5	2.43	26.8	Trace - 52
Momfluorothrin	Insecticide	370	0	0	0.00	NA	NA
Myclobutanil	Fungicide	370	3	0	0.81	86.0	64 - 129
Norflurazon	Herbicide	370	4	3	1.08	27.0	Trace - 27
Norflurazon desmethyl	Herbicide	370	2	2	0.54	NA	NA
Novaluron	Insecticide	370	12	7	3.24	11.2	Trace - 20
Omethoate	Insecticide	347	7	0	2.02	1,220.3	210 - 3,350
Oxamyl	Insecticide	370	0	0	0.00	NA	NA
Oxyfluorfen	Herbicide	370	3	2	0.81	388.0	Trace - 388
Parathion	Insecticide	17	0	0	0.00	NA	NA
Parathion ethyl	Insecticide	353	0	0	0.00	NA	NA
Parathion methyl	Insecticide	370	0	0	0.00	NA	NA
Penconazole	Fungicide	370	0	0	0.00	NA	NA
Pendimethalin	Herbicide	370	41	24	11.08	49.4	Trace - 168
Penthiopyrad	Fungicide	359	77	2	21.45	16.6	Trace - 203
Permethrin	Insecticide	330	9	6	2.73	1,952.7	Trace - 3,360
Phenothrin	Insecticide	370	0	0	0.00	NA	NA
Phorate	Insecticide	370	0	0	0.00	NA	NA
Phosalone	Insecticide	370	0	0	0.00	NA	NA
Phosmet	Insecticide	370	1	1	0.27	NA	NA
Phosmet oxon	Insecticide	227	0	0	0.00	NA	NA
Picoxystrobin	Fungicide	370	2	0	0.54	5.0	5 - 5
Piperonyl butoxide	Insecticide	370	214	169	57.84	189.9	Trace - 2,620
Prallethrin	Insecticide	370	2	0	0.54	400.0	385 - 415
Prodiamine	Herbicide	370	2	0	0.54	40.5	21 - 60
Profenofos	Insecticide	370	0	0	0.00	NA	NA
Prometon	Herbicide	370	29	2	7.84	21.3	Trace - 293
Prometryn	Herbicide	370	3	2	0.81	8.0	Trace - 8
Pronamide	Herbicide	370	0	0	0.00	NA	NA
Propachlor	Herbicide	284	0	0	0.00	NA	NA

Propamocarb hydrochloride	Fungicide	353	22	1	6.23	15.4	Trace - 127
Propanil	Herbicide	370	2	1	0.54	6.0	Trace - 6
Propargite	Insecticide	370	165	54	44.59	62.3	Trace - 713
Propazine	Herbicide	370	0	0	0.00	NA	NA
Propetamphos	Insecticide	353	0	0	0.00	NA	NA
Propiconazole	Fungicide	370	90	4	24.32	41.7	Trace - 1,050
Pymetrozine	Insecticide	370	0	0	0.00	NA	NA
Pyraclostrobin	Fungicide	370	143	18	38.65	27.8	Trace - 415
Pyridaben	Insecticide	370	9	5	2.43	34.3	Trace - 96
Pyrimethanil	Fungicide	370	56	9	15.14	50.3	Trace - 1,080
Pyriproxyfen	Insecticide	370	15	0	4.05	7.5	2 - 30
Quinoxifen	Fungicide	370	7	3	1.89	4.8	Trace - 9
Quintozene	Fungicide	370	0	0	0.00	NA	NA
Resmethrin	Insecticide	17	0	0	0.00	NA	NA
Resmethrin, cis	Insecticide	210	0	0	0.00	NA	NA
Resmethrin, trans	Insecticide	210	0	0	0.00	NA	NA
Sethoxydim	Herbicide	370	0	0	0.00	NA	NA
Simazine	Herbicide	370	0	0	0.00	NA	NA
Spinetoram	Insecticide	308	13	12	4.22	16.0	Trace - 16
Spinosad	Insecticide	308	21	15	6.82	22.8	Trace - 40
Spirodiclofen	Varroacide	370	54	22	14.59	7.1	Trace - 17
Spiromesifen	Insecticide	350	1	0	0.29	42.0	42 - 42
Spirotetramat	Insecticide	370	2	1	0.54	4.0	Trace - 4
Sulfoxaflor	Insecticide	370	1	1	0.27	NA	NA
Tebuconazole	Fungicide	370	63	14	17.03	45.6	Trace - 201
Tebufenozide	Insecticide	370	32	4	8.65	17.4	Trace - 196
Tebuthiuron	Herbicide	370	0	0	0.00	NA	NA
Tefluthrin	Insecticide	370	0	0	0.00	NA	NA
Tetraconazole	Fungicide	370	0	0	0.00	NA	NA
Tetradifon	Insecticide	370	0	0	0.00	NA	NA
Tetramethrin	Insecticide	370	6	0	1.62	10,891.8	901 - 30,600
Thiabendazole	Fungicide	370	4	3	1.08	25.0	Trace - 25
Thiacloprid	Insecticide	370	2	1	0.54	15.0	Trace - 15
Thiamethoxam	Insecticide	370	0	0	0.00	NA	NA
THPI	Fungicide	370	1	0	0.27	1,880.0	1,880 - 1,880
Thymol	Varroacide	370	314	28	84.86	12,996.3	Trace - 1,440,000
Tolfenpyrad	Insecticide	370	0	0	0.00	NA	NA
Triadimefon	Fungicide	370	0	0	0.00	NA	NA
Triadimenol	Fungicide	370	0	0	0.00	NA	NA
Triazophos	Insecticide	370	0	0	0.00	NA	NA
Tribufos	Herbicide	370	0	0	0.00	NA	NA

Trifloxystrobin	Fungicide	370	133	1	35.95	13.1	Trace - 257
Triflumizole	Fungicide	370	24	0	6.49	5.5	1 - 24
Trifluralin	Herbicide	370	13	10	3.51	21.7	Trace - 27
Triticonazole	Fungicide	370	0	0	0.00	NA	NA
Vinclozolin	Fungicide	370	0	0	0.00	NA	NA

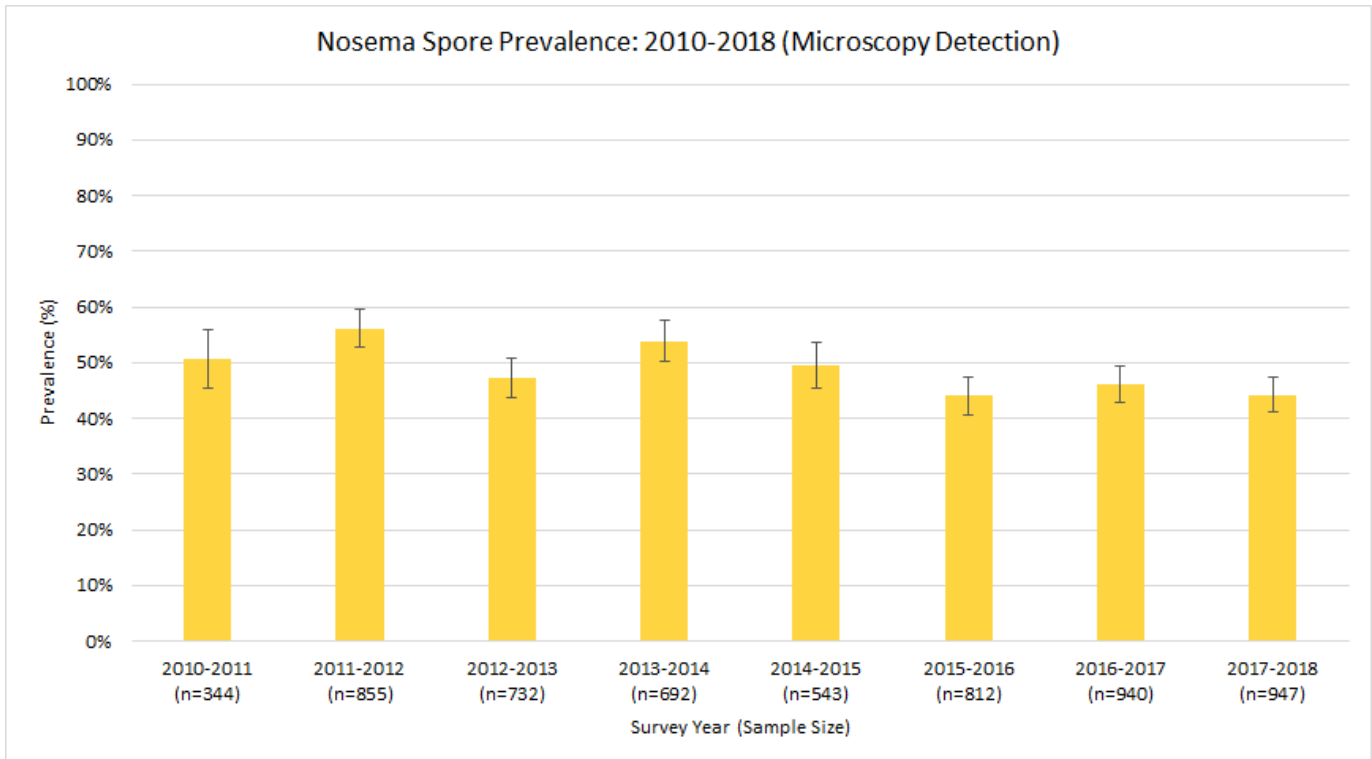


Figure 1: *Nosema* prevalence by survey year (95% confidence intervals shown)

