2014 – 2015 National Honey Bee Disease Survey Report

Shayne Madella[1], Karen Rennich[1], Jay Evans[2], Samuel Abban[2], Heather Eversole[1], Ashrafun Nessa[1], Rachel Fahey[1], Dawn Lopez[2], Todd Waters[1], Anthony Nearman[1], Jeff Pettis[2], Dennis vanEnglesdorp[1] and Robyn Rose[3]

[1]University of Maryland, College Park, [2]USDA Agricultural Research Service, [3]USDA Animal and Plant Health Inspection Service

Disclaimer

For the purposes of this report, the 2014 – 2015 USDA APHIS National Honey Bee Survey (NHBS) is comprised of samples that were collected from July 2014 to June 2015. After the survey has commenced, state survey specialists have one year to collect and submit all samples. Extensions were granted to a select few states that were collecting samples voluntarily. Results for samples collected after July 2015 will be included in the following survey summary report for the 2015 – 2016 APHIS NHBS.

Executive Summary

The 2014 – 2015 USDA Animal Plant and Health Inspection Service (APHIS) sponsored National Honey Bee Survey (NHBS) was conducted in collaboration with the University of Maryland (UMD), the USDA Agricultural Research Service (ARS) and the cooperation of 26 states, 2 territories and the island nation of Grenada. The pilot year for the APHIS National Honey Bee Survey began in 2009 in the states of California, Florida and Hawaii. The survey expanded in 2010 to include a larger but still limited 13 states as funding for the survey increased. In subsequent years, the survey expanded to 34 states in 2011, and 32 states in both 2012 and 2013. The continued operation and expansion of the APHIS NHBS has allowed for the creation of a large and comprehensive honey bee disease and pathogen database.

Through the collection of pest and disease data in the national database, a baseline health measure for honey bees in the United States has been established. The primary focus of the survey is to verify the absence of potentially invasive and harmful honey bee pests and pathogens within the United States such as the parasitic mite *Tropilaelaps* spp., the Asian honey bee A*pis cerana*, and slow bee paralysis virus (SBPV). Confirming the continued absence of these exotic pests and pathogens that can harm the health of the U.S. honey bee supply is the primary objective of the NHBS. The threat of an exotic pest such as *Tropilaelaps spp.* becoming introduced leads to the potential for increased colony losses that are already above beekeeper self-reported acceptable levels (Seitz et al., 2016). With honey bees contributing to more than $14 billion in U.S. crop production, ensuring the continued absence of honey bee pests and diseases becomes an issue of agricultural economics and national food security. To prevent the expansion of exotic honey bee diseases into the United States, a need exists to quickly detect these diseases if they were to enter. In response to the dangers imposed by invasive pests and diseases, a draft *Tropilaelaps* response plan is being developed if such an event were to occur.

An additional objective of the survey was to determine the incidence of known honey bee diseases in the U.S. The results from this survey are used to gauge the overall health of colonies, to create a baseline disease level, and to facilitate interpretation of ongoing and future epidemiological studies. By creating a baseline disease level for colonies across the country we are able to give context to samples sent in by beekeepers that describe their disease levels relative to the rest of the country. These baseline data, including historic data from research institutions such as the ARS and other ongoing field sampling and management surveys, have been incorporated into a single database as part of the Bee Informed Partnership (BIP) (https://beeinformed.org/), originally funded by a 5 year USDA National Institute of Food and Agriculture (NIFA) grant and now a non-profit organization.

Introduction

The 2014-2015 Survey Year included collection of samples from 26 states and 2 territories: Alabama, Arkansas, California, Connecticut, Delaware, Florida, Georgia, Guam, Hawaii, Illinois, Indiana, Michigan, Minnesota, Montana, North Carolina, North Dakota, New Jersey, New York, Oregon, Pennsylvania, Puerto Rico, South Dakota, Tennessee, Texas, Utah, Virginia, Wisconsin, and West Virginia. In addition, Grenada was sampled for *Varroa* and *Nosema* spp. as part of the national survey.

This survey was designed to be representative of the managed honey bees across the broad geography of the U.S and the survey was open to any state wishing to participate. Beekeeper participation was completely voluntary, and the beekeeper was not required to be present nor assist with the sampling. The results can be considered as representative of the distribution of pests and pathogens present in the U.S.

Milestones and Project Timelines

As the survey has evolved from its pilot year in 2009, various changes in the protocol and diseases tested have been implemented. These changes were made as knowledge of honey bee pests and diseases improved and more effective protocols became known:

- Tracheal mites (*Acarapis woodi*) were removed in 2011 as there were no detections from 2009 through 2010.
- 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.

- Absolute quantification of viral targets via qRT-PCR was added to the results in 2013 enabling direct comparison to standardized European results.
- 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 operations within their state except California which received kits to sample 48 apiaries. The 48 kits in California were used to collect samples from 24 operations who stay in California year round and 24 beekeepers who migrate to California during almond pollination.

Apiary Specialists conducted an aggregate sampling from previously identified commercial, migratory, sideliner and backyard beekeepers with at least 8 colonies per apiary. In most cases, apiaries consisted of at least 10 colonies. A single aggregate sample was collected from 8 randomly selected colonies per apiary per operation (APHIS National Honey Bee Survey Sampling Protocol). In each state, apiaries were chosen through opportunity sampling with an attempt to give as close to an equal representation of the entire state as possible. Ideally, a state was sectioned into 4 quadrants with apiaries randomly chosen within a quadrant. When possible, ten queen producers were sampled. Of the remaining sampled apiaries, 1/2 were from migratory operations (operations that move out of the state and return prior to sampling) and 1/2 were from stationary operations (operations that only move within the state or do not move at all). Additional apiaries occurring near ports or other areas that could be considered high risk were also considered for sampling (APHIS NHBS Project Plan 2014 – 2015). Three distinct collection methods were used to sample each apiary: 1. Brood cell bump sample; 2. Alcohol preserved samples; 3. Live bee samples.

Brood cell bump samples were analyzed for the following:

1. *Tropilaelaps* spp.

The alcohol preserved samples were analyzed for the following:

1. *Nosema* spp. spore loads
2. *Varroa destructor* loads
3. *Apis cerana*

Live bee samples were analyzed for the following viruses:

1. Acute bee paralysis virus (ABPV)
2. Deformed wing virus (DWV)
3. Israeli acute paralysis virus (IAPV)
4. Kashmir bee virus (KBV)
5. Chronic bee paralysis virus (CBPV)
6. Lake Sinai virus-2 (LSV-2)
7. Slow bee paralysis virus (SBPV)

The alcohol preserved samples were collected from a brood frame which contained both capped an uncapped brood and ¼ cup of nurse bees were taken from each of the 8 colonies that were sampled in the apiary. These bees were put into a bottle of 70% ethanol solution for preservation and sent to the University of Maryland for analysis.

The brood cell bump sample was taken from debris dislodged by 'bumping' sampled brood frames over a collection pan. The brood cell debris was then collected in a filter cloth in placed in a bottle filled with alcohol. The brood cell bump sample focused on monitoring for *Tropilaelaps* but also included any mites, beetles and other hive debris filtered from bumping the brood frames.

The live bee sample was collected for viral analysis from the same brood frame as the alcohol preserved sample and frozen at -80°C and 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. By updating the protocol, new high-performance assays have been implemented for more effective and stream-lined sample processing in the molecular analysis. 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.

All participating beekeepers, as well as State Apiarists/Inspectors, received a single report for each sample taken. The report provides detail of the results for *Varroa* load, *Nosema* load, exotic pests and pathogens and the presence of viruses. They also noted the presence or absence of *A. cerana* and *Tropilaelaps* spp. This report is sent within 3-6 months of receipt of samples. Reports also included the national prevalence for viruses as well as specific beekeeper percentile rankings of *Varroa* load, *Nosema* spore load, and viral copy load.

Using the U.S. Postal Service (USPS), live bee shipments were mailed to USDA/ARS and survivability was tracked for all live bee shipments. The results of this analysis, previously proven to be a robust and suitable alternative for shipping bees on dry ice by the Pilot and Limited Survey, continued to work well. In some states, a small number of live bee samples were degraded, such that no molecular data could be obtained from these samples. Overall 99.6% of all samples arrived in suitable condition for viral analysis with only 2 samples received with excessive mortality rates.

Results

The primary objective of the NHBS is to confirm the absence of *Tropilaelaps* spp., *Apis cerana* and slow bee paralysis virus (SBPV) have shown that these potentially invasive pests and pathogens are

not present. The absence of these exotic pests and pathogens suggest that the current policies to prevent their introduction into the United States has been successful.

At the start of this survey year, a total of 682 live bee kits were sent out (22 States at 24 kits per state, plus 24 for Puerto Rico, 10 for Grenada and an additional 24 for California). At the conclusion of the survey year, 552 live bee kits were returned representing 80.9% of all live bee kits sent to the states. Of the live bee samples that were received, 99.6% of all samples were analyzed for viruses. Two live bee samples were insufficient for analyses. Reasons for a sample to be insufficient for analysis can include live bees dying in transit, loss of sample in long term storage or low quality RNA due to insufficient nucleic acid extraction. Moving forward, we initiated a standard operating protocol (SOP) for the arrival of the live bee sample to minimize loss/damage in storage.

