2015 – 2016 National Honey Bee Disease Survey Report

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Executive Summary

The 2015 – 2016 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 36 states 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, and 28 states in 2014. 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., the Asian honey bee, *Apis cerana*, and Slow Bee Paralysis Virus (SBPV). *Tropilaelaps* spp. is a parasitic mite native to Asia which, like *Varroa*, feeds on honey bee brood and vectors viruses (Chantawannakul et al., 2018). Because of its faster reproduction cycle, *Tropilaelaps* dominates in regions where it coexists with *Varroa* (Guzman et al., 2017). *Apis cerana* is a species of honey bee found in south and southeastern Asia, which resemble the western honey bee in that they both build nests in cavities. This similar lifestyle might explain why pathogens and parasite affecting *Apis cerana* have the potential to jump host to the western honey bee in their shared geographical area. *Apis cerana* was the original host of *Varroa destructor* and *Nosema ceranae* (Fries, 1993; Rosenkranz et al., 2010). SPBV is one of the viruses that can be 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).

If exotic honey bee pests like *Tropilaelaps* spp. were to be introduced to the United States it would threaten managed honey bee colonies in the United States which are already facing unsustainably high colony loss rates (Kulhanek et al., 2017). With honey bees contributing $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 Survey confirms the absence of such exotic honey bee pests, and allows us to deny importation of honey bees from other nations unless the exporting nation can confirm absence of *Tropilaelaps* spp., *Apis cerana*, and Slow Bee Paralysis Virus (SBPV).

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 over the years 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 have been incorporated into a single database, the nationwide Bee Informed Partnership (BIP) database. The Bee Informed Partnership is now a non-profit 501(c)(3) and 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/ and viral data provided here: https://bip2.beeinformed.org/state_reports/viruses/).

Introduction

The 2015 – 2016 Survey Year included sample collection in 36 states and one U.S. territory, Puerto Rico. The participating states include: Alabama, Arkansas, California, Connecticut, Florida, Georgia, Hawaii, Iowa, Idaho, Illinois, Indiana, Kentucky, Maryland, Michigan, Minnesota, Mississippi, Montana, North Carolina, North Dakota, 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 network of surveillance that maximizes the chances of detecting the arrival of the exotic pests while being representative of the managed honey bee colonies of the US. The survey was open to any state wishing to participate. In cooperating states, the state department of Agriculture (where applicable) was tasked with randomly selecting beekeeping operations to be sampled from each quadrant of their state, with particular attention to queen breeders and those areas considered higher risks of invasion (such as ports). When possible, half of the samples were collected from migratory operations and half from stationary, both commercial and small-scale operations. Beekeeper participation within the states was voluntary and any identifiable information was kept strictly confidential in any resulting report and publication. With sampling occurring throughout the majority of the US, this stratified random survey offers one of the most systematic and comprehensive representation of the pests and disease levels in US 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 till 2014-15) were published in that effect in Traynor et al. (2016).

Milestones and Project Timelines

The survey design is constantly evolving to reflect the recommendations of scientific experts in order to best fit the objectives of the program based on the most updated scientific knowledge available. These protocols or targets are likely to continue to change as new threats 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). Since 2012, all participating states sent in 10 bee bread samples for pesticide detection and quantification.

- 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.

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.

Apiary Specialists conducted an aggregate sampling from previously identified commercial, migratory, side-liner and backyard beekeepers with at least 8 colonies per apiary. In most cases, apiaries included at least 10 colonies. Under guidelines provided to the Apiary Specialists, they selected apiaries to be sampled with 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 a quadrant. 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 occurring near deep water shipping ports or other areas that could be at risk of exotic pest or disease invasion were also considered for sampling.

In each selected apiary, a single aggregate sample was collected from 8 randomly selected colonies. Three distinctive collection methods were used to sample each apiary: 1. Live bee sampling, 2. Alcohol preserved sampling of bees, and 3. Brood frame bump sampling.

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

The live bee sample was collected from a brood frame with contained both capped and uncapped brood. ¼ cup of nurse bees were taken from each of the 8 colonies and collected in an aggregate sample contained in a live bee shipping box. Using the U.S. Postal Service (USPS), live bee shipments were mailed to USDA/ARS where they were promptly frozen at -80°C. The frozen bees were then 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.

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 be analyzed for *Varroa destructor* loads, *Nosema* spp. spore loads, and presence or absence of *Apis cerana.*

The brood bump sample was taken from debris dislodged by 'bumping' sampled brood frames over a collection pan. The brood frame debris were then 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.

Bee bread was also collected in a subsample of 10 apiaries from 12 states. Bee bread is pollen honey bees have gathered from flowers, fermented and stored within the hive. A minimum of 3 grams of bee bread was collected from the same 8 colonies, preferably in the same brood area, from the other three samples described above. These samples were shipped to University of Maryland where they were catalogued by UMD personnel and sent to the USDA AMS Lab in Gastonia, NC for pesticide analysis.

In the 2015-2016 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. Slow bee paralysis virus (SBPV)

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

The brood bump samples were analyzed for:

1. *Tropilaelaps* spp. presence or absence

The bee bread samples were analyzed for:

1. 174 different pesticides measured in parts per billion (ppb) which included varroacides, insecticides, herbicides, and fungicides (list of analytes is determined by the USDA AMS lab and is depicted in Figure 14)

All participating beekeepers, as well as Apiary Specialists, State Survey Coordinators, State Plant Regulatory Officials, and APHIS State Plant Health Directors, receive 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.

Results

The APHIS National Survey has confirmed the absence, as of 2016, of *Tropilaelaps* spp., *Apis cerana*, and Slow Bee Paralysis Virus (SBPV). The absence of these exotic pests and pathogens in the 2015 – 2016 Survey suggest that the current policies to prevent their introduction into the United States have been successful.

At the start of this survey year, a total of 912 sampling kits were sent out (36 states at 24 kits per state, plus 24 for Puerto Rico and an extra 24 for California). At the conclusion of the survey year, 814 live bee boxes were returned (89.3% return rate), 811 alcohol samples (88.9% return rate) and 809 Tropilaelaps bump samples (88.7% return rate).

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 811 alcohol samples that were analyzed for *Nosema* spp. spore load, 358 (44%) tested positive (Figure 1). The average *Nosema* spore load was 0.49 million spores per bee for samples that tested positive (Figure 2). Of all samples that were processed for *Nosema* spp. spores, 5.1% (42) exceeded the threshold thought to cause damage (more than 1 million spores per bee). This result shows a small decrease from the 2014-2015 APHIS National Survey when 7.8% of all samples processed exceeded the threshold. Figures 1 and 2 illustrate *Nosema* spp. prevalence, and *Nosema* spp. spore load from 2010 to 2016. Average *Nosema* spp. spore load (Figure 3) varies throughout the year, 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 811 alcohol samples that were analyzed for *Varroa* load during the 2015-2016 APHIS National Survey, 744 (92%) were positive for mites (Figure 4). This is an increase in prevalence from the 2014-2015 survey year where 86% tested positive. 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 irreparable damage to a colony of honey bees. This threshold was exceeded in 41.7% (342) of all samples analyzed. The average *Varroa* load was found to be 4.14 mites per 100 bees for samples that tested positive (Figure 5). Figure 6 illustrates the dynamic nature and seasonality of mite populations across all years of the APHIS National Honey Bee Survey. Generally, *Varroa* increases exponentially in the late summer and peaks in the fall.

Viral Load and Prevalence

Of the 814 live bee boxes that were received, 779 (95.7%) of all samples were analyzed for viruses. The other 35 live bee samples were insufficient for analyses. Reasons for a sample to be insufficient can include live bees dying in transit, loss of sample in long term storage or low quality RNA due to insufficient nucleic acid extraction. Figure 7 illustrates the viral prevalence of all targets that were tested from 2010 to 2016 (ABPV, BQCV, CBPV, DWV, IAPV, KBV and LSV-2). The most prevalent virus detected

in the 2015 – 2016 survey year was deformed wing virus (DWV) found in 92% (714) of all samples. This is an increase from previous years of the survey which average at 85% prevalence for the virus. *Varroa destructor* is known to be a vector of deformed wing virus, transferring the virus from one bee to another (Bowen-Walker et al., 1999). Support of this can been found in the APHIS National Survey by the association between prevalence of DWV and *Varroa* (Figure 8).