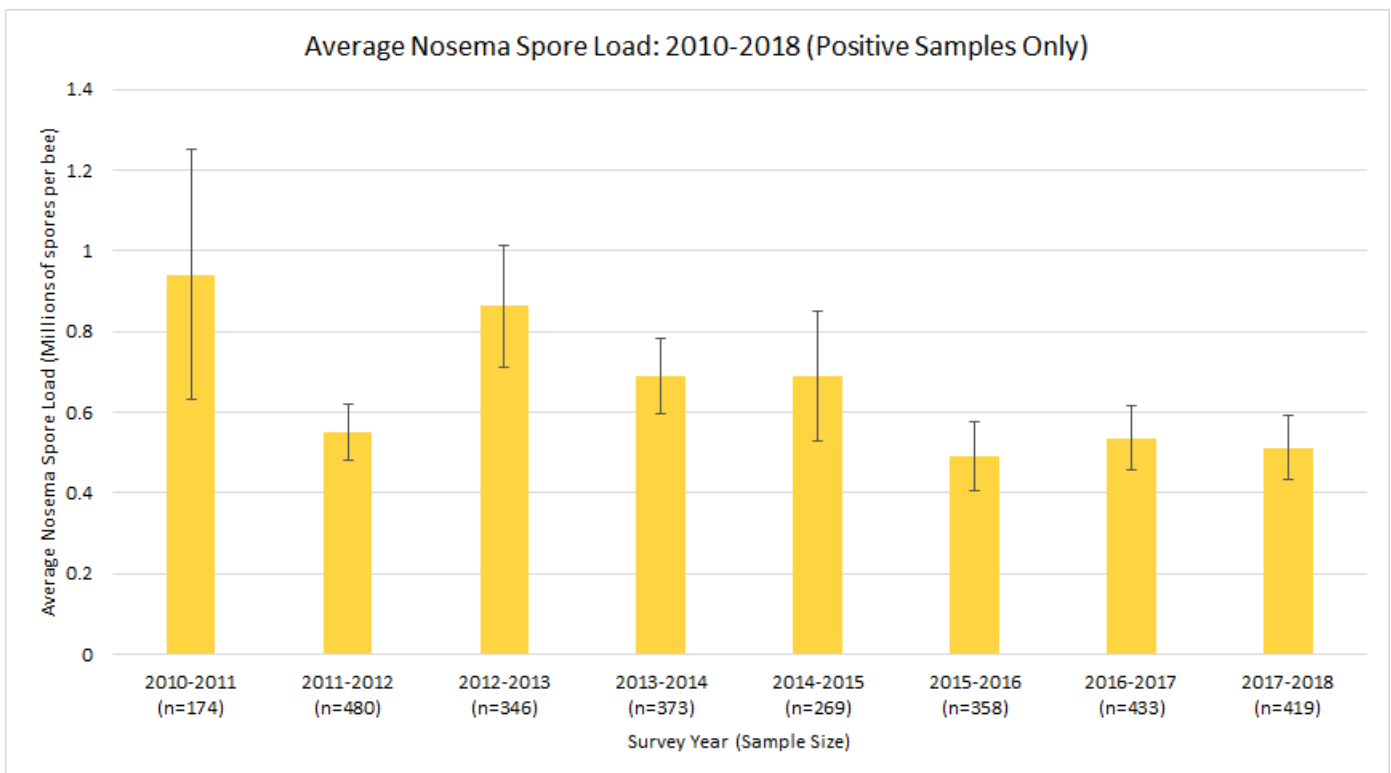


Figure 2: *Nosema* spore load by survey year (95% confidence intervals shown)



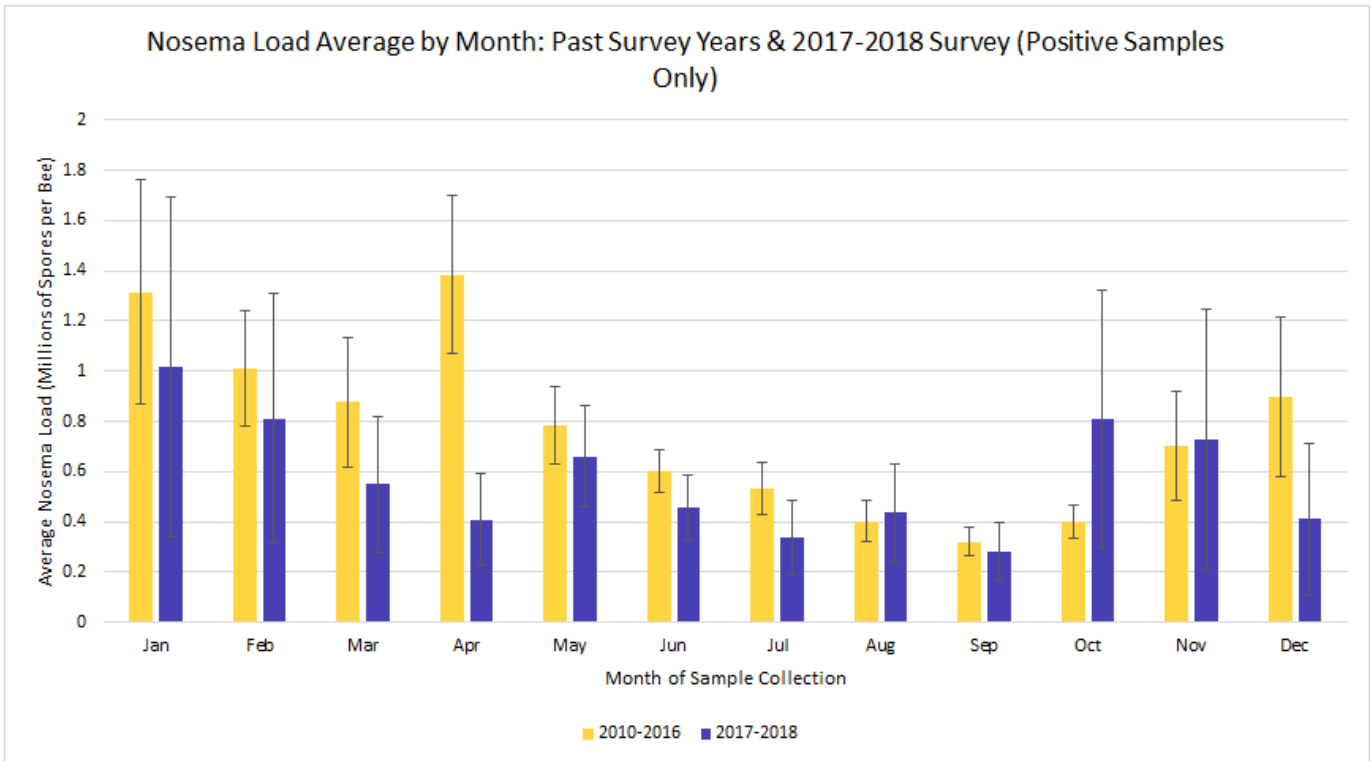


Figure 3: Average *Nosema* spore load by month for all years of the survey (95% confidence intervals shown)

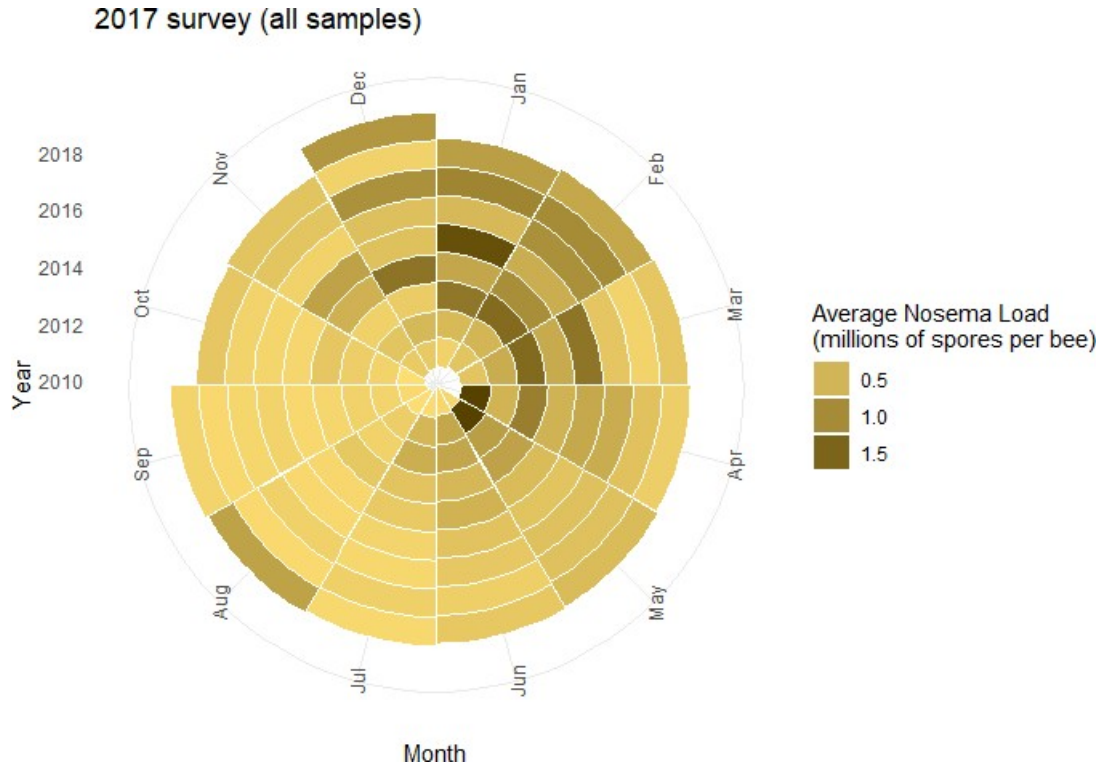


Figure 4: Monthly averages of *Nosema* loads (millions of spores per bee) for all survey years as a time spiral (from center to edge).