For the samples stored in alcohol, 710 samples were mailed at the onset of the survey. At the conclusion of the survey, 552 alcohol sampling kits had been returned or 77.7% of all samples, representing 4,416 colonies that were analyzed for *Varroa*, *Nosema*, and *Tropilaelaps*. The proportion of samples returned is significantly less than the previous year which had 88.3% of all live bee samples returned and 93.3% of all alcohol sampling kits returned. The 2014 – 2015 NHBS was conducted on a nationwide volunteer basis where states did not receive funding to conduct the sampling events except for a select few which may explain the decrease in compliance with the sampling protocol.

Nosema spp. Spore Load and Prevalence

Of the 551 alcohol samples that were sent in for *Nosema* spp. spore load analysis, 277 (50.3%) tested positive (Figure 1). The average *Nosema* spore load was 0.69 million spores/bee for samples that tested positive (Figure 2). Of all samples that were processed for *Nosema* spp. spores, 7.8% exceeded the threshold thought to cause damage (>1,000,000 spores per bee). This result shows a decrease from the previous year's NHBS when 13.0% of all samples processed exceeded the threshold. Figures 1 and 2

illustrate *Nosema* spp. prevalence, and *Nosema* spp. spore load from all 5 years of the survey. *Nosema* spp. spore load (Figure 3) has a strong relationship to seasonality with the highest loads occurring in the winter and early spring periods followed by a sharp decline in summer months.

Varroa Load and Prevalence

For *Varroa* prevalence in the 2014- 2015 survey year, 86.0% of all samples tested positive for *Varroa*. This marks a drop in prevalence from 88.4% in the previous survey year and the 2011 – 2012 survey year when the prevalence was at 92.3% (Figure 4). 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 colony damage. In the 2014-2015 survey year, the average *Varroa* load was found to be 4.0 mites per 100 bees for samples that tested positive (Figure 5). The proportion that has exceeded the threshold of 3.0 mites per 100 bees is 38.8% of all samples processed. Although there has been a decrease in the *Varroa* load and proportion exceeding the threshold from the 2013 – 2014 survey year (5.0 mites/100 bees and 50.2% respectively) these results are still concerning for colony health. Figure 6 illustrates the dynamic nature of *Varroa* populations over the course of the survey, demonstrating the seasonality of *Varroa* populations of exponential growth beginning in late summer and peaking in the fall.

Viral Load and Prevalence

Figure 7 illustrates the viral prevalence of targets that were tested in past survey years (SBPV, KBV, ABPV, IAPV, DWV, LSV-2, CBPV and BQCV) and the 2014 – 2015 survey year. A notable shift has occurred in the prevalence of Kashmir bee virus (KBV). KBV has replaced chronic bee paralysis virus as the least common virus found. The prevalence of KBV for the most recent survey was 12.5% and CBPV was 15.2%. In the previous year the prevalence for KBV and CBPV was 15.4% and 9.3% respectively.

The largest change in the viral assessments has been the increasing incidence of CBPV. In the 2010 – 2011 survey CBPV prevalence was at 1.4% and it continued to remain low with a prevalence of 0.8% in the 2011 – 2012 survey year (Figure 7). The prevalence of CBPV began to increase to 6% in 2012 – 2013, 8.9% in 2013 – 2014 and 15.2% in 2014 – 2015 (Figure 7). Some of the increased incidence of CBPV could be attributed to increased primer sensitivity since the implementation of the new SLU primers; however, this is not thought to be the major driver since the increase in the incidence of CBPV began in 2012 and has continued each successive year.

Deformed wing virus (DWV) remains the most common honey bee virus that we test for as part of the NHBS. The prevalence for DWV for the 2014-2015 survey was at 82.2% which is consistent with previous years of the survey (Figure 7). Figures 8 – 14 display the monthly prevalence of the viral targets for the NHBS from 2010 – 2015 and highlight the seasonality of these pathogens. In the 2013 – 2014 survey, the analysis of BQCV was discontinued as it was determined to be ubiquitous in colonies and not as beneficial in following as an indicator of colony health as other viral targets. The viral target that replaced BQCV was Lake Sinai virus 2 (LSV-2).

Lake Sinai virus was discovered in 2011 near Lake Sinai in South Dakota and was incorporated into the NHBS beginning in the 2013 – 2014 survey. The analysis of LSV-2 in the NHBS survey has shown that there is a strong seasonality to the virus. The time period with the highest viral load for LSV-2 is in the spring season peaking in May at 58.8% in the 2013 – 2014 NHBS and April at 85.7% in the 2014 – 2015 NHBS (Figure 13). Prevalence for LSV-2 in the months of February and March in the most recent survey are not displayed as there were not enough samples taken during these months for a statistical analysis.

For Israeli acute paralysis virus (IAPV) and acute bee paralysis virus (ABPV) a correlation appears to be occurring (Figures 10 and 11) and there may be an inverse relationship between IAPV and ABPV.

IAPV prevalence is highest in early summer months, peaking in the 2014 – 2015 survey in June at 35.6% and lowest in September at 27.8%. ABPV prevalence is highest in fall months, peaking in the 2014 – 2015 survey in November at 52.6% and lowest in July at 3.92%.

Conclusions

Varroa load decreased from its peak of 5.5 mites per 100 bees in the 2012 – 2013 survey to 4.0 mites per 100 bees in the recent 2014-2015 survey year (Figure 5). The cause behind this change in *Varroa* load is currently not known. An explanation could be that outreach efforts for beekeepers to monitor and treat for mites have been successful and these declines that are being observed are stemming from increased awareness of *Varroa,* and increased implementation of *Varroa* management strategies such as the application of miticides in colonies. An alternative explanation could be that viruses associated with *Varroa* have become more virulent causing increased damage to colonies at lower mite loads. If the viruses associated with *Varroa* have become more virulent, colony loss would occur before mite loads are able to approach the levels recorded in earlier years of the survey. This explanation is supported through research conducted in Hawaii showing how more virulent strains of DWV become strongly selected against more benign variants after the introduction of *Varroa* (Martin et al., 2012). Changes in the viral copy load of DWV between the 2013 – 2014 survey and the 2014 – 2015 survey also indicate this as a possibility with loads increasing from 7.3×10^8 copies per bee to 3.9×10^9 copies per bee (Figure 15). With results from only two years of quantitative data, this increase in DWV cannot be determined to be statistically significant at this time.

Nosema spore prevalence has remained relatively stable since the 2010 – 2011 survey year of 51.3% and 50.3% recorded in the 2014 – 2015 survey year. The high prevalence of *Nosema* spores in the 2009 survey can be attributed to the limited states sampled in the pilot year which included only Florida, California and Hawaii. For *Nosema* spore loads, there was a small decrease in spore load of all positive

samples from the previous 2013 – 2014 survey year of 0.80 million spores/bee to 0.69 million spores/bee in the recent 2014-2015; Although there has been a small decrease in spore load from the previous survey year long term trends suggest that overall spore load has been stable and a further decrease in subsequent years would be necessary to indicate a trend of decreasing spore loads.

The 2014-2015 NHBS was the second year that absolute quantification of viral targets was analyzed. Differences in viral loads of the six viruses known to be in the United States are displayed in Figure 15. The paralysis viruses (KBV, ABPV, and IAPV) have the lowest median viral load, although the quantity for ABPV can be highly variable with some samples having higher loads than all other viral targets. The low median viral load of the paralysis viruses may be attributed to a higher virulence causing them to require fewer copies to induce symptoms. The viral target with the highest median loads is deformed wing virus which may reflect the necessity of many viral copies to induce symptoms.

After two years of collecting absolute viral quantification data a seasonal pattern has been observed for deformed wing virus (Fig. 16). The month-to-month fluctuations for deformed wing virus highly correlate with the month-to-month *Varroa* load fluctuations. High viral loads rise during the summer to their peak in the fall, before decreasing during the winter until the spring season when colony brood production renews. This correlation between *Varroa* and viruses provides observational evidence for their linkage that has been hypothesized.

Results from the 2014 – 2015 APHIS NHBS provide strong evidence for the absence of the exotic pests *Tropilaelaps* spp., slow bee paralysis virus (SBPV) and *Apis cerana*. The absence of these species suggests that the current methods of preventing potentially invasive honey bee pests and pathogens from entering the United States have been successful. Additionally, comprehensive information on known honey bee diseases have allowed a national database of honey bee health to be established. This

database has provided insight into trends and disease load information for *Varroa destructor*, *Nosema* spp. and a collection of viruses.