The least prevalent virus in the 2015-2016 survey year was Kashmir bee virus (KBV) detected in 7% of all samples tested. Although KBV does not appear to be problematic for the U.S. honey bee population, the rising prevalence of Chronic Bee Paralysis Virus (CBPV) may become concerning. When the survey first began in 2010, the incidence of CBPV was quite low, occurring in only 9% of all samples tested. However in recent years (2015-2016 survey year), prevalence of CBPV has risen to 14% (Figure 7). The APHIS National Survey will continue to monitor changes in CBPV incidence.

Another subject 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 9). Incidence of the virus is higher in the spring, peaking in April at 61% in the 2015-2016 survey year. These levels gradually decreased into the fall, and were at their lowest in December at 20%.

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, hovering around 20% detection in all samples tested each survey year (Figure 7).

Chronic Bee Paralysis Virus (CBPV), Israeli Acute Paralysis Virus (IAPV), and Kashmir Bee Virus (KBV), do not seem to exhibit seasonal changes. Results for these viruses can be found in Figures 11-13.

Pesticide Detections in Bee Bread

This year, 12 states (Arkansas, California, Florida, Idaho, Illinois, Kansas, Montana, North Dakota, New Jersey, New York, Oregon and Texas) submitted composite bee bread samples (79 total samples). These samples were tested by USDA AMS in Gastonia, NC through their Apiculture Pesticide Residue Screen, which includes testing for 174 different compounds.

The most prevalent pesticides in bee bread are miticides applied by beekeepers to control infestations of *Varroa destructor.* These miticides, also known as varroacides, include the Amitraz metabolite 2,4 Dimethylphenyl formamide (detected in 40.5% of samples), Fluvalinate (detected in 27.9% of samples), Coumaphos (detected in 12.7% of samples), and Thymol (detected in 10.1% of samples). The most prevalent insecticide detected was Chlorpyrifos, found in 22.8% of samples. The fungicide with highest number of detections was Azoxystrobin, found in 13.9% of samples. The most prevalent herbicide was Atrazine, detected in 8.9% of samples.

On average each sample had 3 different compounds detected with as many as 11 compounds detected in a single sample. The full set of results, grouped by their classification as a varroacide, insecticide, fungicide or herbicide is in Figure 14. The level of detection (LOD), or minimum amount that can be detected, the prevalence (%) within this survey year, the average quantity detected (ppb), and the range of detection (ppb) are provided for each pesticide tested. If a pesticide was detected only once, a single

value is given for the range and is marked with an asterisk. The breakdown in classification of the pesticides detected for the 2015-2016 survey can be found in the pie chart, Figure 15.

Conclusions

Nosema spp. spore prevalence has been historically consistent since the origin of the APHIS National Survey. On average, *Nosema* spores have been detected in 50% of all samples. Although prevalence has remained about the same, the average load of *Nosema* spores appears to be decreasing over time. Currently, the average *Nosema* spore load was 0.49 million spores per bee, which is down from the 2014-2015 survey where the average *Nosema* spore load was 0.69 million spores per bee. This trend will continue to be monitored in subsequent years of the National Survey.

The prevalence of *Varroa destructor* in APHIS National Survey samples has remained relatively the same since 2010, and has been detected in 91% 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 4.1 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.

Results from the 2015-2016 APHIS National Survey provide strong evidence for the absence of *Tropilaelaps* spp., and *Apis cerana*. The absence of these species suggest that the current methods of preventing potentially harmful honey bee pests from entering the United States have been successful.

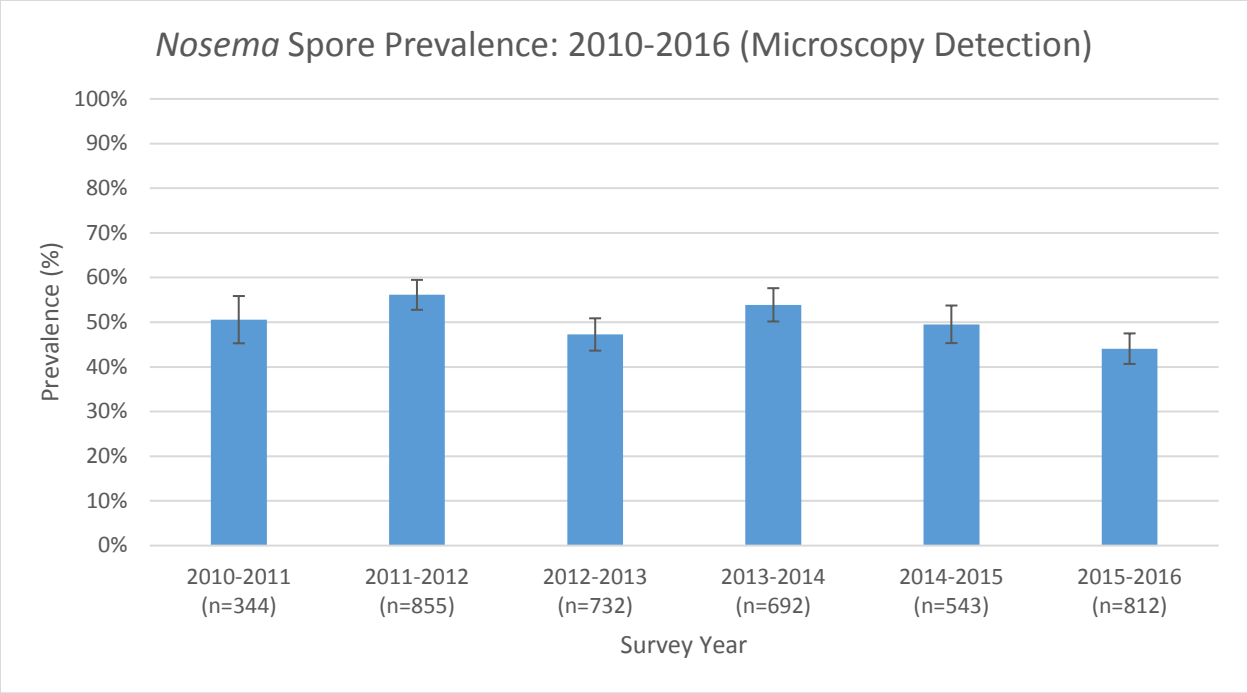

Figure 1: *Nosema* prevalence by survey year (95% confidence intervals shown)

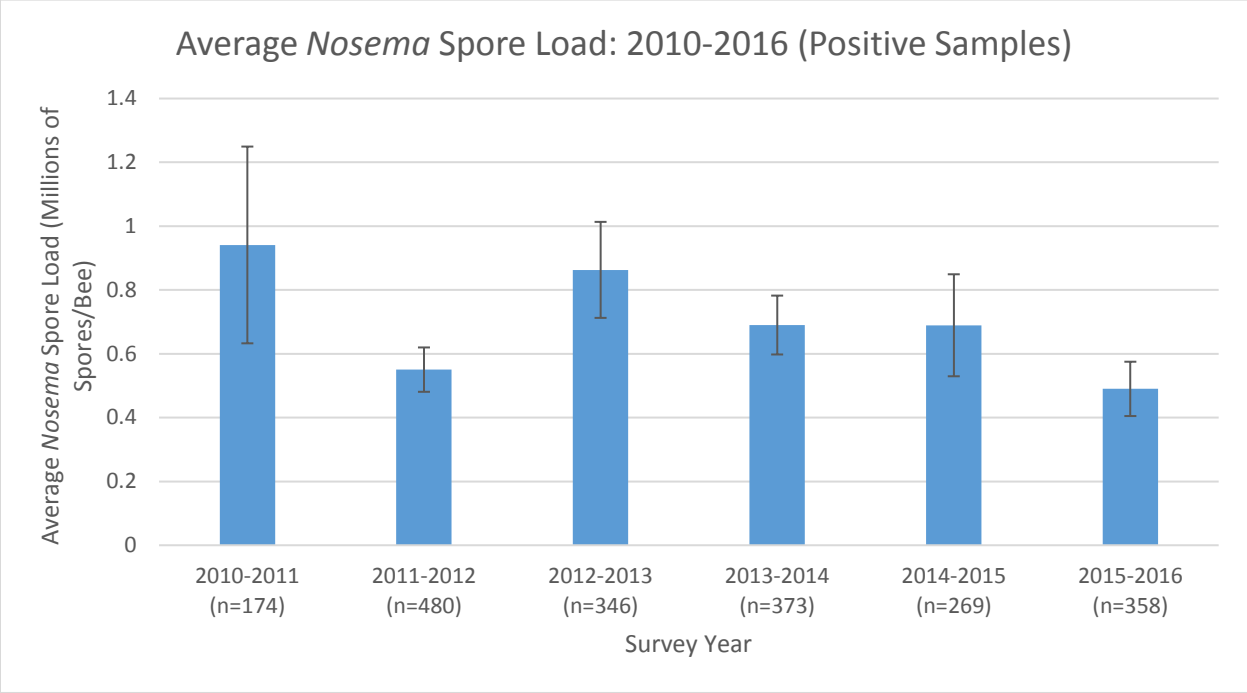

Figure 2: *Nosema* spore load by survey year (95% confidence intervals shown)