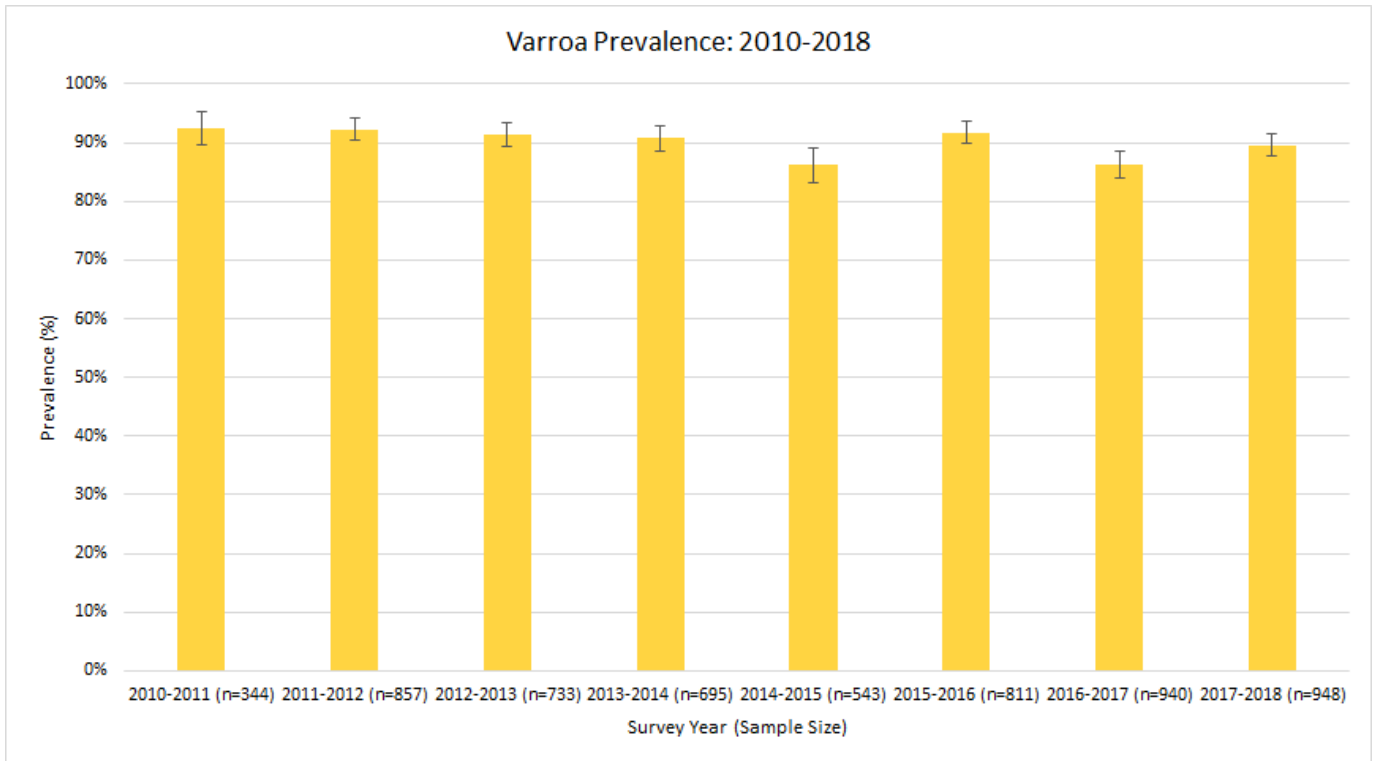


Figure 5: *Varroa* prevalence by survey year (95% confidence intervals shown)

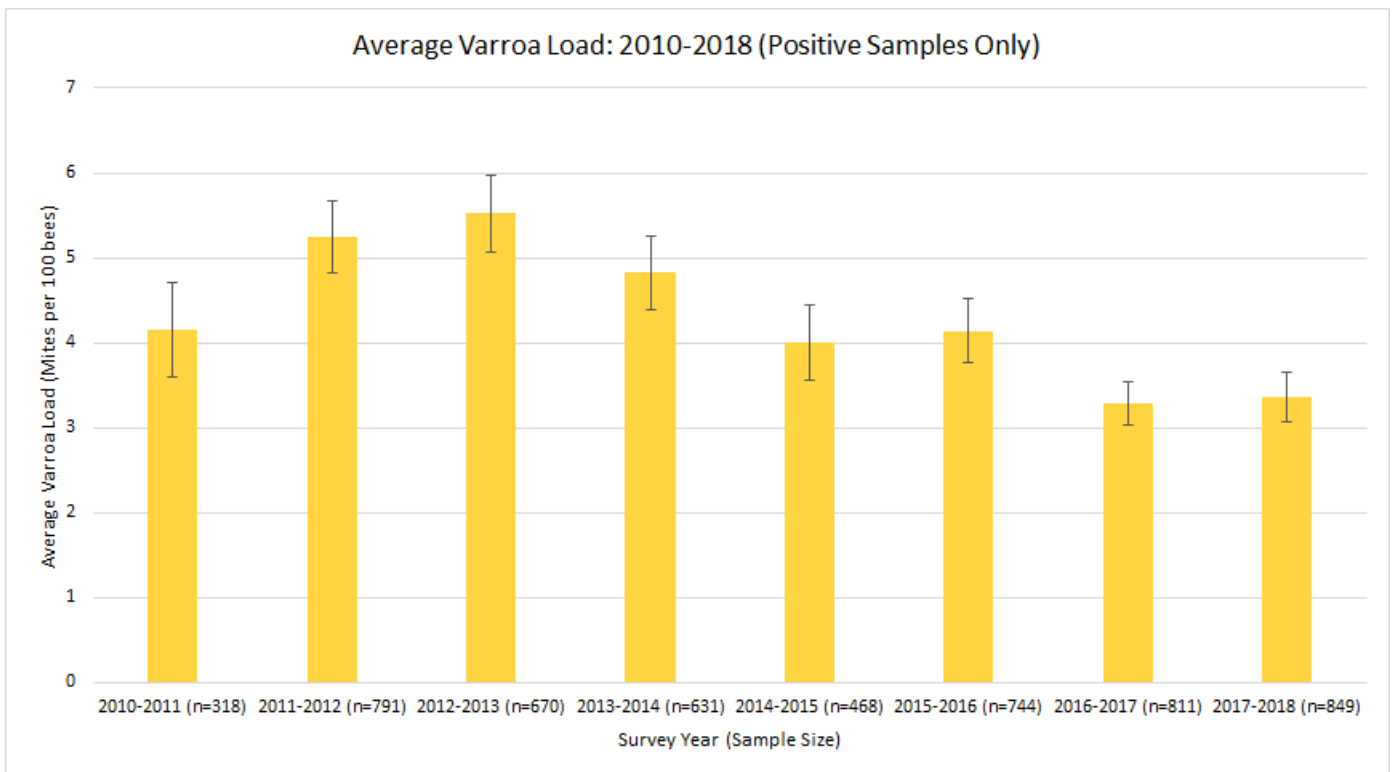


Figure 6: *Varroa* load by survey year (95% confidence intervals shown)

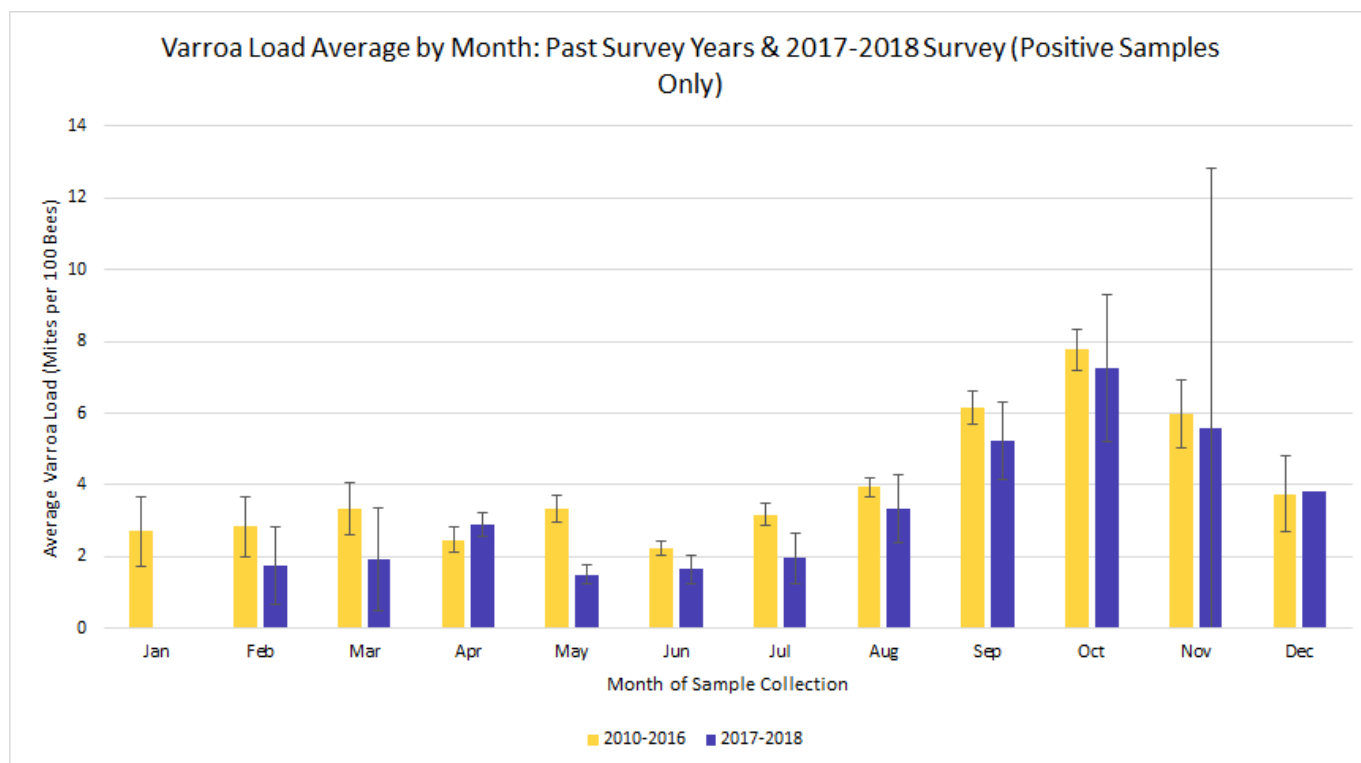


Figure 7: Average *Varroa* load by month for all years of the survey (95% confidence intervals shown)