References

Martin, S. J., Highfield, A. C., Brettell, L., Villalobos, E. M., Budge, G. E., Powell, M., … Schroeder, D. C. (2012). Global Honey Bee Viral Landscape Altered by a Parasitic Mite. *Science*, *336*(6086), 1304–1306. http://doi.org/10.1126/science.1220941

Seitz, N., Traynor, K. S., Steinhauer, N., Rennich, K., Wilson, M. E., Ellis, J. D., … vanEngelsdorp, D. (2016). A national survey of managed honey bee 2014–2015 annual colony losses in the USA. *Journal of Apicultural Research*, *0*(0), 1–12. http://doi.org/10.1080/00218839.2016.1153294

Appendix

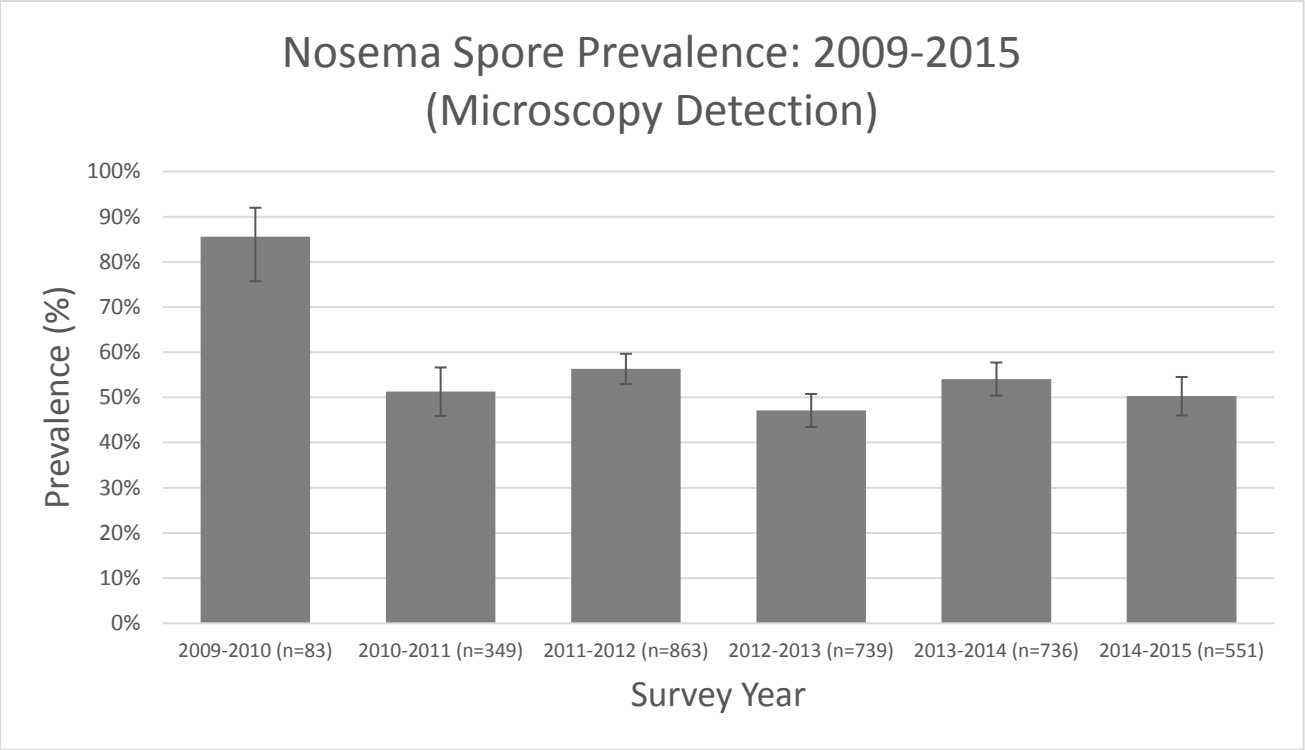

Figure 1: *Nosema* prevalence by survey year (95% confidence intervals shown).

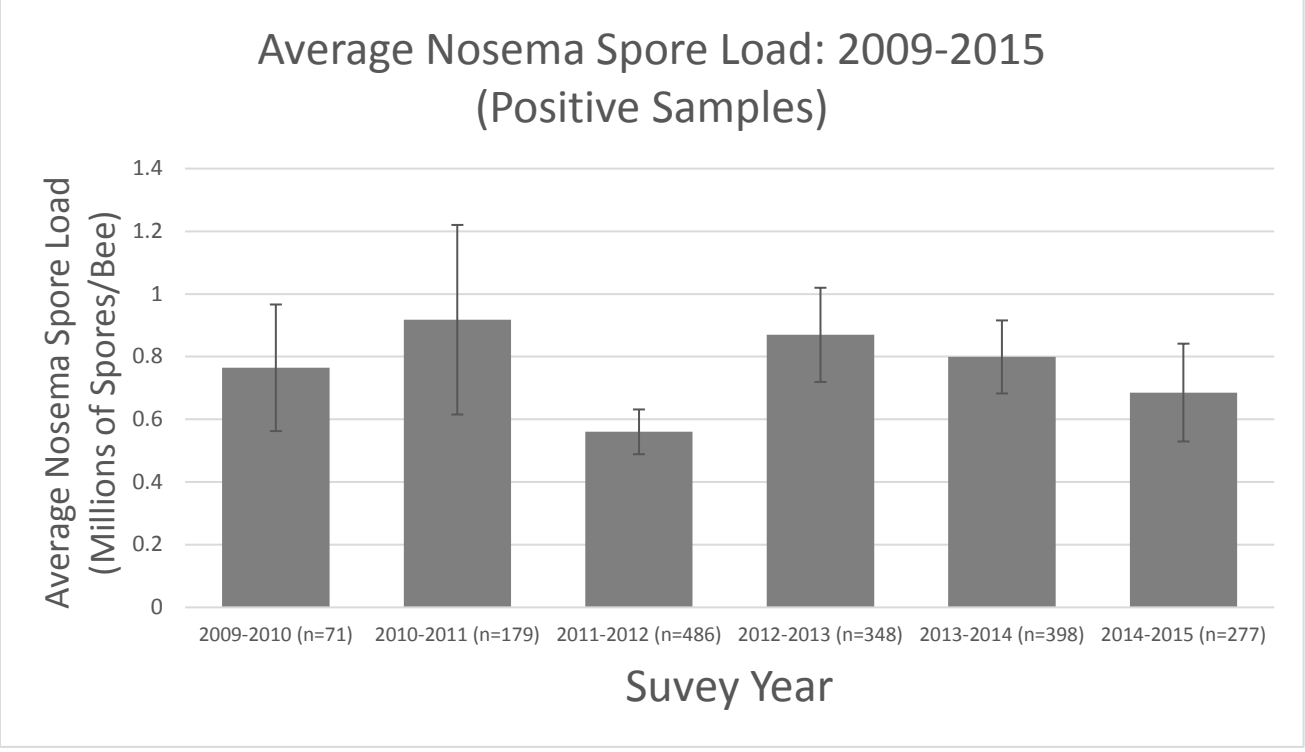

Figure 2: Nosema spore load by survey year (95% confidence intervals shown).

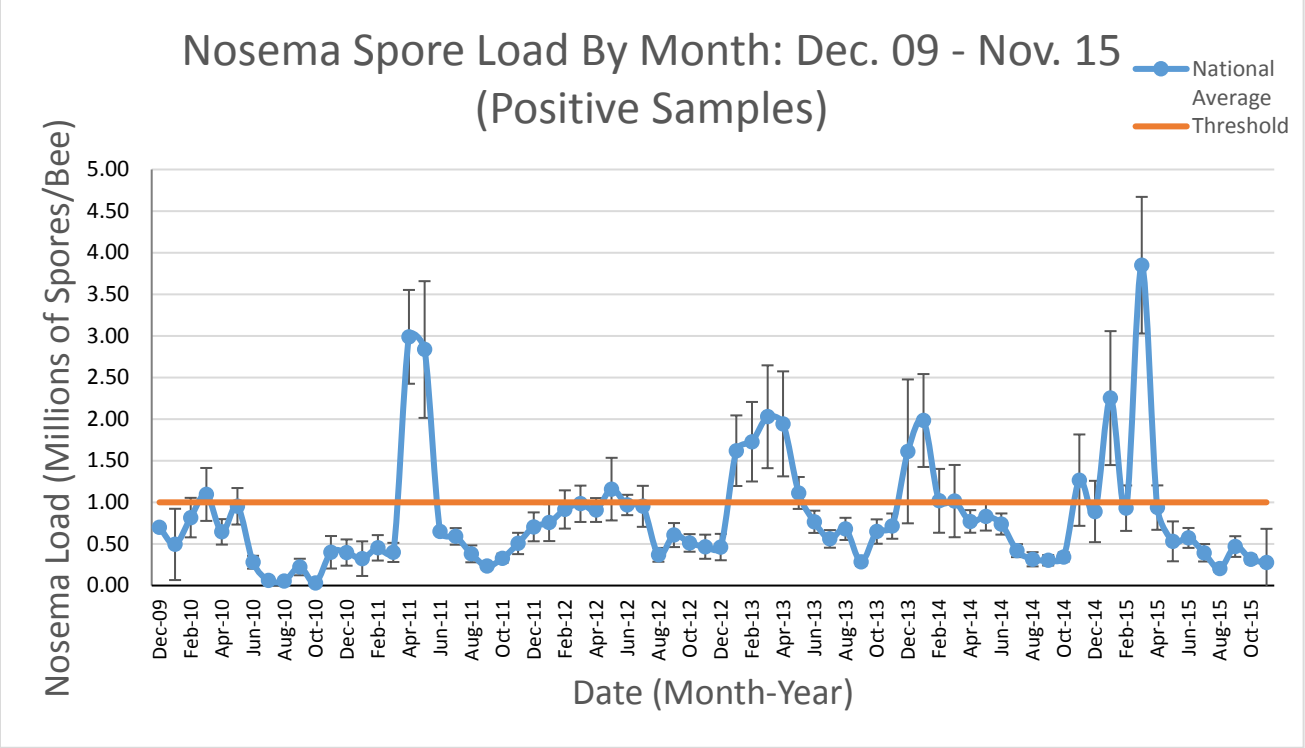

Figure 3: Monthly *Nosema* spp. load from December 2009 to November 2015 (Standard error shown).