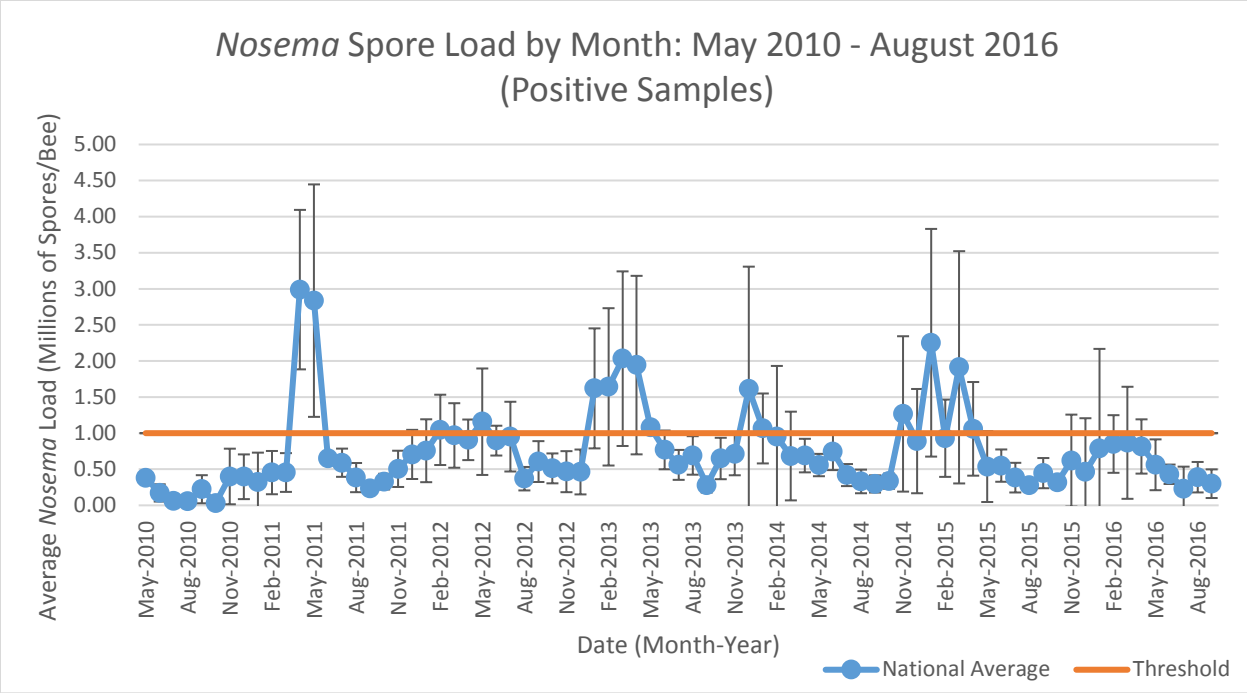

Figure 3: *Nosema* spore load by month from May 2010 to August 2016 (95% confidence intervals shown)

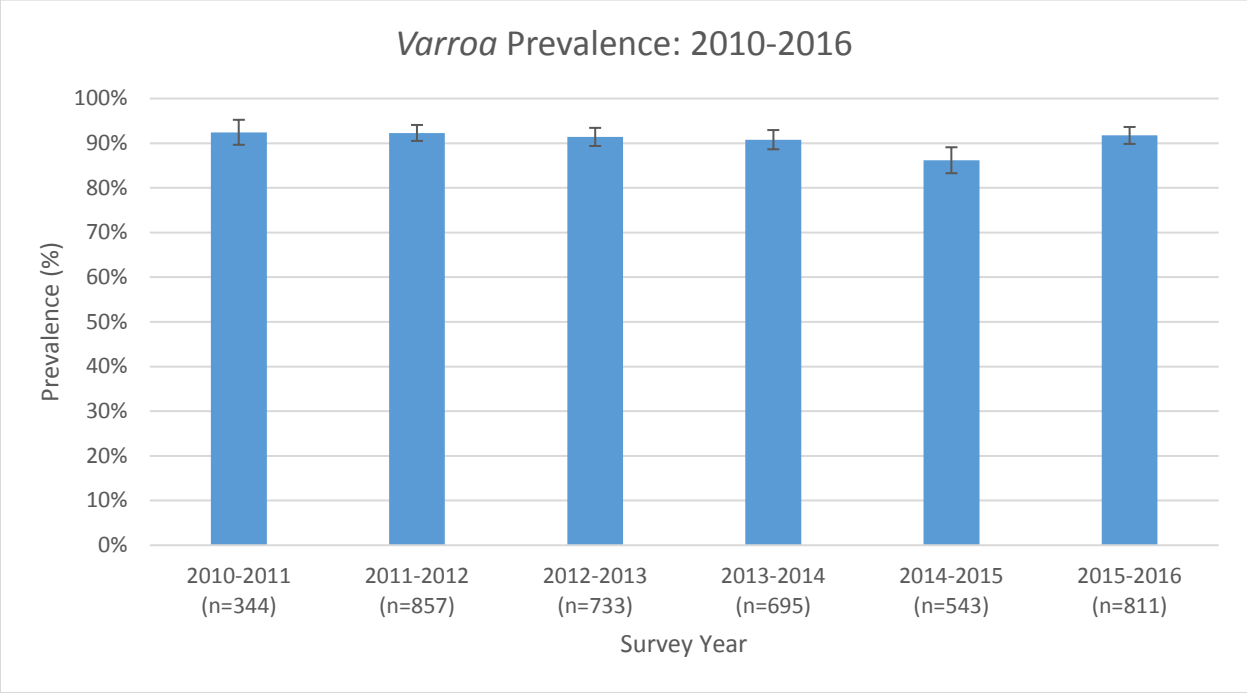

Figure 4: *Varroa* prevalence by survey year (95% confidence intervals shown)