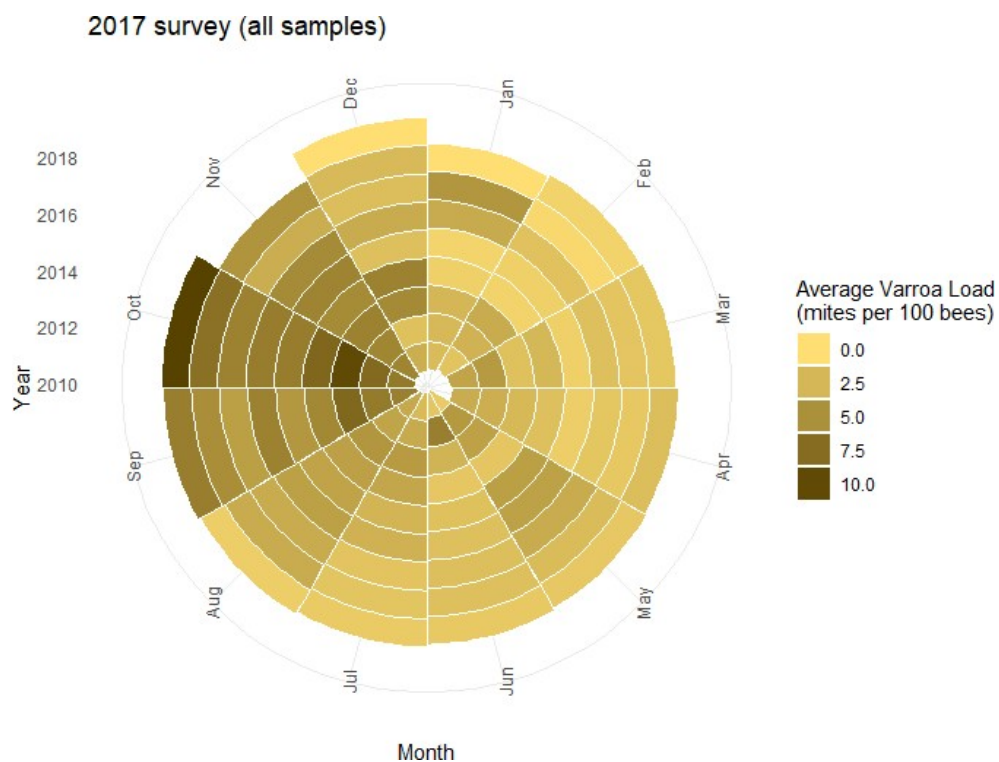


Figure 8: Monthly averages of *Varroa* loads (mites per 100 bees) for all survey years as a time spiral (from center to edge).

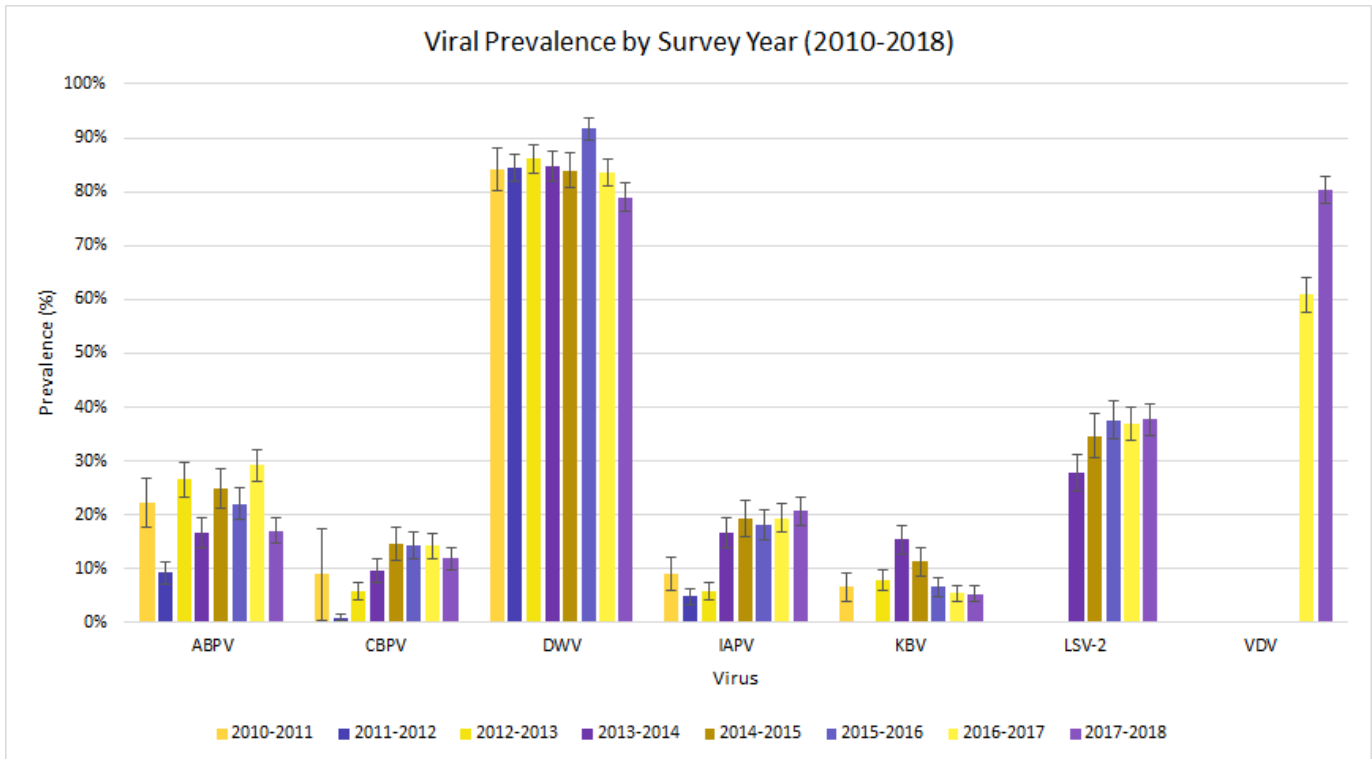


Figure 9: Yearly changes in viral prevalence from 2010 to 2016 (95% confidence intervals shown). VDV was added to the list of viral targets in 2016.

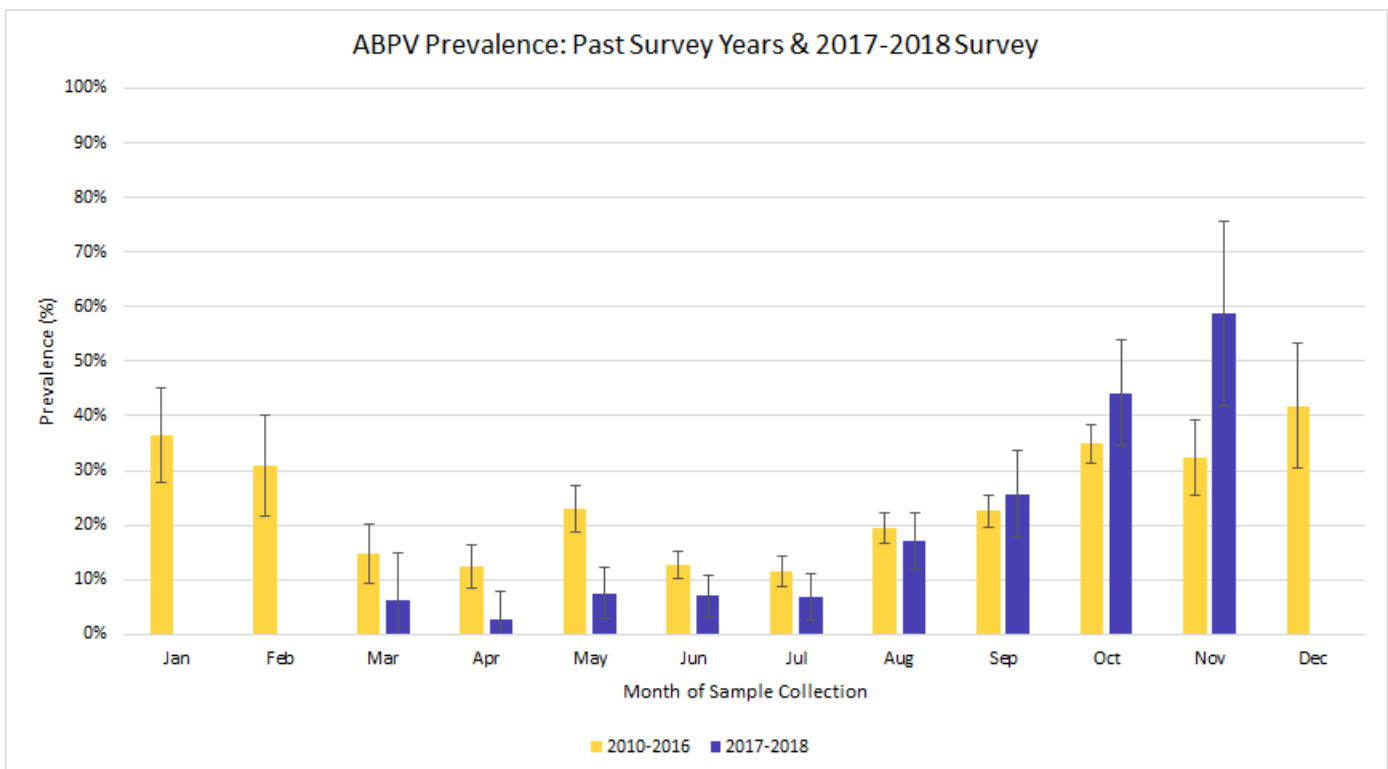


Figure 10: Prevalence of acute bee paralysis virus by month (95% confidence intervals shown)