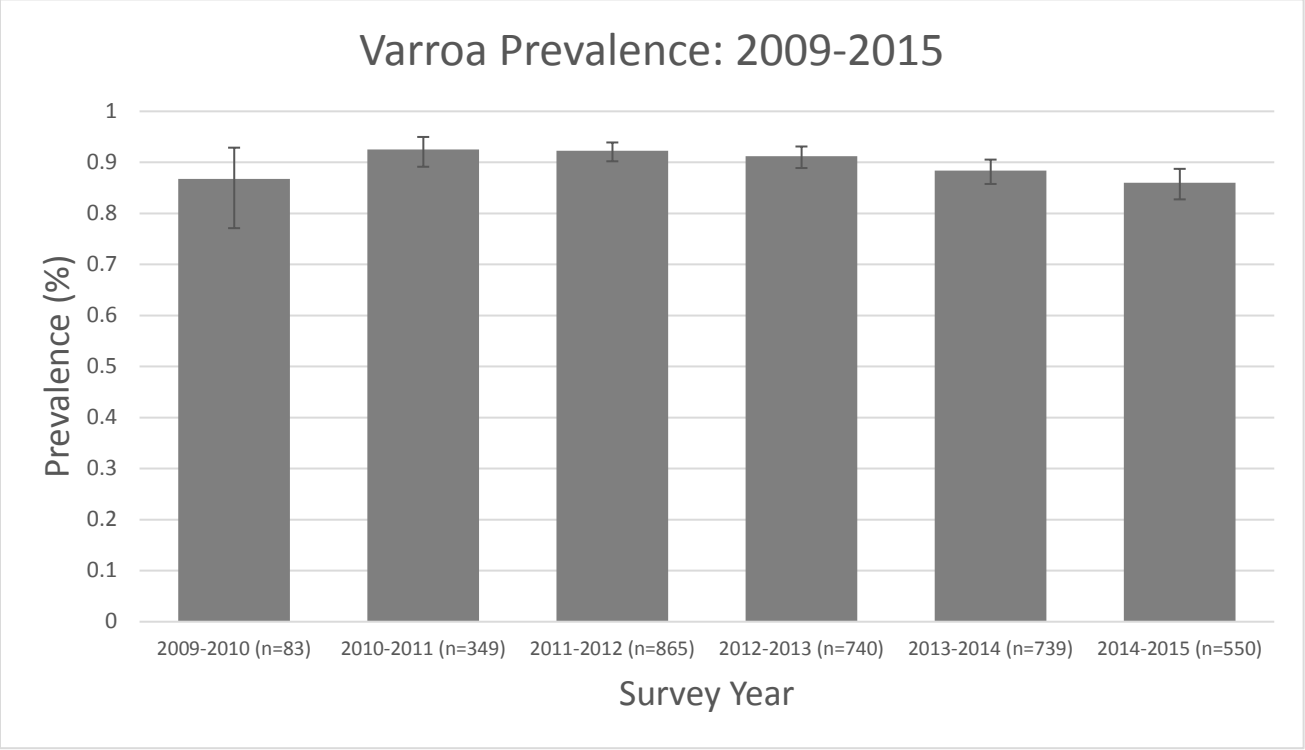

Figure 4: *Varroa* prevalence by survey year (95% confidence Interval shown).

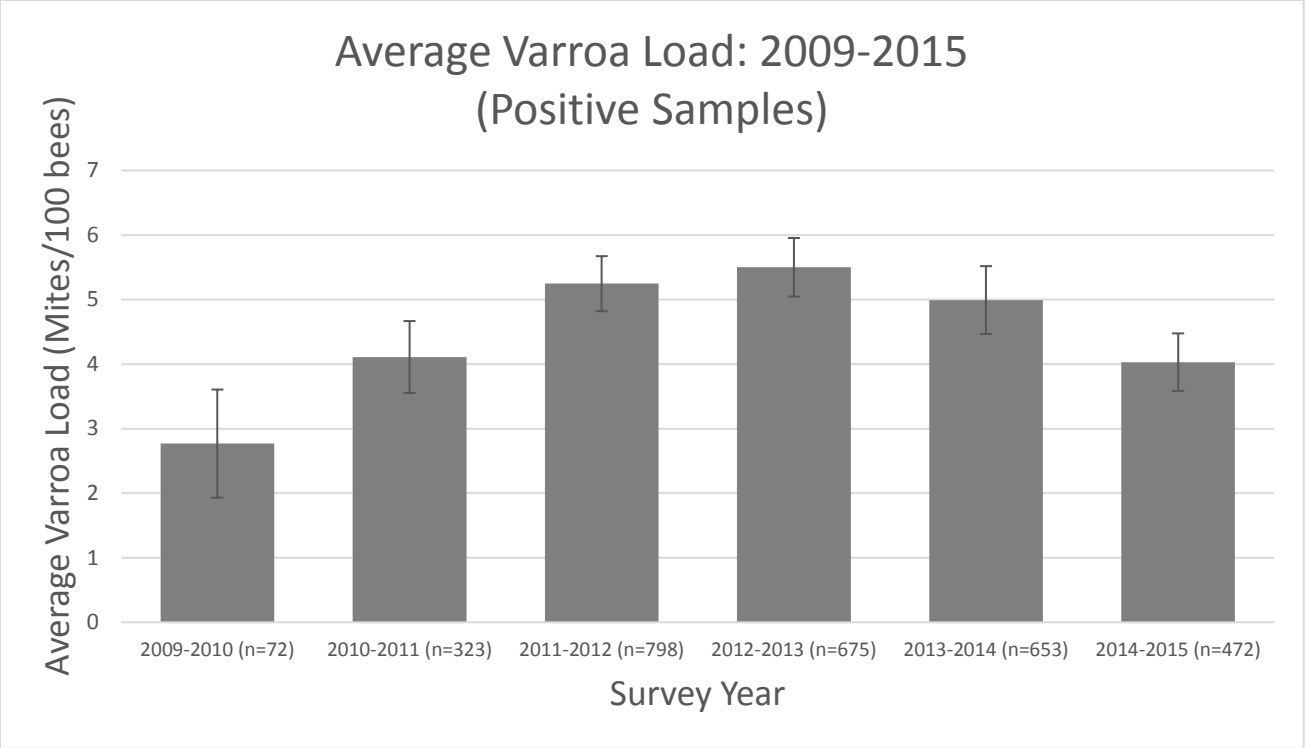

Figure 5: Average *Varroa* load by survey year (95% confidence interval shown).

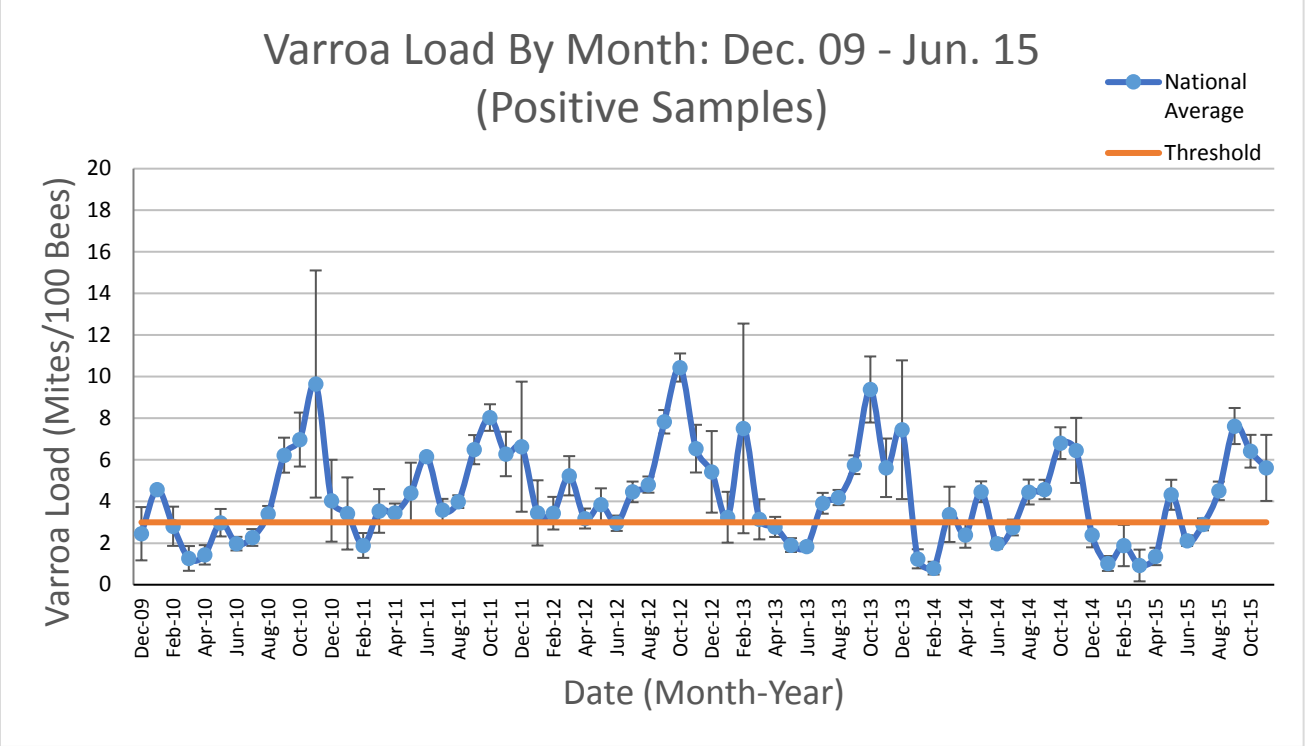

Figure 6: Monthly *Varroa* load from December 2009 to June 2015 (Standard error shown)