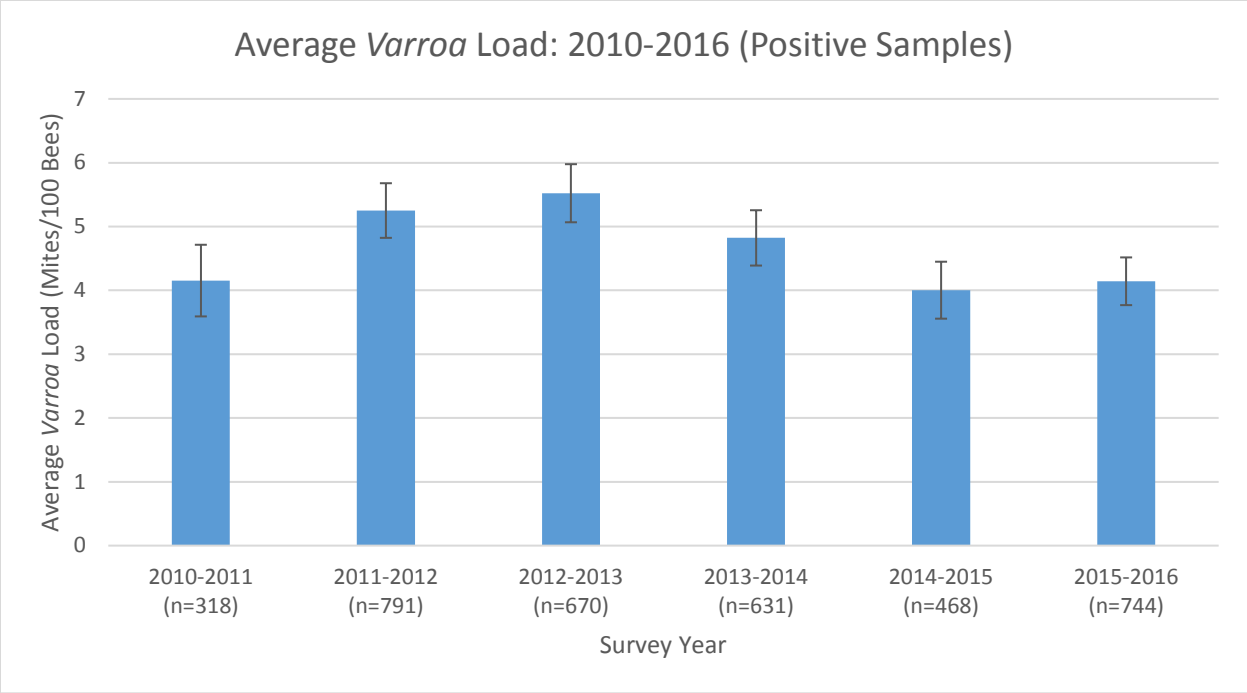

Figure 5: *Varroa* load by survey year (95% confidence intervals shown)

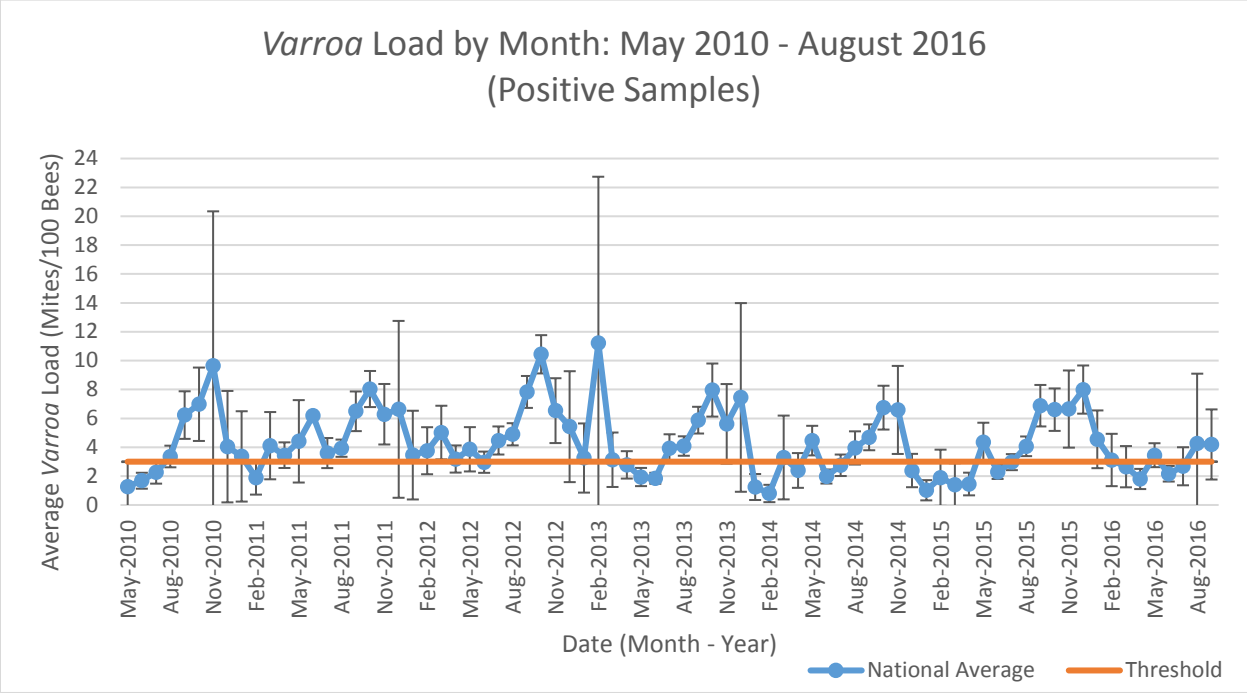

Figure 6: *Varroa* load by month from May 2010 to August 2016 (95% confidence intervals shown)

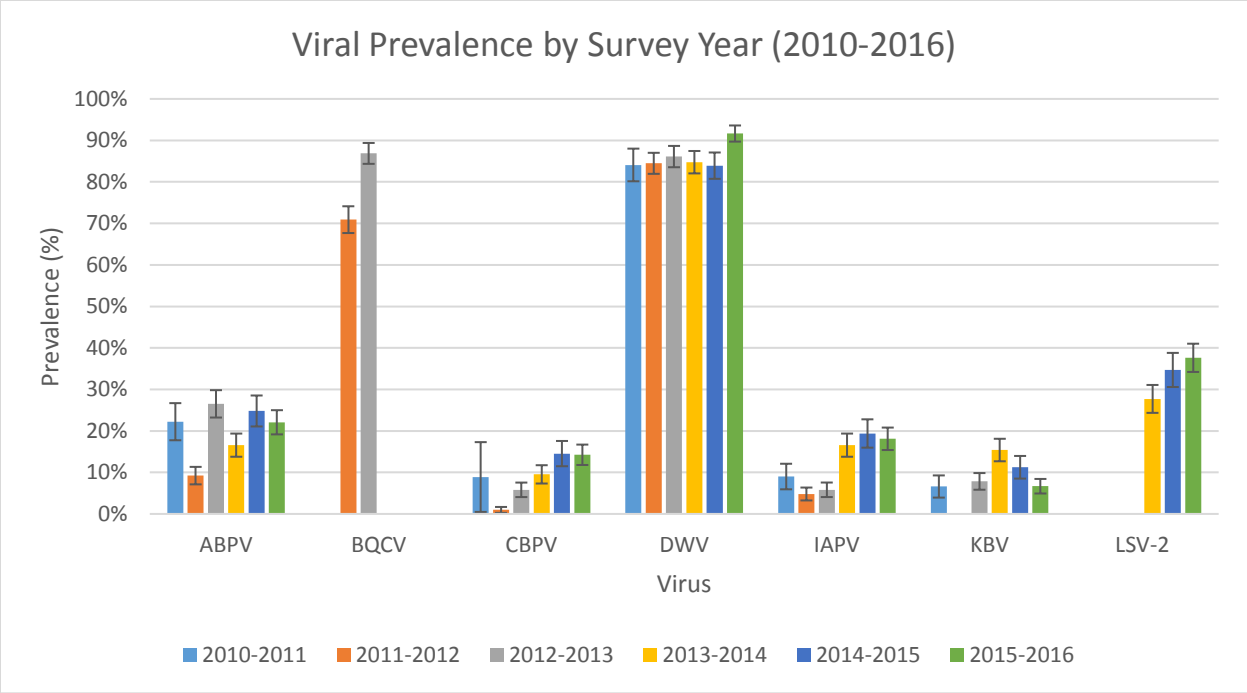

Figure 7: Yearly changes in viral prevalence from 2010 to 2016 (95% confidence intervals shown)