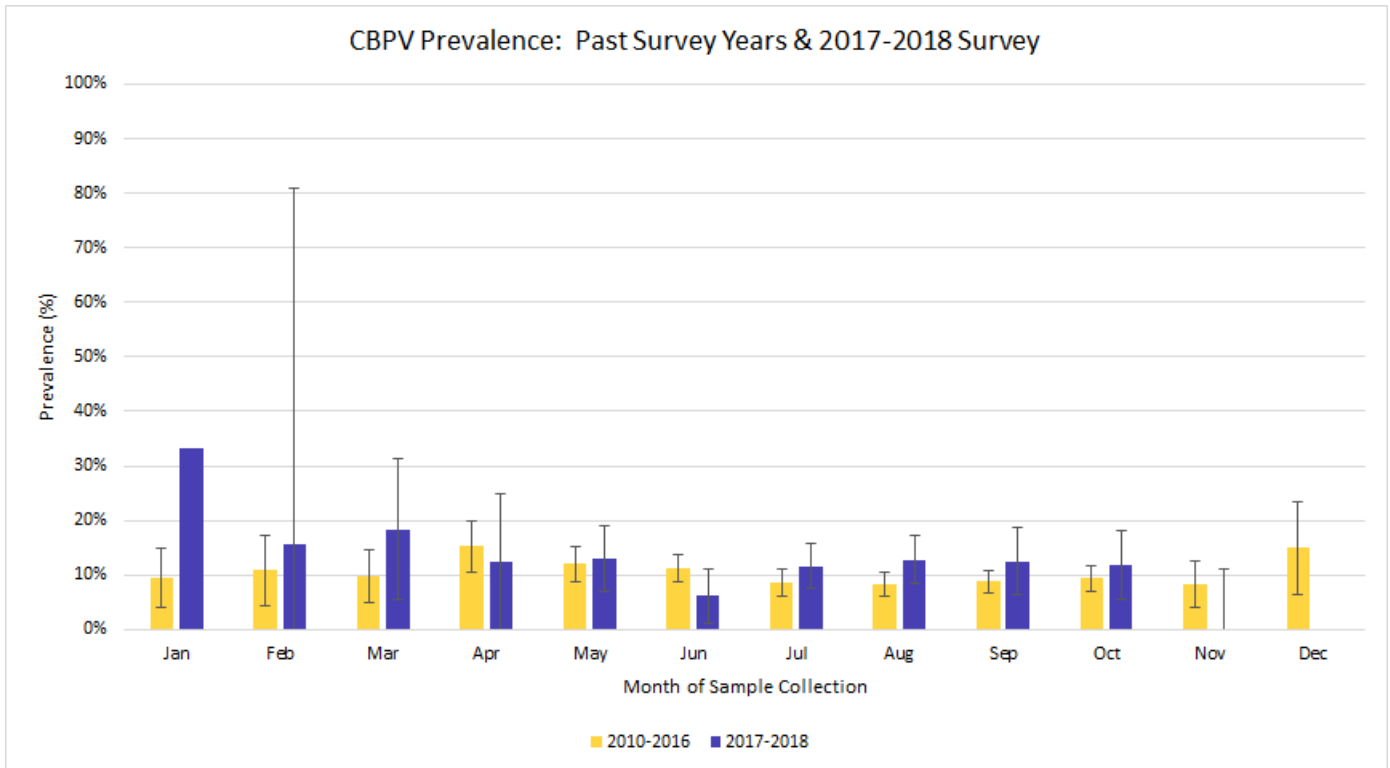


Figure 11: Prevalence of chronic bee paralysis virus by month (95% confidence intervals shown)

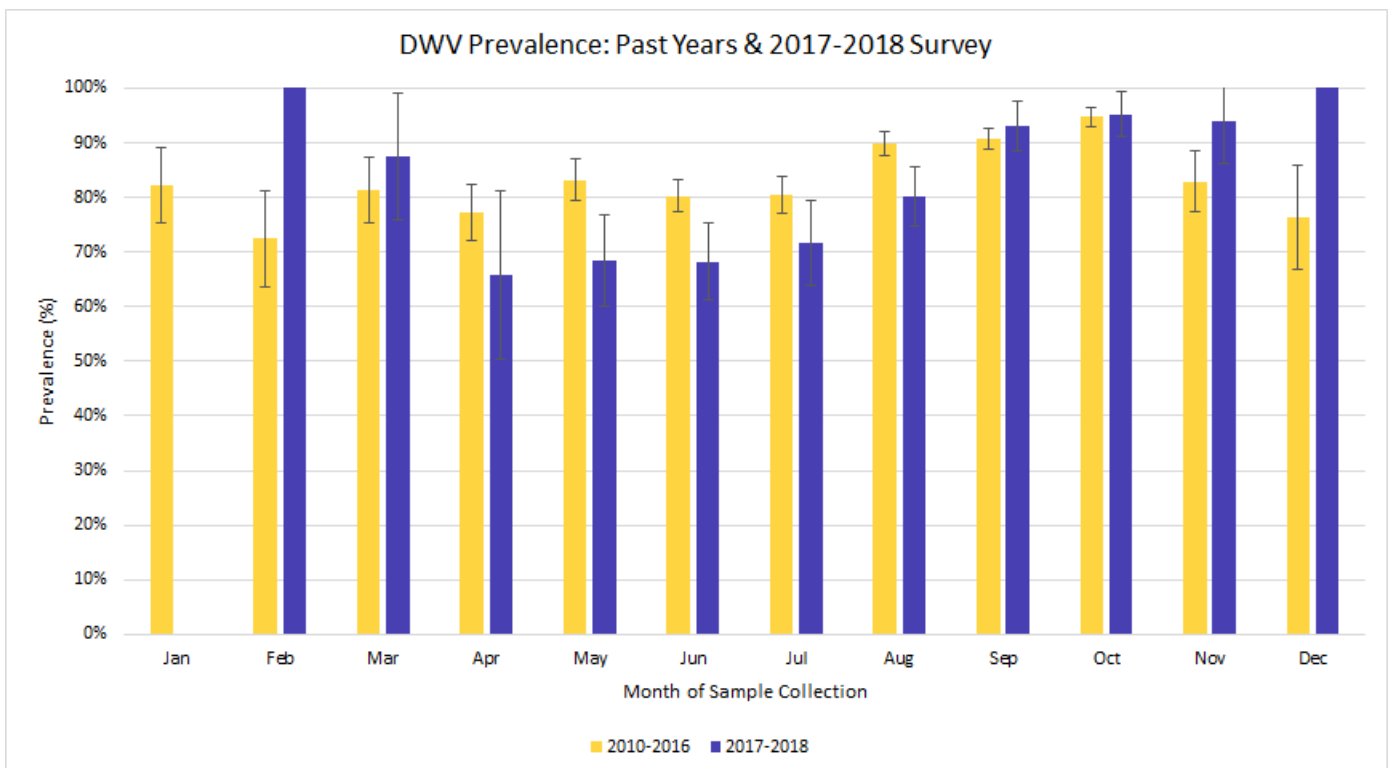


Figure 13: Prevalence of deformed wing virus by month (95% confidence intervals shown)