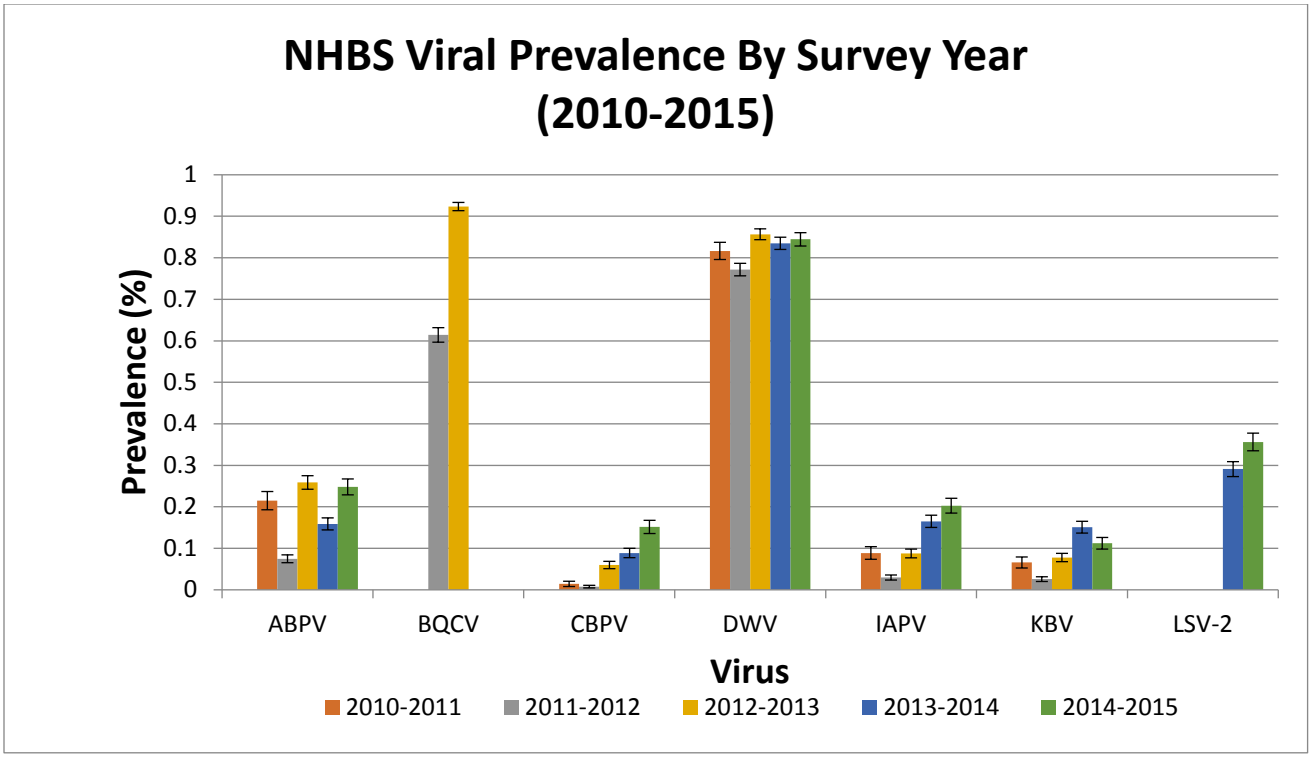

Figure 7: Yearly changes in viral prevalence from 2009 to 2015 by survey year (95% confidence interval shown).

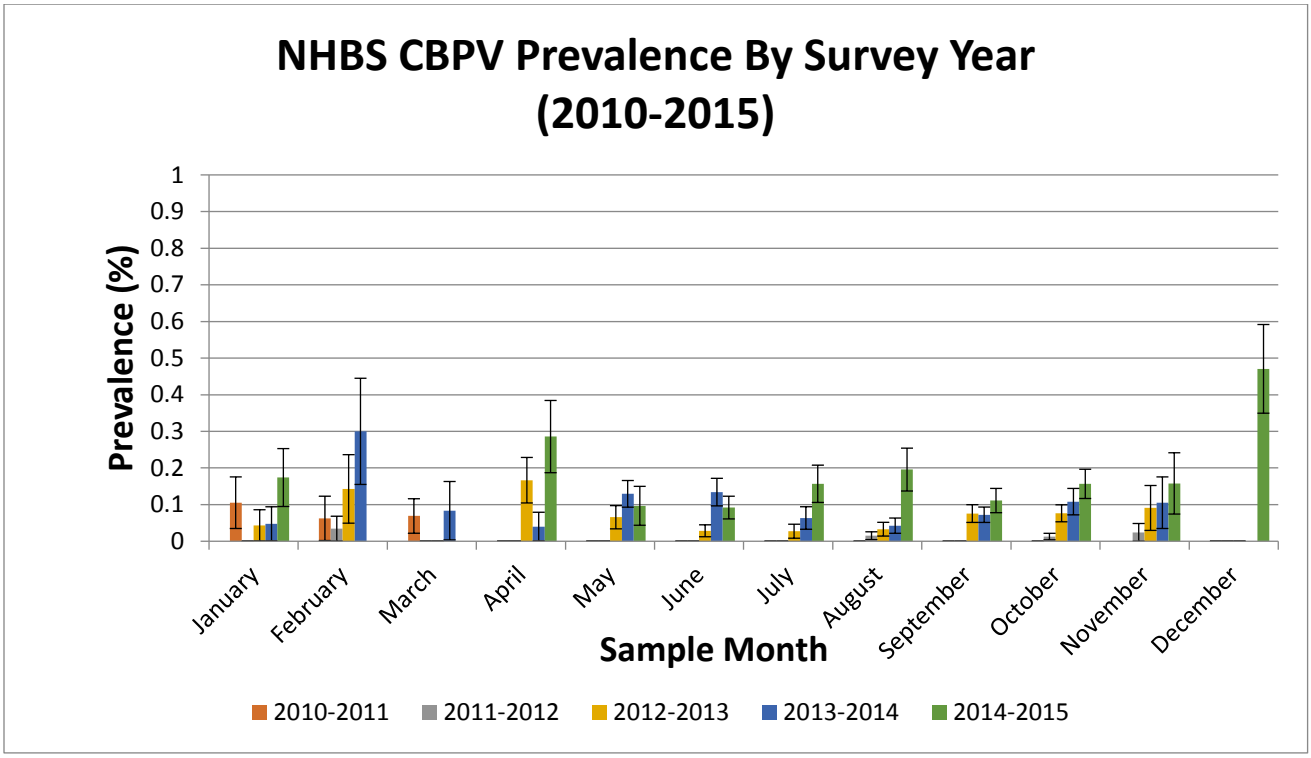

Figure 8: Monthly changes in viral prevalence of CBPV by survey year (95% confidence interval shown).