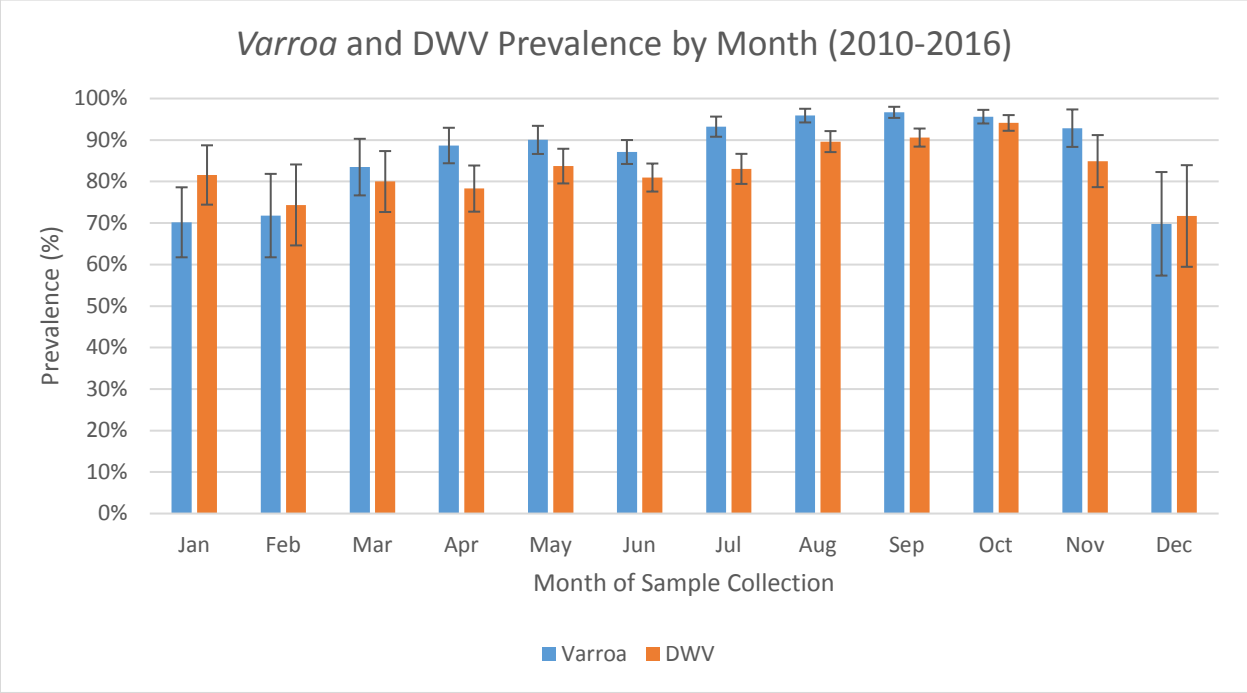

Figure 8: Prevalence of *Varroa* and deformed wing virus (DWV) by month (95% confidence intervals shown)

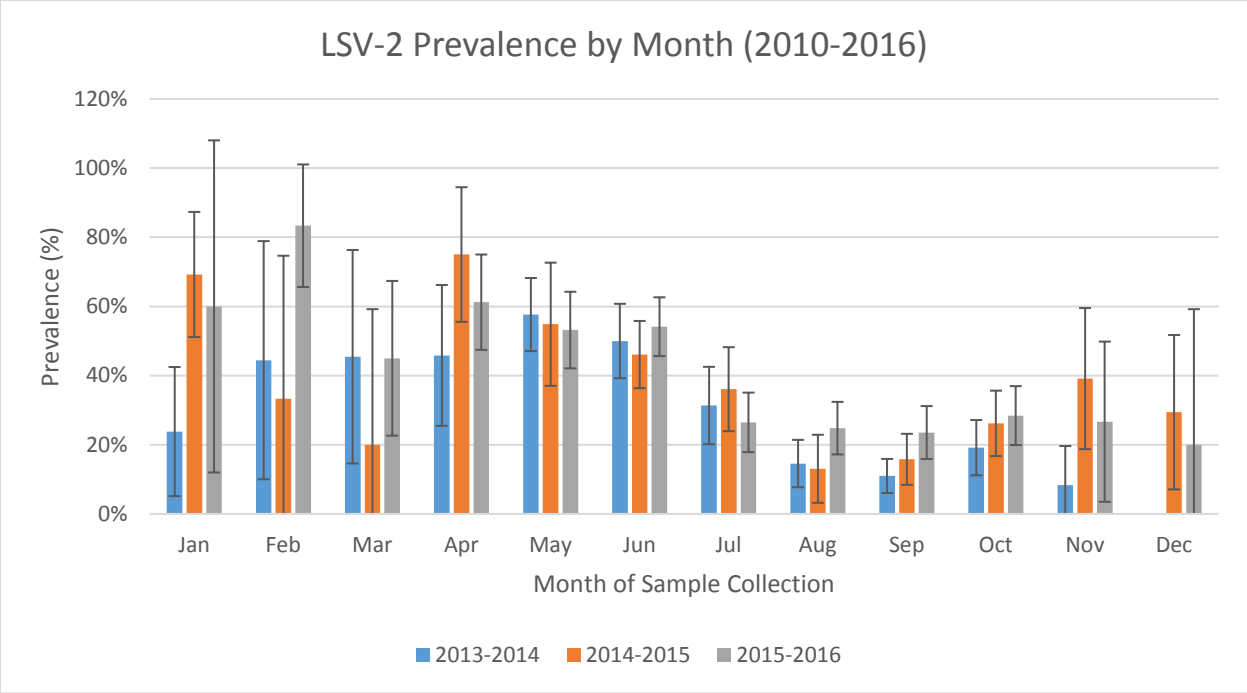

Figure 9: Prevalence of Lake Sinai virus 2 by month (95% confidence intervals shown)

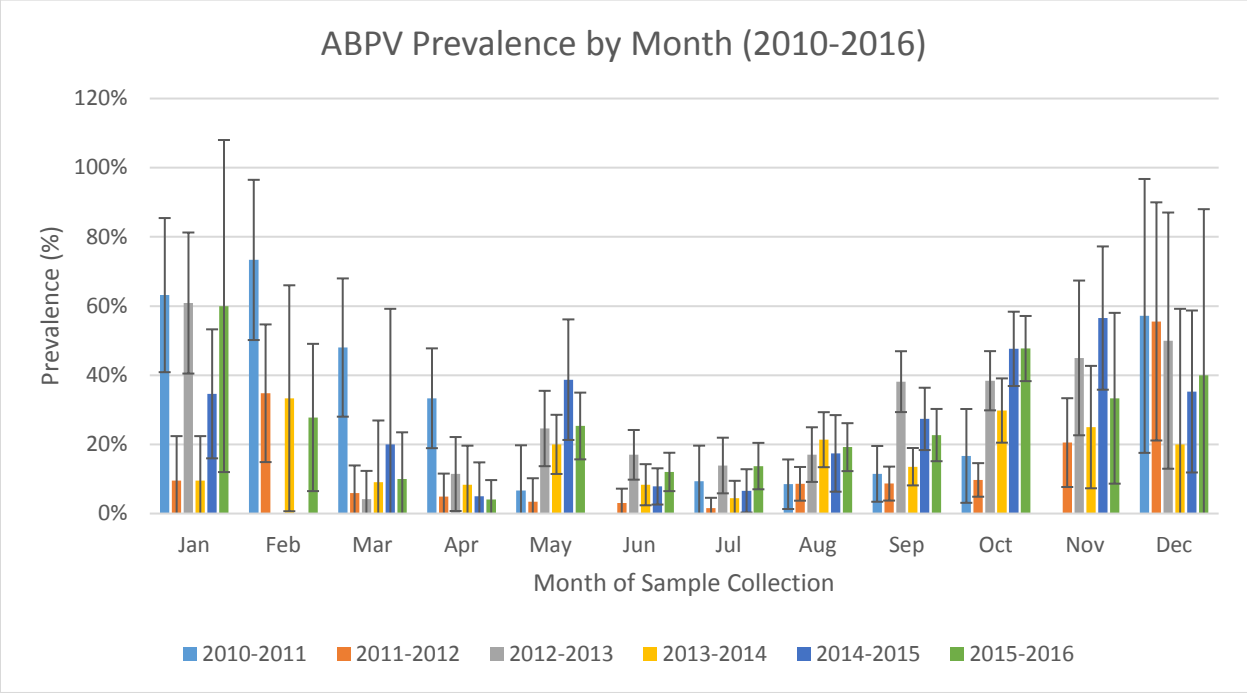

Figure 10: Prevalence of acute bee paralysis virus by month (95% confidence intervals shown)