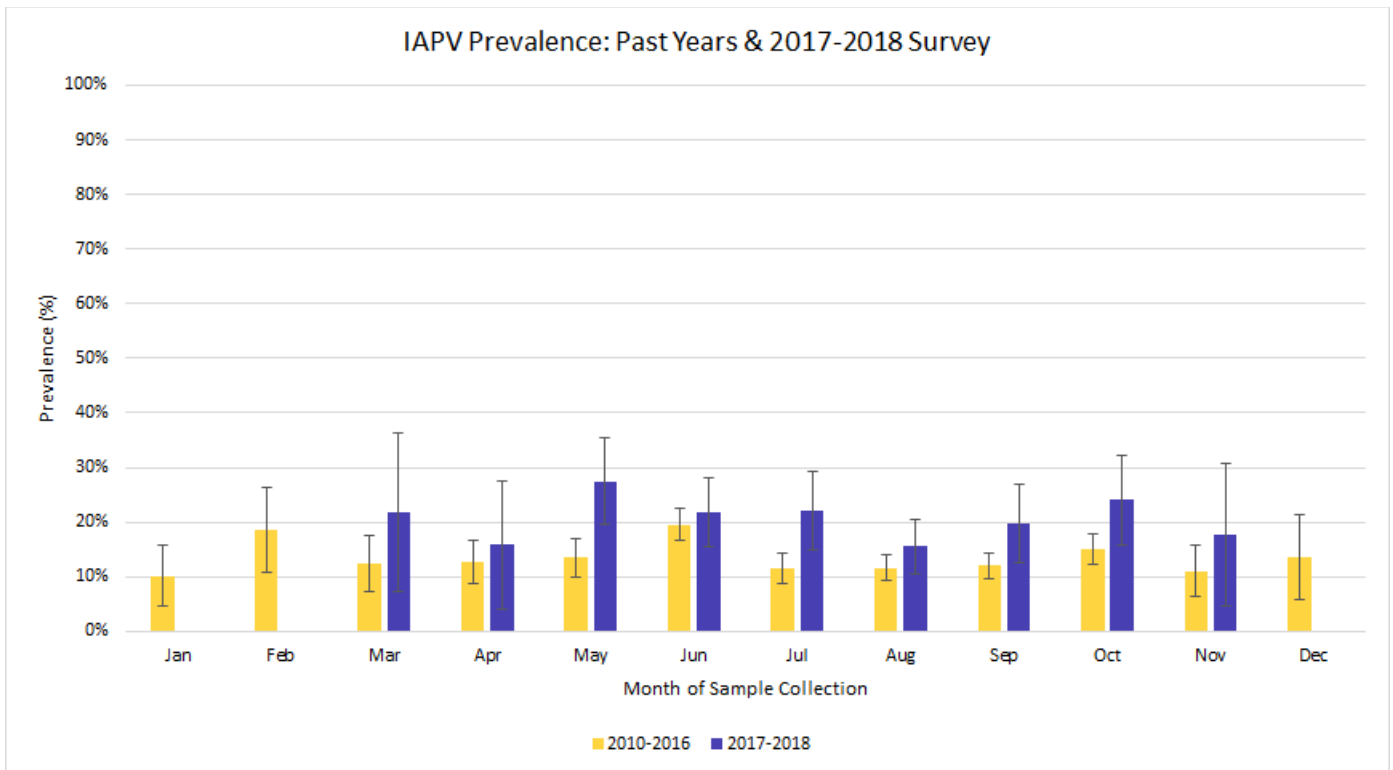


Figure 14: Prevalence of Israeli acute paralysis virus by month (95% confidence intervals shown)

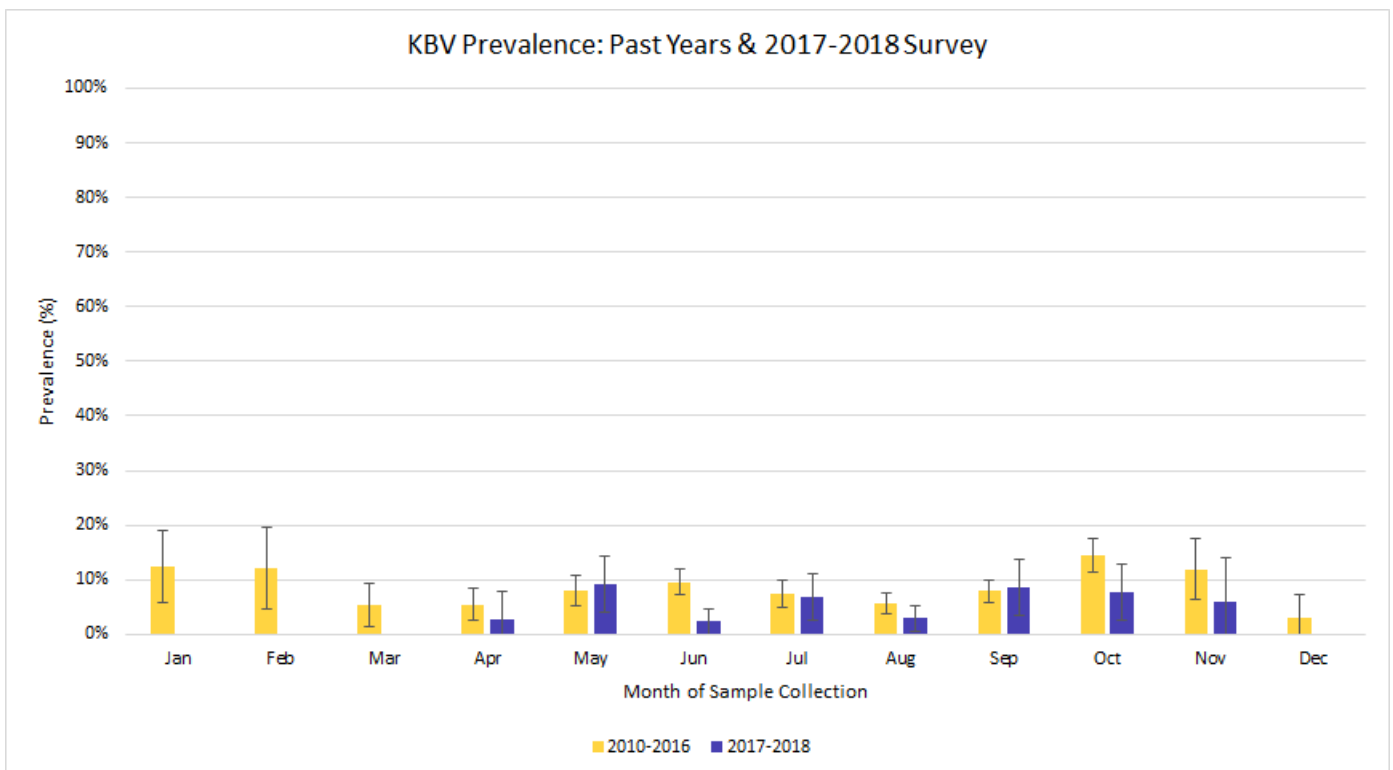


Figure 15: Prevalence of Kashmir bee virus by month (95% confidence intervals shown)

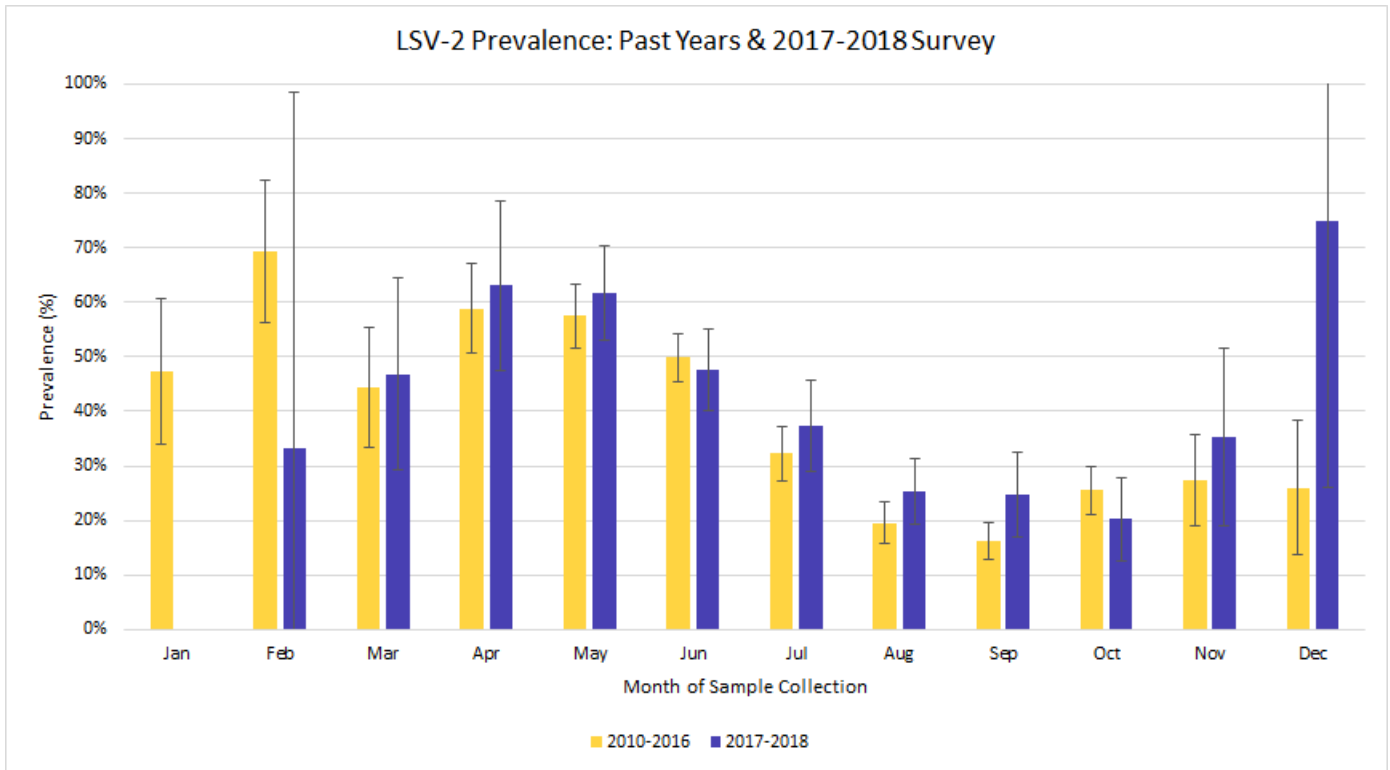


Figure 16: Prevalence of Lake Sinai virus 2 by month (95% confidence intervals shown)