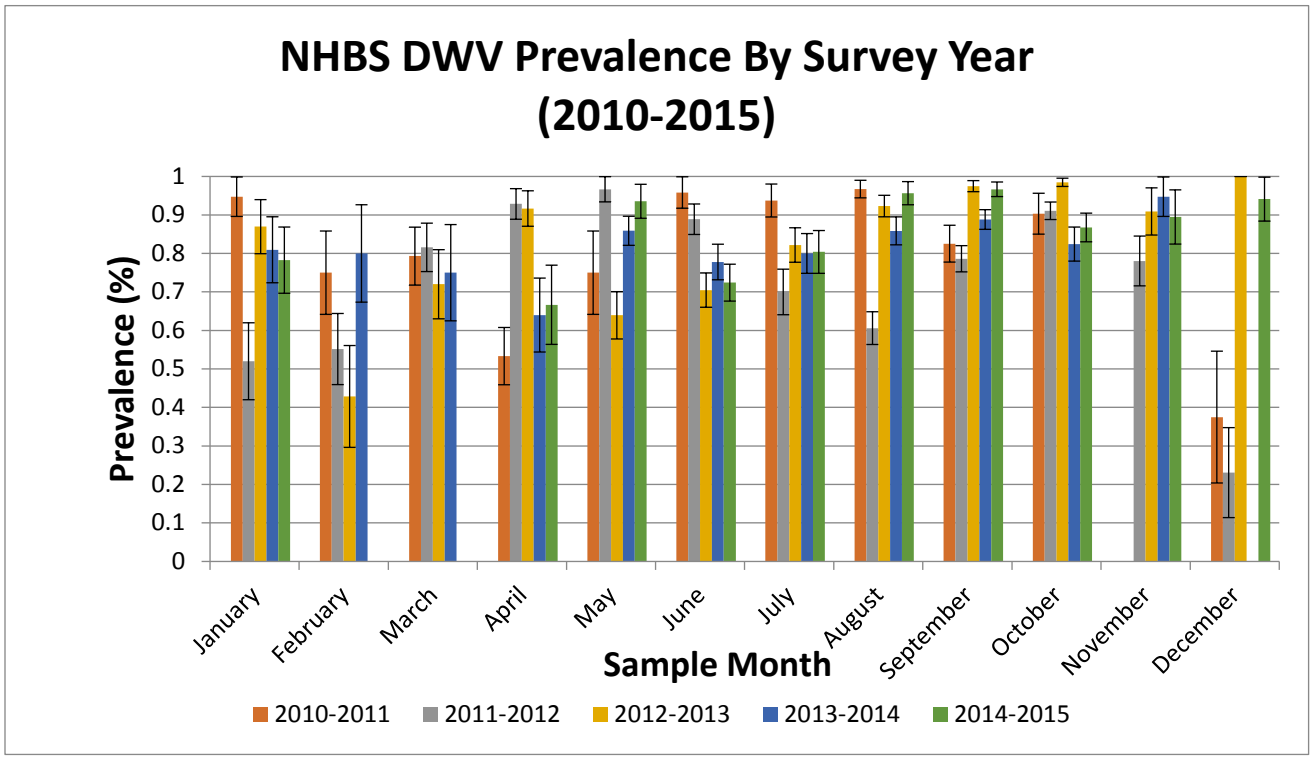

Figure 9: Monthly changes in viral prevalence of DWV by survey year (95% confidence interval shown).

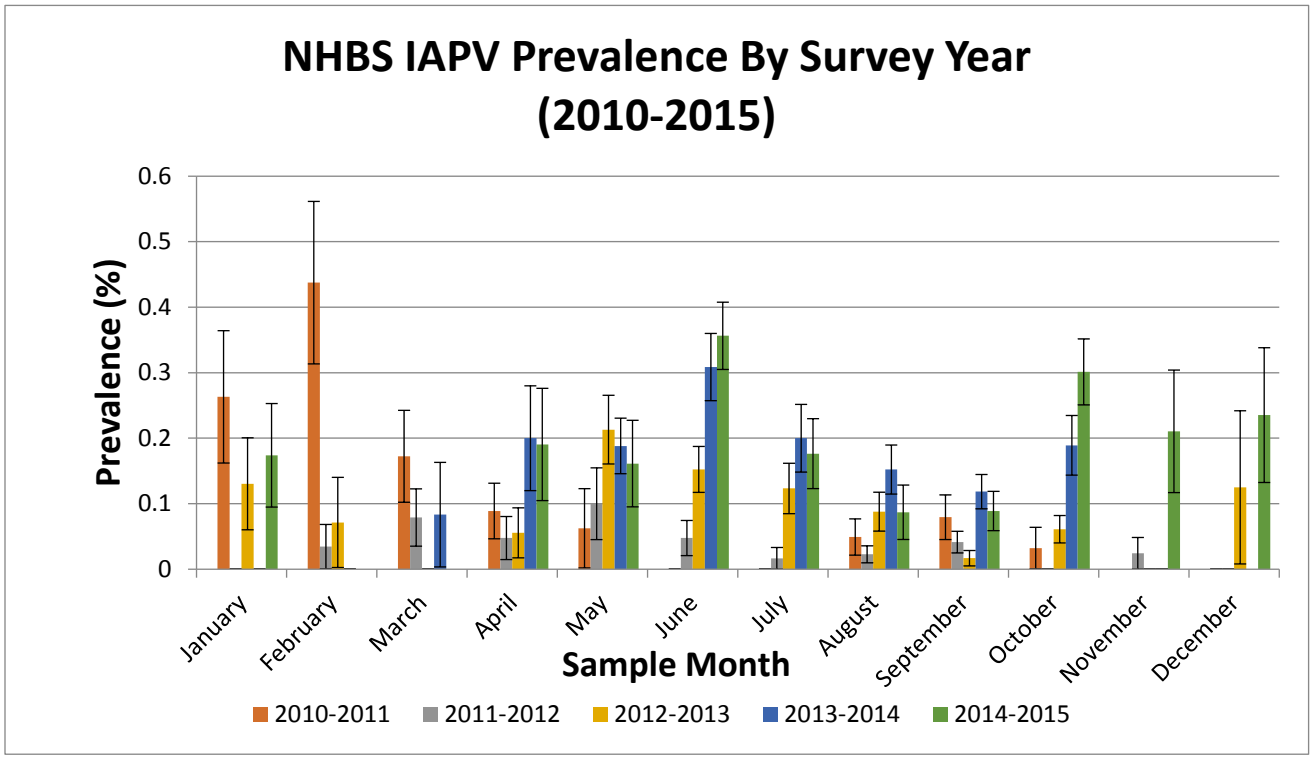

Figure 10: Monthly changes in viral prevalence of IAPV by survey year (95% confidence interval shown).

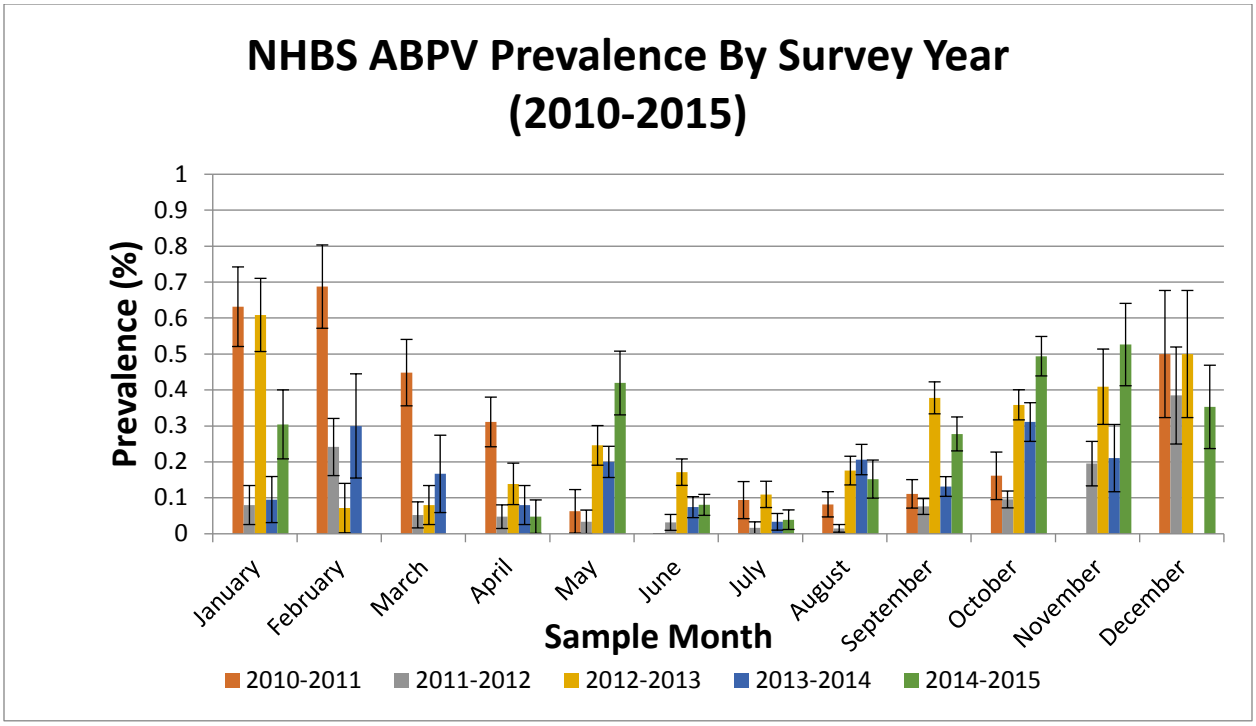

Figure 8: Monthly changes in viral prevalence of ABPV by survey year (95% confidence interval shown).