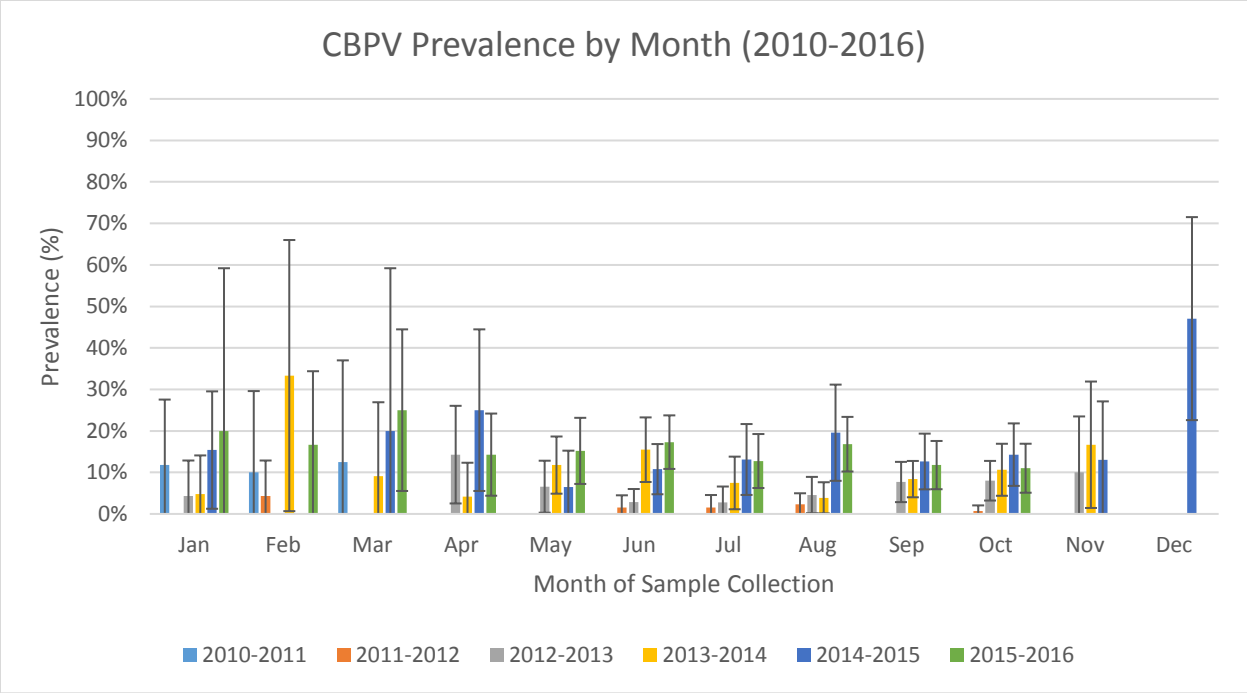

Figure 11: Prevalence of chronic bee paralysis virus by month (95% confidence intervals shown)

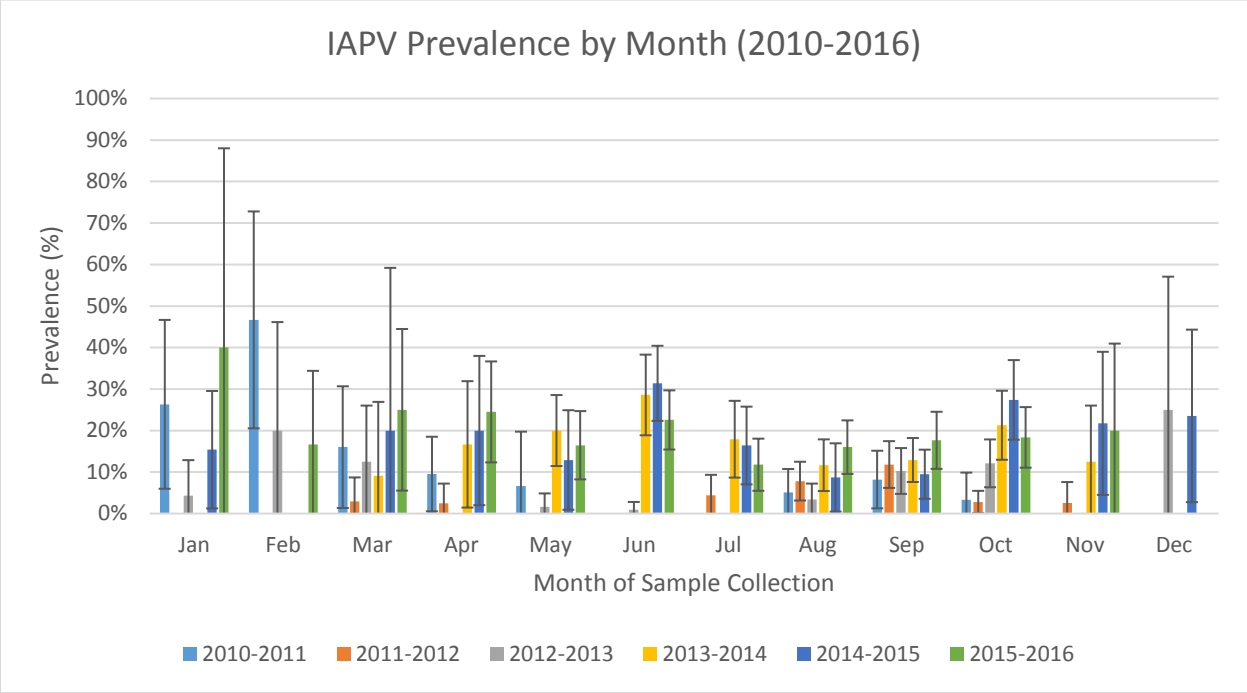

Figure 12: Prevalence of Israeli acute paralysis virus by month (95% confidence intervals shown)

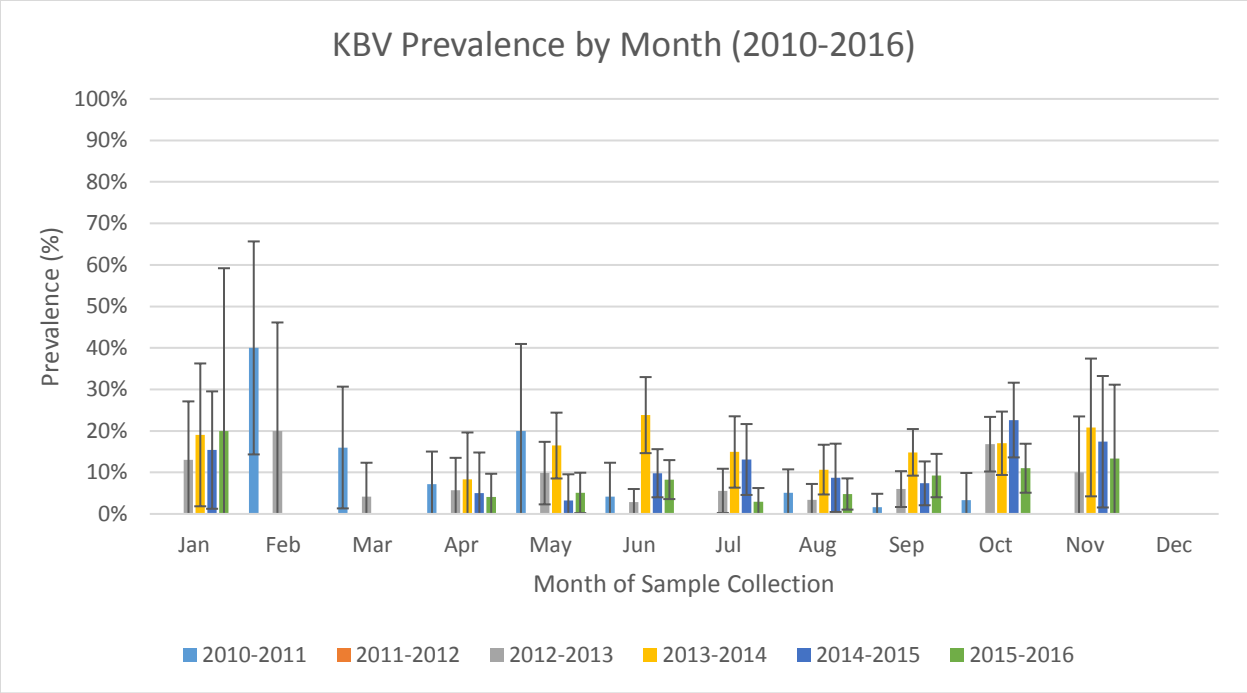

Figure 13: Prevalence of Kashmir bee virus by month (95% confidence intervals shown)