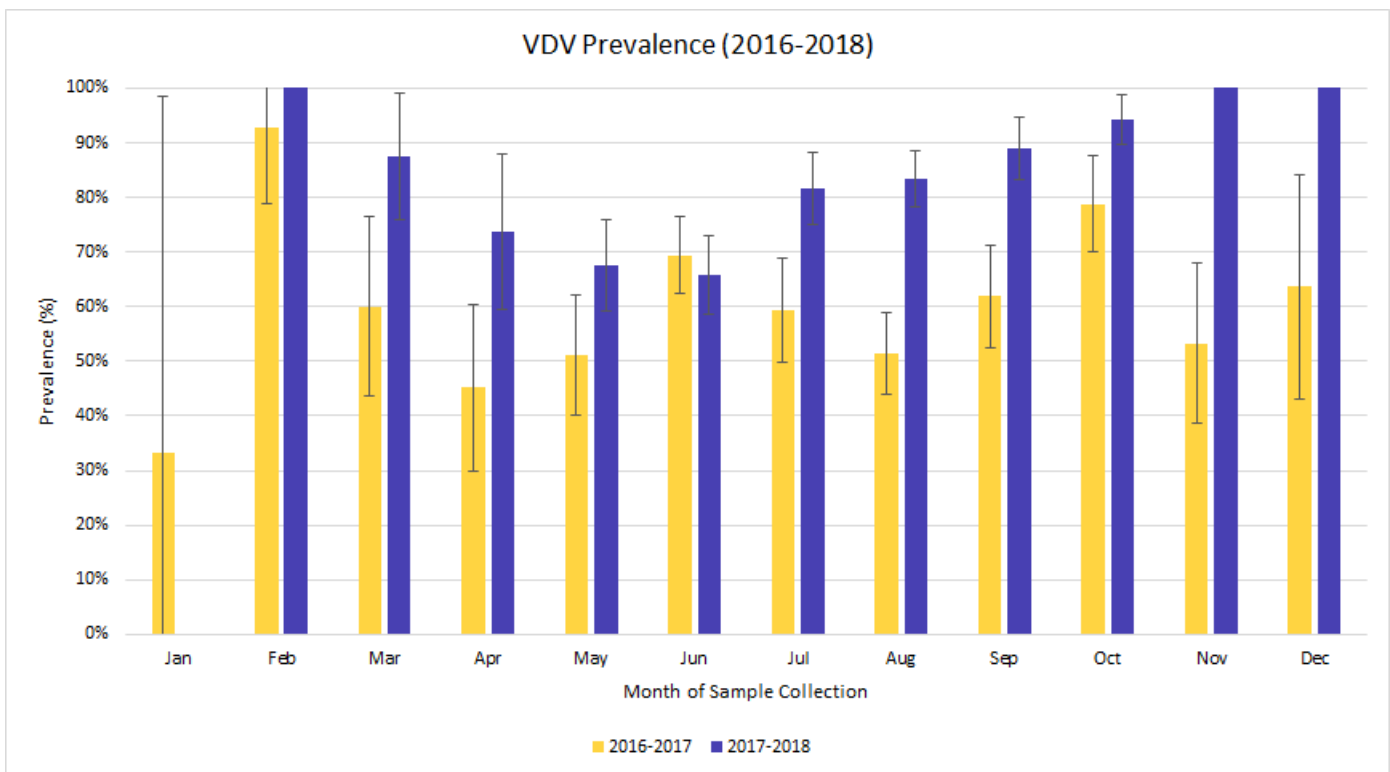


Figure 17: Prevalence of Varroa destructor virus by month (95% confidence intervals shown)

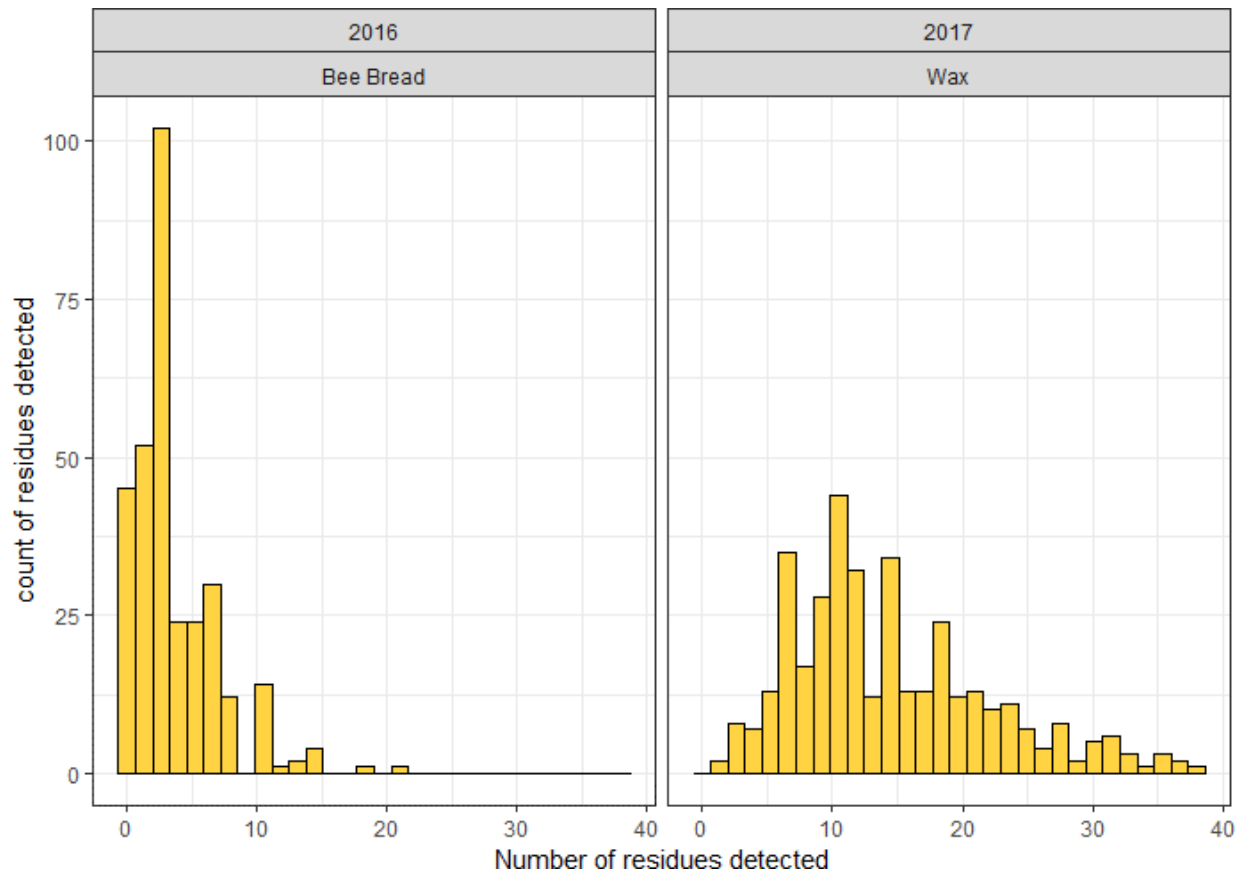


Figure 18: Histogram of number of residues detected per sample in bee bread (2016-17 survey year) and wax (2017-18 survey year)



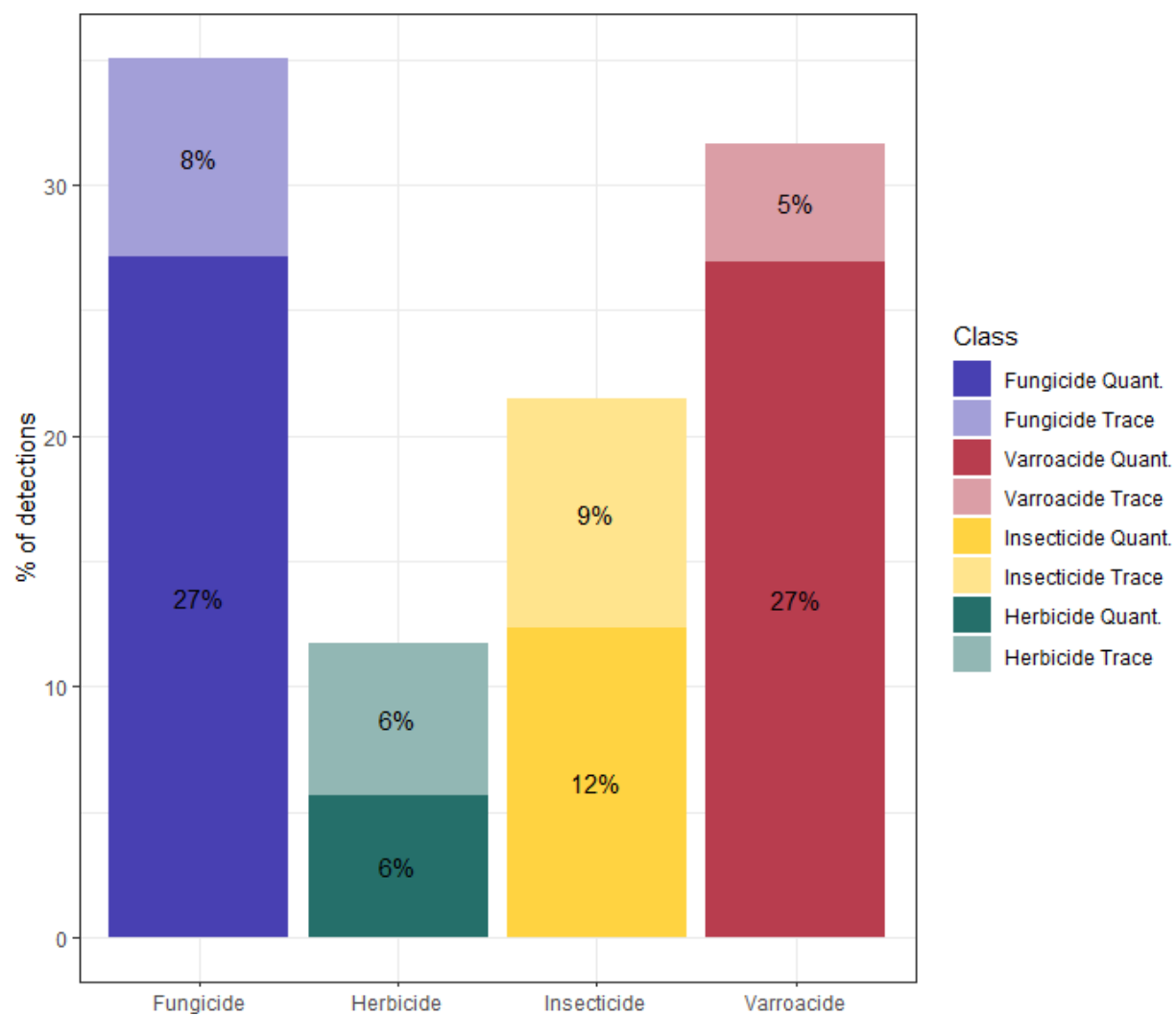


Figure 19: The relative frequency of each type of pesticide residue detected in wax (2017-18 survey year)