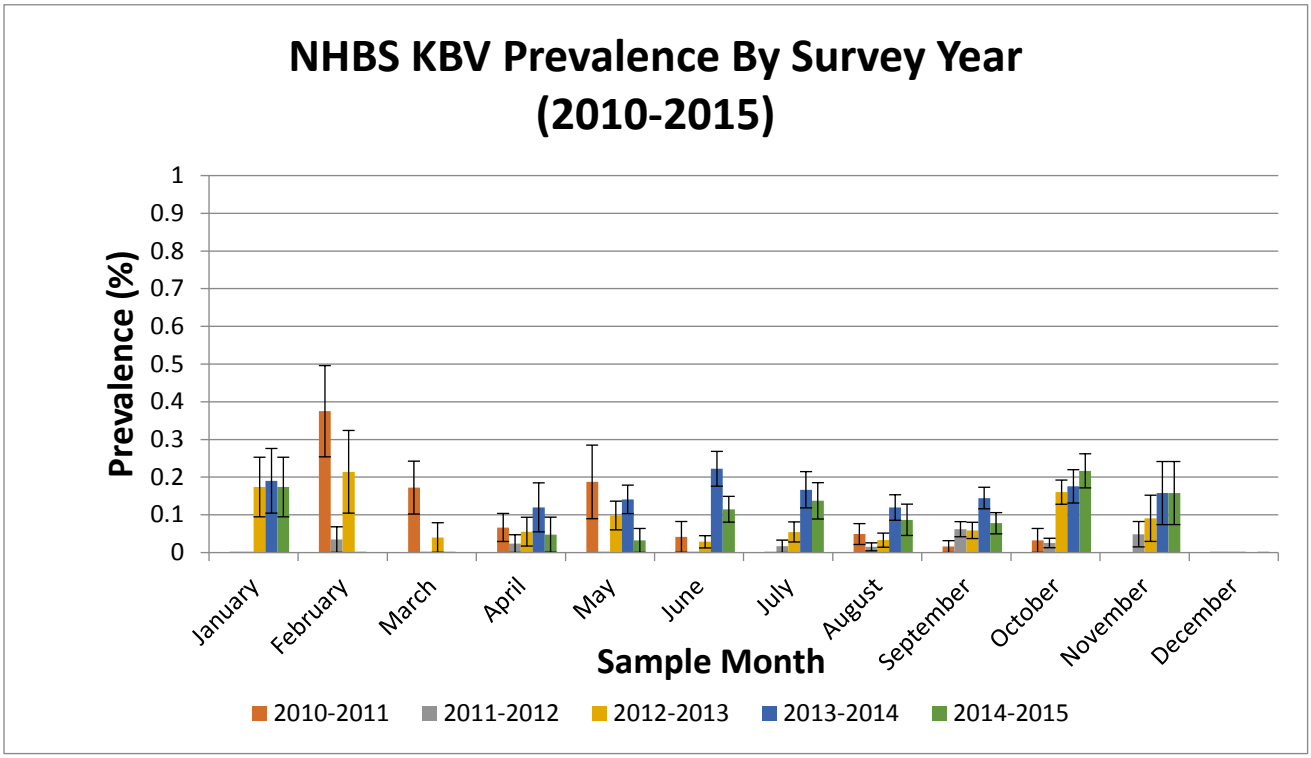

Figure 9: Monthly changes in viral prevalence of KBV by survey year (95% confidence interval shown).

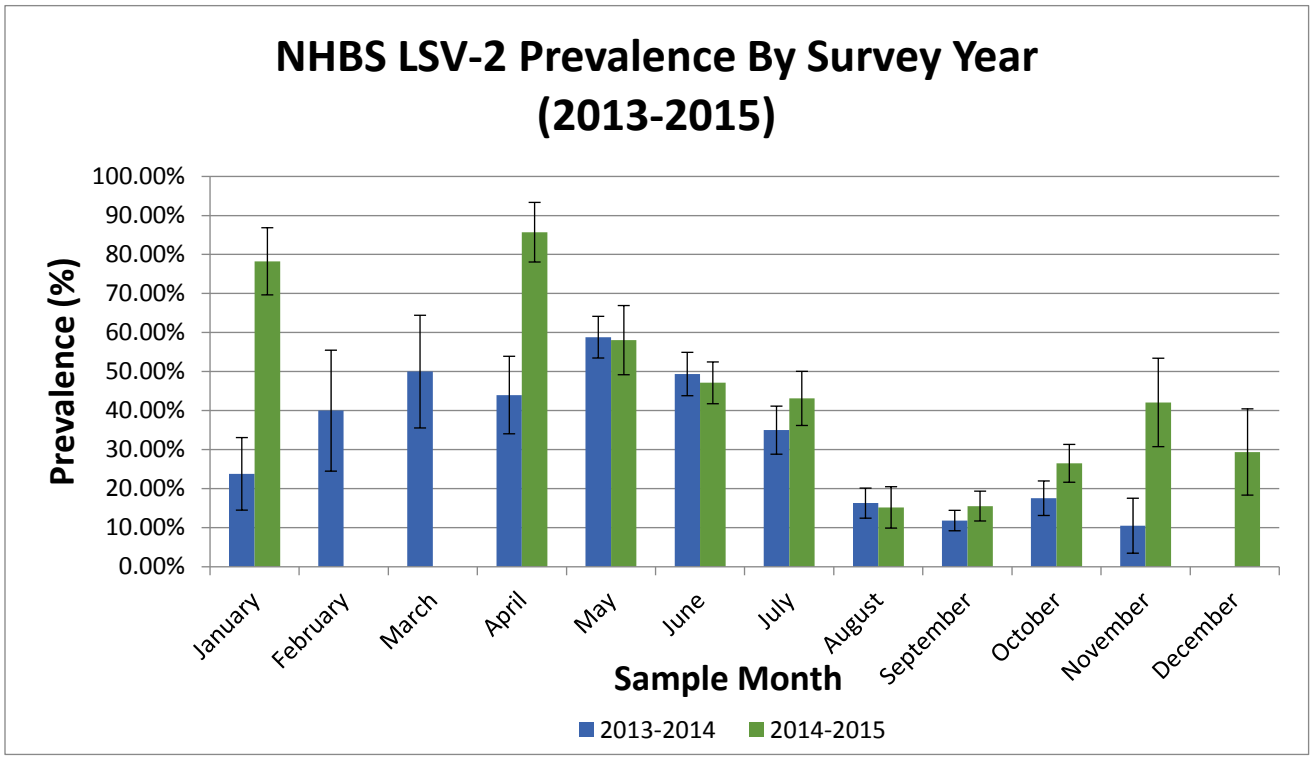

Figure 10: Monthly changes in viral prevalence of LSV-2 by survey year (95% confidence interval shown).

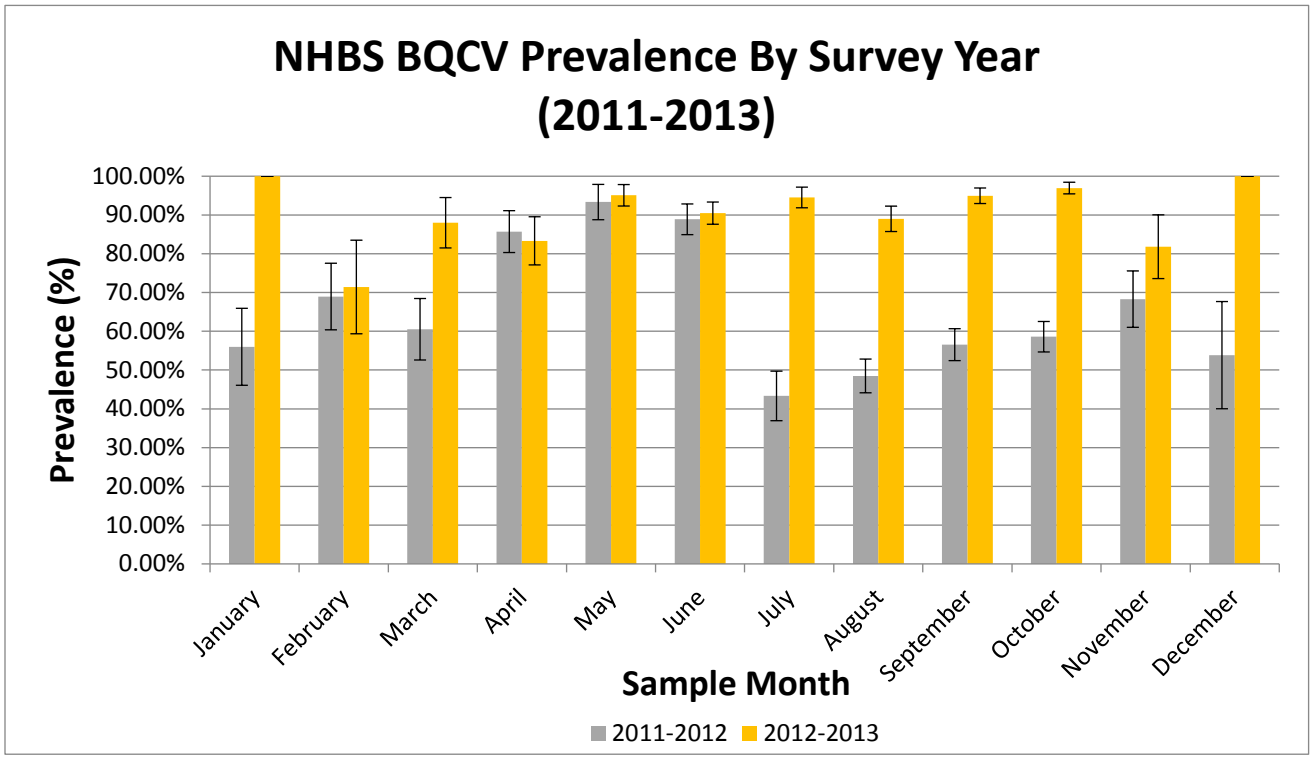

Figure 11: Monthly changes in viral prevalence of BQCV by survey year (95% confidence interval shown).

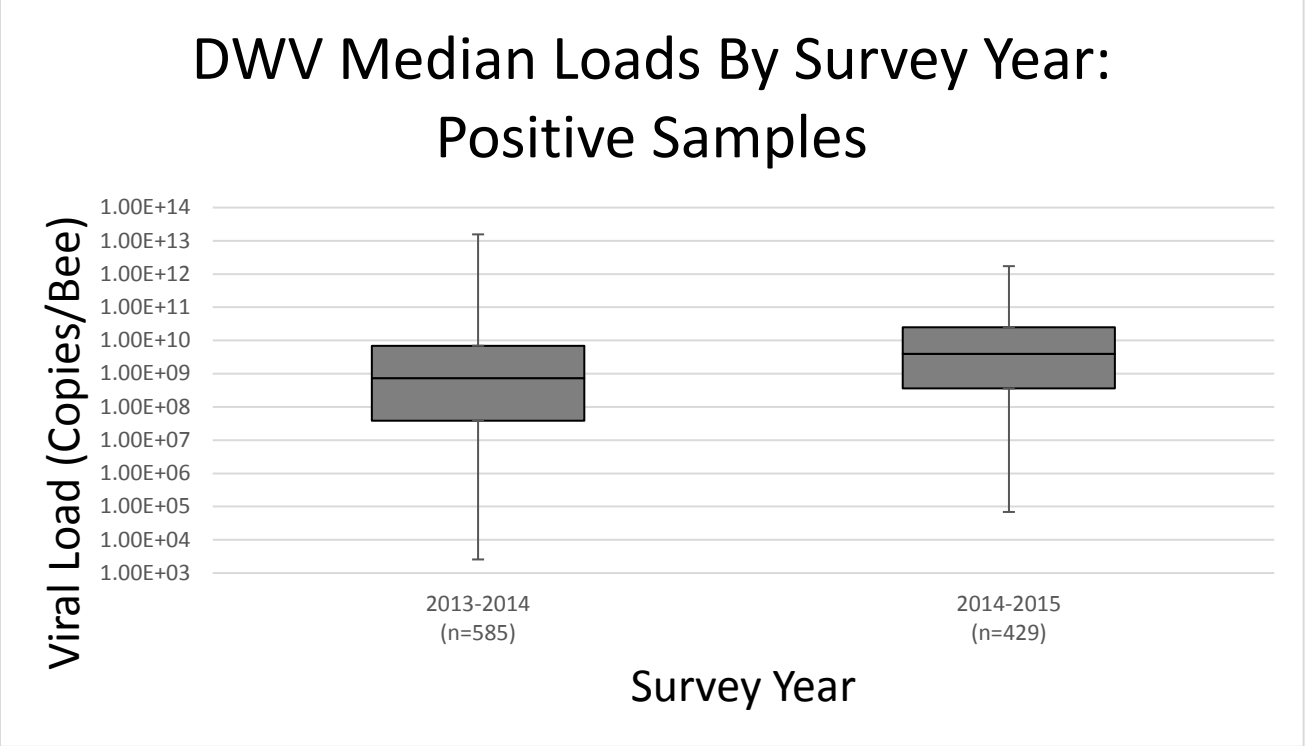

Figure 12: Median DWV viral load between the 2013 – 2014 survey year and 2014 – 2015 survey year (Displayed as box and whisker chart)

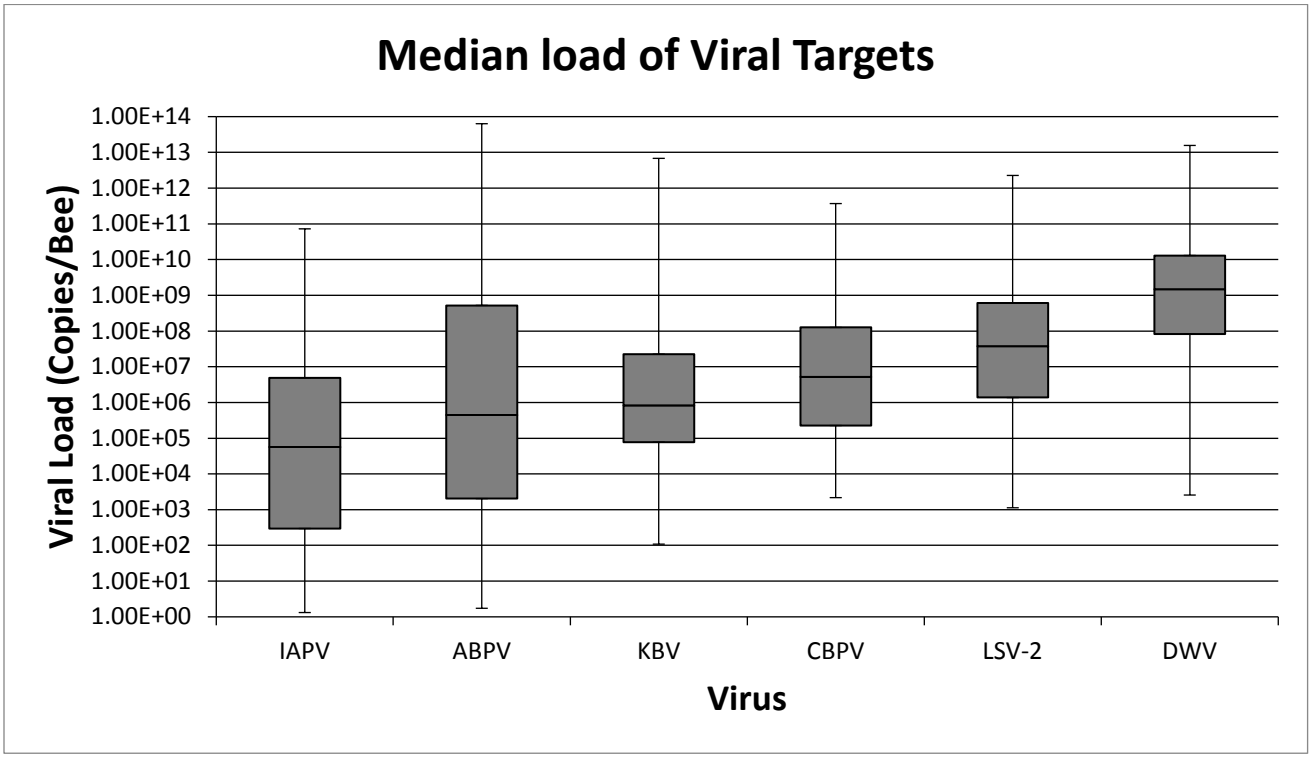

Figure 13: Median Viral loads from the 2013 – 2015 (displayed as box and whisker chart).

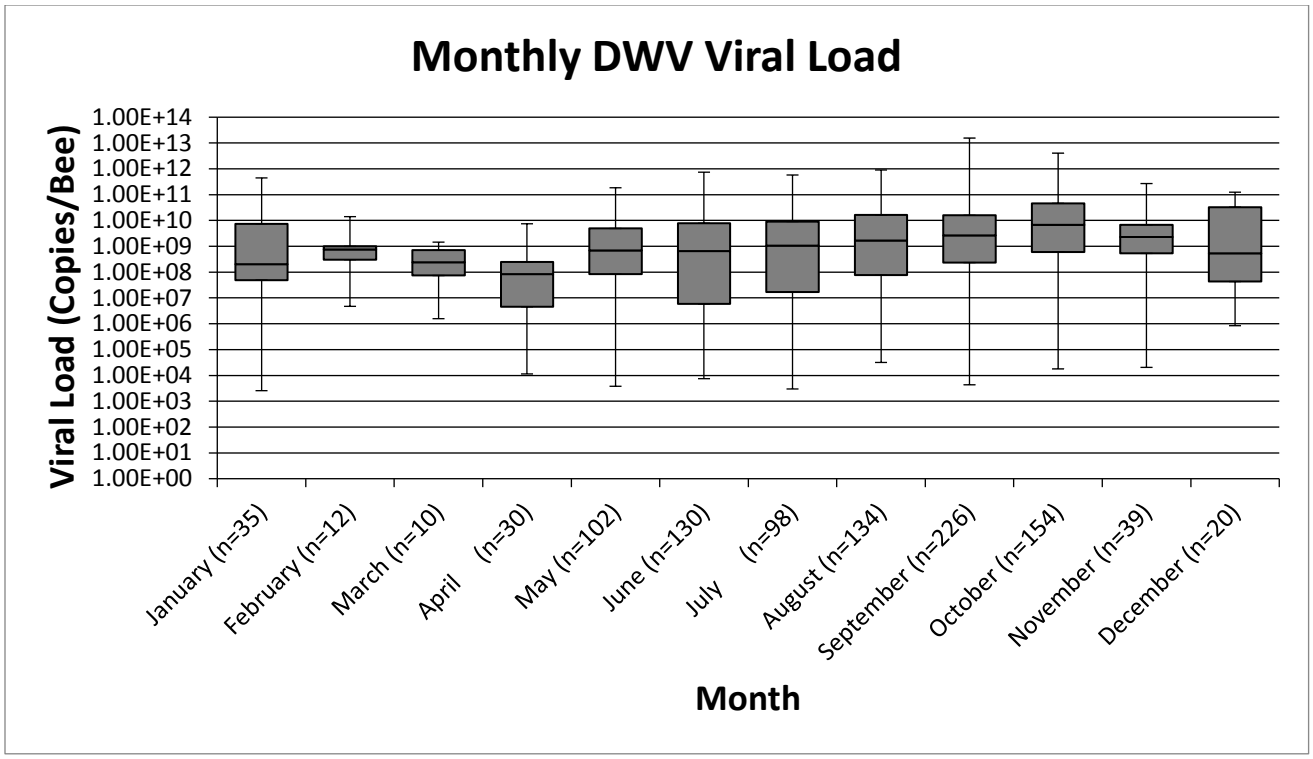

Figure 14: Monthly changes in DWV median viral from the 2013 – 2014 and 2014 – 2015 survey year (displayed as box and whisker chart).