Pesticide	Type	LOD (ppb)	Prevalence %	Average detection if positive for target (ppb)	Range if positive for target (ppb)
1-Naphthol	Insecticide	50	N/A	N/A	N/A
2,4 Dimethylaniline	Varroacide	250	2.53%	2245	1150 - 3340
2,4 Dimethylphenyl formamide (DMPF)	Varroacide	5	40.51%	47.7	5 - 123
3-Hydroxycarbofuran	Insecticide	10	N/A	N/A	N/A
4-Hydroxychlorothalonil	Fungicide	10	N/A	N/A	N/A
4,4 Dibromobenzophenone	Insecticide	0	N/A	N/A	N/A
Acephate	Insecticide	50	N/A	N/A	N/A
Acetamiprid	Insecticide	4	1.27%	81	81*
Acetochlor	Herbicide	15	1.27%	88.3	88.3*
Alachlor	Herbicide	25	N/A	N/A	N/A
Aldicarb	Insecticide	25	N/A	N/A	N/A
Aldicarb sulfone	Insecticide	15	N/A	N/A	N/A
Aldicarb sulfoxide	Insecticide	25	N/A	N/A	N/A
Aldrin	Insecticide	30	N/A	N/A	N/A
Allethrin	Insecticide	10	N/A	N/A	N/A

Amicarbazone	Insecticide	15	N/A	N/A	N/A
Amitraz	Varroacide	0	N/A	N/A	N/A
Atrazine	Herbicide	4	8.86%	29.7	11.1 - 72.1
Azinphos methyl	Insecticide	15	N/A	N/A	N/A
Azoxystrobin	Fungicide	5	13.92%	43.6	11.4 - 106
Bendiocarb	Insecticide	10	N/A	N/A	N/A
Benoxacor	Herbicide	15	N/A	N/A	N/A
BHC alpha	Insecticide	15	N/A	N/A	N/A
Bifenazate	Insecticide	15	N/A	N/A	N/A
Bifenthrin	Insecticide	10	1.27%	41.4	41.4*
Boscalid	Fungicide	10	7.59%	294.6	1 - 1630
Bromuconazole	Fungicide	50	N/A	N/A	N/A
Buprofezin	Insecticide	60	N/A	N/A	N/A
Captan	Fungicide	50	8.86%	803.5	45.5 - 5180
Carbaryl	Insecticide	2	5.06%	15	Trace - 15
Carbendazim	Fungicide	5	6.33%	37.8	1 - 126
Carbofuran	Insecticide	10	N/A	N/A	N/A
Carboxin	Fungicide	15	N/A	N/A	N/A
Carfentrazone ethyl	Herbicide	5	N/A	N/A	N/A
Chlorantraniliprole	Insecticide	15	2.53%	90.3	Trace - 90.3
Chlorfenopyr	Insecticide	5	N/A	N/A	N/A
Chlorfenvinphos	Insecticide	10	N/A	N/A	N/A
Chlorothalonil	Insecticide	100	3.80%	463.5	51.4 - 1070
Chlorpropham (CIPC)	Insecticide	10	N/A	N/A	N/A
Chlorpyrifos	Insecticide	5	22.78%	28.3	3.8 - 233
Chlorpyrifos methyl	Insecticide	5	N/A	N/A	N/A
Clofentezine	Insecticide	6	N/A	N/A	N/A
Clothianidin	Insecticide	15	1.27%	40	40*
Coumaphos	Varroacide	3	12.66%	53.1	1 - 268
Cyfluthrin	Insecticide	10	N/A	N/A	N/A
Cyhalothrin total	Insecticide	5	1.27%	7.5	7.5*
Cypermethrin	Insecticide	10	N/A	N/A	N/A
Cyphenothrin	Insecticide	100	N/A	N/A	N/A
Cyprodinil	Fungicide	10	10.13%	1339.8	Trace - 5800
DDD p,p'	Insecticide	5	N/A	N/A	N/A
DDT p,p'	Insecticide	5	N/A	N/A	N/A
Deltamethrin	Insecticide	50	N/A	N/A	N/A
Diazinon	Insecticide	15	N/A	N/A	N/A
Dichlorvos (DDVP)	Insecticide	15	N/A	N/A	N/A
Dicloran	Fungicide	5	N/A	N/A	N/A
Dicofol	Insecticide	5	1.27%	47	47*

Dieldrin	Insecticide	10	N/A	N/A	N/A
Difenoconazole	Fungicide	10	N/A	N/A	N/A
Diflubenzuron	Insecticide	5	N/A	N/A	N/A
Dimethenamid	Herbicide	10	N/A	N/A	N/A
Dimethoate	Insecticide	15	N/A	N/A	N/A
Dimethomorph	Fungicide	25	1.27%	197	197*
Dinotefuran	Insecticide	10	N/A	N/A	N/A
Diphenamid	Herbicide	3	N/A	N/A	N/A
Endosulfan I	Insecticide	10	N/A	N/A	N/A
Endosulfan II	Insecticide	10	N/A	N/A	N/A
Endosulfan sulfate	Insecticide	10	N/A	N/A	N/A
Endrin	Insecticide	25	N/A	N/A	N/A
Epoxiconazole	Fungicide	5	N/A	N/A	N/A
Esfenvalerate	Insecticide	5	N/A	N/A	N/A
Ethion	Insecticide	15	N/A	N/A	N/A
Ethofumesate	Herbicide	20	N/A	N/A	N/A
Etoxazole	Insecticide	5	1.27%	2.9	2.9*
Etridiazole	Fungicide	5	N/A	N/A	N/A
Famoxadone	Fungicide	25	N/A	N/A	N/A
Fenamidone	Fungicide	30	N/A	N/A	N/A
Fenbuconazole	Fungicide	15	11.39%	177.7	Trace - 809
Fenhexamid	Fungicide	30	N/A	N/A	N/A
Fenoxaprop-ethyl	Herbicide	15	N/A	N/A	N/A
Fenpropathrin	Insecticide	10	N/A	N/A	N/A
Fenpyroximate	Varroacide	4	5.06%	61.9	1 - 214
Fenthion	Insecticide	15	N/A	N/A	N/A
Fipronil	Insecticide	50	N/A	N/A	N/A
Flonicamid	Insecticide	15	N/A	N/A	N/A
Flubendiamide	Insecticide	10	N/A	N/A	N/A
Fludioxonil	Fungicide	60	1.69%	53.8	53.8*
Fluopyram	Fungicide	5	3.39%	Trace	Trace
Fluoxastrobin	Fungicide	5	N/A	N/A	N/A
Fluridone	Herbicide	5	N/A	N/A	N/A
Flutolanil	Fungicide	15	N/A	N/A	N/A
Fluvalinate	Varroacide	5	27.85%	58.3	4.8 - 413
Heptachlor	Insecticide	15	N/A	N/A	N/A
Heptachlor epoxide	Insecticide	15	N/A	N/A	N/A
Hexachlorobenzene (HCB)	Insecticide	5	N/A	N/A	N/A
Hexythiazox	Fungicide	15	N/A	N/A	N/A
Hydroprene	Insecticide	100	N/A	N/A	N/A
Imazalil	Fungicide	20	N/A	N/A	N/A