## References

- Bowen-Walker, P., Martin, S., Gunn, A., 1999. The Transmission of Deformed Wing Virus between Honeybees (*Apis mellifera* L.) by the Ectoparasitic Mite *Varroa jacobsoni* Oud. *J. Invertebr. Pathol.* 73, 101–106. <https://doi.org/10.1006/jipa.1998.4807>
- Carreck, N.L., Ball, B.V., Martin, S.J., 2010. Honey bee colony collapse and changes in viral prevalence associated with *Varroa destructor*. *J. Apic. Res.* 49, 93–94. <https://doi.org/10.3896/IBRA.1.49.1.13>
- Chantawannakul, P., Ramsey, S., vanEngelsdorp, D., Khongphinitbunjong, K., Phokasem, P., 2018. *Tropilaelaps* mite: an emerging threat to European honey bee. *Curr. Opin. Insect Sci.* 26, 69–75. <https://doi.org/10.1016/j.cois.2018.01.012>
- de Miranda, J.R., Dainat, B., Locke, B., Cordonni, G., Berthoud, H., Gauthier, L., Neumann, P., Budge, G.E., Ball, B.V., Stoltz, D.B., 2010. Genetic characterization of slow bee paralysis virus of the honeybee (*Apis mellifera* L.). *J. Gen. Virol.* 91, 2524–2530. <https://doi.org/10.1099/vir.0.022434-0>
- Fries, I., 1993. *Nosema apis* - a parasite in the honey bee colony - International Bee Research Association [WWW Document]. URL <http://www.ibra.org.uk/articles/Nosema-apis-a-parasite-in-the-honey-bee-colony> (accessed 12.1.13).
- Guzman, D., I, L., Williams, G.R., Khongphinitbunjong, K., Chantawannakul, P., 2017. Ecology, Life History, and Management of *Tropilaelaps* Mites. *J. Econ. Entomol.* 110, 319–332. <https://doi.org/10.1093/jee/tow304>
- Kulhanek, K., Steinhauer, N., Rennich, K., Caron, D.M., Sagili, R.R., Pettis, J.S., Ellis, J.D., Wilson, M.E., Wilkes, J.T., Tarpy, D.R., Rose, R., Lee, K., Rangel, J., vanEngelsdorp, D., 2017. A national survey of managed honey bee 2015–2016 annual colony losses in the USA 56, 328–340.
- Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of *Varroa destructor*. *J. Invertebr. Pathol.* 103, Supplement, S96–S119. <https://doi.org/10.1016/j.jip.2009.07.016>
- Traynor, K.S., Rennich, K., Forsgren, E., Rose, R., Pettis, J., Kunkel, G., Madella, S., Evans, J., Lopez, D., vanEngelsdorp, D., 2016. Multiyear survey targeting disease incidence in US honey bees. *Apidologie* 47, 325–347. <https://doi.org/10.1007/s13592-016-0431-0>

## Appendix

### Appendix 1: Sample data sheet

National Honey Bee Disease and Pest Survey Apiary Data Information Sheet									
Sample Identification:					PLACE SAMPLE ID STICKER HERE				
Collection date: _____									
Sampler name: _____					Sampler phone #: _____				
Sampler address: _____					Beekeeper phone #: _____				
Beekeeper name: _____					Beekeeper email address: _____				
Beekeeper address: _____					GPS – use decimal degrees, e.g. dd.ddddddd				
_____					Latitude: _____				
Sampling Address: _____					Longitude: _____				
_____					Name all locations (cities and states) where hive was located within the <u>past year</u> : _____				
Sampling County: _____									
State Origin of Hive: _____					Which of the following best describes the primary function of the sampled apiary?				
Is the sampled apiary part of a migratory operation?					[ ] honey production [ ] pollination [ ] queen production				
[ ] Yes [ ] No					[ ] Other (please specify): _____				
Please place a check (✓) or an 'X' in the colonies where the disease/pest/condition is observed. If there are no signs of the disease or pest, please write a "0" in the box unless otherwise directed. See back for additional guidance in completing this form.									
	Colony #								
Brood disease	1	2	3	4	5	6	7	8	Total
AFB									
EFB									
Sac Brood									
Chalkbrood									
Parasitic Mite Syndrome (PMS)/Snotty brood									
Adult disease									
Deformed wing virus									
Black shiny bees									
Pest infestation									
Small hive beetle - larvae or adult									
Wax moth - larvae or adult									
Queen condition									
Queen cells present									
Drone laying queen									
Queen right (queen or eggs are viewed)									
Queenless (no eggs or queen viewed)									
No. colonies in apiary: _____					No. colonies sampled: _____				
Comments:									

## Appendix 2: List of pesticides residues analyzed by AMS during the survey year 2017-2018

1-Naphthol, 2,4 Dimethylphenyl formamide (DMPF), 2,6-Dichlorobenzamide (BAM), 3-Hydroxycarbofuran, 4-Hydroxychlorothalonil, Acephate, Acetamiprid, Acetochlor, Acrinathrin, Alachlor, Aldicarb, Aldicarb sulfone, Aldicarb sulfoxide, Ametoctradin, Atrazine, Avermectin, Azinphos-methyl, Azoxystrobin, Bensulide, Bentazon, Bifenazate, Bifenthrin, Boscalid, Bromacil, Bromopropylate, Bromuconazole, Buprofezin, Captan, Carbaryl, Carbendazim, Carbofuran, Carfentrazone-ethyl, Chlorantraniliprole, Chlorfenapyr, Chlorfenvinphos, Chlorothalonil, Chlorpropham, Chlorpyrifos, Chlorpyrifos methyl, Chlorthal-dimethyl (DCPA), Clofentezine, Clothianidin, Coumaphos, Coumaphos oxon, Cyantraniliprole, Cyazofamid, Cyflufenamid, Cyflumetofen, Cyfluthrin, Cyhalothrin, cyhalothrin lambda, Cymiazole, Cymoxanil, Cypermethrin, Cyphenothrin, Cyprodinil, Cyromazine, DDE, p,p', DEET, Deltamethrin, Diazinon, Diazinon oxon, Dichlorvos (DDVP), Dicloran, Dicofol, Difenconazole, Diflubenzuron, Dimethenamid, Dimethoate, Dimethomorph, Dinotefuran, Diphenamid, Diphenylamine, Diuron, Emamectin Benzoate, Endosulfan I, Endosulfan II, Endosulfan sulfate, Epoxiconazole, Esfenvalerate, Esfenvalerate/Fenvalerate, Ethion, Ethofumesate, Etofenprox, Etoxazole, Famoxadone, Fenamidone, Fenarimol, Fenazaquin, Fenbuconazole, Fenhexamid, Fenoxaprop-p-ethyl, Fenpropathrin, Fenpyroximate, Fipronil, Fipronil sulfide, Fipronil sulfone, Flonicamid, Fludioxonil, Fluometuron, Fluopicolide, Fluopyram, Fluoxastrobin, Flupyradifurone, Fluridone, Flutriafol, Fluvalinate, Fluxapyroxad, Hexazinone, Hexythiazox, Imazalil, Imidacloprid, Indoxacarb, Iprodione Kresoxim-methyl, Linuron, Malathion, Mandipropamide, Metalaxyl, Metconazole, Methamidophos, Methidathion, Methomyl, Methoprene, Methoxyfenozide, Metolachlor, Metribuzin, MGK-264, Momfluorothrin, Myclobutanil, Norflurazon, Norflurazon desmethyl, Novaluron, Omethoate, Oxamyl, Oxyfluorfen, Parathion, Parathion ethyl, Parathion methyl, Penconazole, Pendimethalin, Penthioopyrad, Permethrin, Phenothrin, Phorate, Phosalone, Phosmet, Phosmet oxon, Picoxystrobin, Piperonyl butoxide, Prallethrin, Prodiamine, Profenofos, Prometon, Prometryn, Pronamide, Propachlor, Propamocarb hydrochloride, Propanil, Propargite, Propazine, Propetamphos, Propiconazole, Pymetrozine, Pyraclostrobin, Pyridaben, Pyrimethanil, Pyriproxyfen, Quinoxifen, Quintozene, Resmethrin, Resmethrin, cis, Resmethrin, trans, Sethoxydim, Simazine, Spinetoram, Spinosad, Spirodiclofen, Spiromesifen, Spirotetramat, Sulfoxaflor, Tebuconazole, Tebufenozide, Tebuthiuron, Tefluthrin, Tetraconazole, Tetradifon, Tetramethrin, Thiabendazole, Thiacloprid, Thiamethoxam, THPI, Thymol, Tolfenpyrad, Triadimefon, Triadimenol, Triazophos, Tribufos, Trifloxystrobin, Triflumizole, Trifluralin, Triticonazole, Vinclozolin