Imidacloprid	Insecticide	6	2.53%	12	5 - 18.9
Imidacloprid 5-hydroxy	Insecticide	150	N/A	N/A	N/A
Imidacloprid olefin	Insecticide	50	N/A	N/A	N/A
Indoxacarb	Insecticide	30	N/A	N/A	N/A
Iprodione	Fungicide	50	N/A	N/A	N/A
Lindane	Insecticide	10	N/A	N/A	N/A
Linuron	Herbicide	15	N/A	N/A	N/A
Malathion	Insecticide	10	1.27%	Trace	Trace*
Metalaxyl	Fungicide	5	1.27%	42	42*
Metconazole	Fungicide	10	1.27%	114	114*
Methamidophos	Insecticide	5	N/A	N/A	N/A
Methidathion	Insecticide	5	N/A	N/A	N/A
Methomyl	Insecticide	25	N/A	N/A	N/A
Methoprene	Insecticide	80	N/A	N/A	N/A
Methoxyfenozide	Insecticide	5	3.80%	22.7	11.4 - 36
Metolachlor	Herbicide	5	N/A	N/A	N/A
Metribuzin	Herbicide	5	N/A	N/A	N/A
MGK-264	Insecticide	25	N/A	N/A	N/A
MGK-326	Insecticide	30	N/A	N/A	N/A
Myclobutanil	Fungicide	15	N/A	N/A	N/A
Norflurazon	Herbicide	15	N/A	N/A	N/A
Oxamyl	Insecticide	15	N/A	N/A	N/A
Oxyfluorfen	Herbicide	5	1.27%	21	21*
Paradichlorobenzene	Insecticide	250	N/A	N/A	N/A
Parathion methyl	Insecticide	10	N/A	N/A	N/A
Pendimethalin	Herbicide	15	6.33%	246.3	31.6 - 892
Permethrin total	Insecticide	25	N/A	N/A	N/A
Phenothrin	Insecticide	30	N/A	N/A	N/A
Phorate	Insecticide	25	N/A	N/A	N/A
Phosalone	Insecticide	15	N/A	N/A	N/A
Phosmet	Insecticide	50	2.53%	6.1	1 - 11.2
Piperonyl butoxide	Insecticide	15	N/A	N/A	N/A
Pirimiphos methyl	Insecticide	15	N/A	N/A	N/A
Prallethrin	Insecticide	20	N/A	N/A	N/A
Pronamide	Herbicide	5	N/A	N/A	N/A
Propachlor	Herbicide	25	N/A	N/A	N/A
Propanil	Herbicide	10	N/A	N/A	N/A
Propargite	Insecticide	15	N/A	N/A	N/A
Propazine	Herbicide	10	N/A	N/A	N/A
Propetamphos	Insecticide	20	N/A	N/A	N/A
Propham	Herbicide	15	N/A	N/A	N/A

Propiconazole	Fungicide	15	N/A	N/A	N/A
Pymetrozine	Insecticide	30	N/A	N/A	N/A
Pyraclostrobin	Fungicide	5	5.06%	178.5	22.6 - 569
Pyrethrins	Insecticide	250	N/A	N/A	N/A
Pyridaben	Insecticide	5	3.80%	32.1	19.5 - 52.6
Pyrimethanil	Fungicide	15	5.06%	10	10 - 10
Pyriproxyfen	Insecticide	5	N/A	N/A	N/A
Quinoxyfen	Fungicide	15	N/A	N/A	N/A
Quintozene (PCNB)	Fungicide	5	N/A	N/A	N/A
Resmethrin	Insecticide	30	N/A	N/A	N/A
Sethoxydim	Herbicide	10	N/A	N/A	N/A
Simazine	Herbicide	50	N/A	N/A	N/A
Spinosad	Insecticide	15	N/A	N/A	N/A
Spirodiclofen	Insecticide	5	N/A	N/A	N/A
Spiromesifen	Insecticide	50	N/A	N/A	N/A
Tebuconazole	Fungicide	5	6.33%	47.9	Trace - 73.6
Tebufenozide	Insecticide	5	1.27%	1	1*
Tebuthiuron	Herbicide	15	1.27%	2.2	2.2*
Tefluthrin	Insecticide	5	N/A	N/A	N/A
Tetrachlorvinphos	Insecticide	15	N/A	N/A	N/A
Tetraconazole	Fungicide	15	N/A	N/A	N/A
Tetradifon	Insecticide	5	N/A	N/A	N/A
Tetramethrin	Insecticide	30	N/A	N/A	N/A
Thiabendazole	Fungicide	5	N/A	N/A	N/A
Thiacloprid	Insecticide	5	N/A	N/A	N/A
Thiamethoxam	Insecticide	10	N/A	N/A	N/A
THPI	Fungicide	15	5.06%	559.3	410 - 728
Thymol	Varroacide	50	10.13%	3291.1	345 - 8040
Triadimefon	Fungicide	10	N/A	N/A	N/A
Triadimenol	Fungicide	25	N/A	N/A	N/A
Tribufos (DEF)	Fungicide	10	N/A	N/A	N/A
Trifloxystrobin	Fungicide	10	2.53%	493.5	406 - 581
Triflumizole	Fungicide	40	N/A	N/A	N/A
Trifluralin	Herbicide	5	3.80%	Trace	Trace - Trace
Triticonazole	Fungicide	30	N/A	N/A	N/A
Vinclozolin	Fungicide	5	N/A	N/A	N/A

Figure 14: Pesticide detection in the 2015 – 2016 survey year (79 samples) (*denotes single detection only) (positive detections are highlighted in yellow)

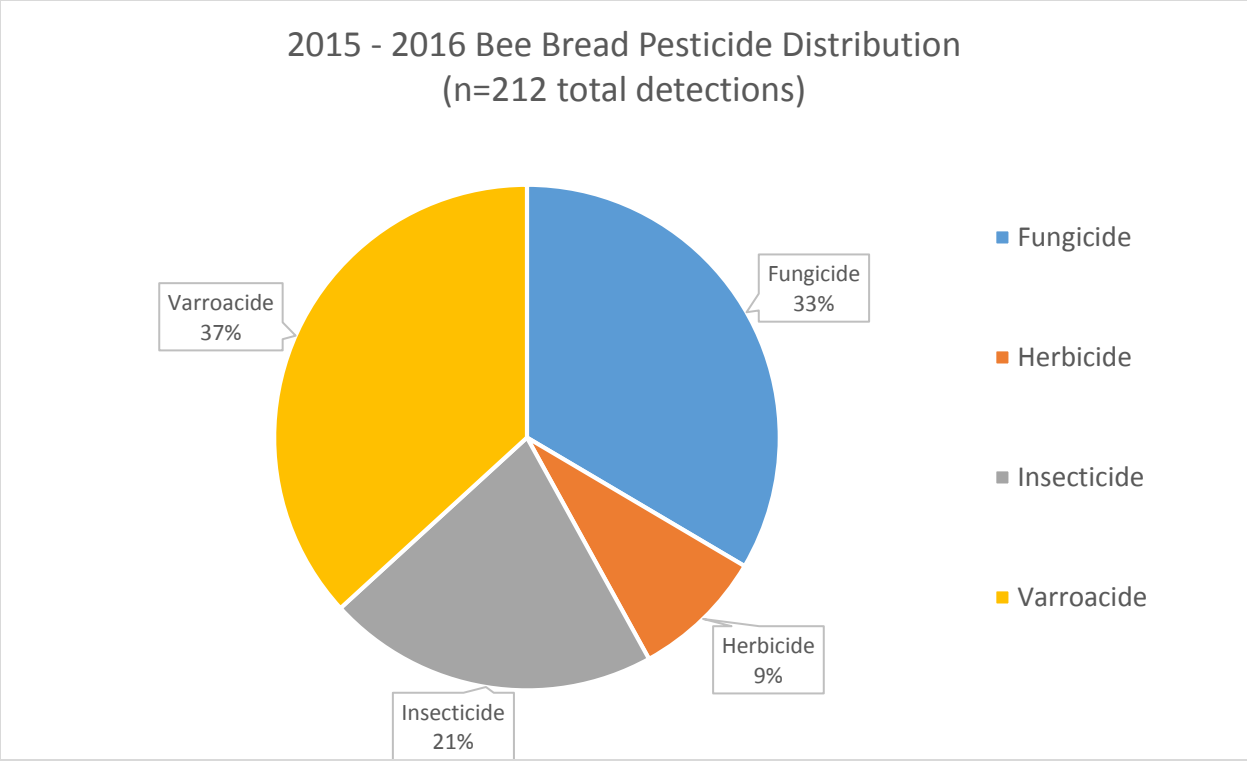

Figure 15: Classification of pesticide type detected in the 2015 – 2016 survey year.

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Appendix

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.dddddd**

_____ Latitude: _____

Sampling Address: _____ Longitude: _____

_____ Name all locations (cities and states) where hive was located within the **past year**:

Sampling County: _____ _____

State Origin of Hive: _____ **Which of the following best describes the primary function of the sampled apiary?**
[ ] honey production [ ] pollination [ ] queen production

Is the sampled apiary part of a migratory operation?
[ ] 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: