2010-2011 National Honey Bee Pests and Diseases Survey Report

KAREN RENNICH[1], JEFF PETTIS[2], DENNIS VANENGELSDORP[1],
JERRY HAYES[3], MICHAEL ANDRE[1], ROB SNYDER[1], KAREN ROCCASECCA[1], NATHAN RICE[2],
JAY EVANS[2], DAWN LOPEZ[2], VIC LEVI[2], MARGARET SMITH[2], NISHIT PATEL[1] and ROBYN ROSE[4]

[1]Pennsylvania State University, [2]USDA Agricultural Research Service, [3]Florida Department of Agriculture and Consumer Services, [4]USDA Animal and Plant Health Inspection Service

Executive summary

The 2010 Limited National survey, focusing on 13 states, was performed to expand and augment the baseline pest and pathogen data collected from the pilot study conducted in 2009. It is the most comprehensive U.S. honey bee pest and disease survey to date. The primary focus of this survey was to verify the absence of the parasitic mite *Tropilaelaps* and other exotic threats to the U.S. bee population (e.g., *Apis cerana*). Under current international trade agreements, the U.S. cannot deny import permits from other nations unless the exporting nation has a disease, parasite, or pest of honey bees that is not found in the U.S. Establishing the absence of threats to honey bee populations not thought to be present in the U.S. was the primary objective of this effort.

To capitalize on the information gathered from this survey, samples were analyzed for other honey bee diseases and parasites known to be present in the U.S. The survey results are used to gauge the overall health of colonies and to help create a disease level baseline to help interpret ongoing and future epidemiological studies. The 2010-2011 National Survey effort was limited to collection of samples from 13 states including Alabama, California, Florida, Georgia, Hawaii, Indiana, Michigan, New York, Pennsylvania, South Dakota, Tennessee, Texas and Washington. A total of 349 samples representing over 2,700 colonies were collected. A further expansion of this survey is planned for 2011/2012 with the number of participating states increasing to 33.

The survey samples were analyzed for 11 known honey bee viruses, pests and pathogens including a DNA test for any occurrence of *Apis cerana*, the Asian honey bee. Molecular primers and a restriction enzyme test diagnostic for mitochondrial DNA of *A. cerana* were created for this survey and a broad sample representing all states was tested without a single detection. Slow Paralysis Virus (SPV), the only virus included in this year's testing that is not currently found in the U.S., was examined in all samples and no detection was made. No diseases or parasites of bees not already known to exist in the country were discovered. Only one virus, Deformed Wing Virus (DWV), was found in all 13 states. Also common to all states were the parasitic microsporidian *Nosema ceranae*, and the trypanosome *Crithidia*. It is not known at this time if *Crithidia* negatively or positively affect colony health. As in the pilot study

of 2009-2010, *N. ceranae* was identified in all samples positive for *Nosema* spp. while *Nosema apis* were not found in any state. For the second year, we saw no evidence of *Tropilaelaps* mites, nor honey bee tracheal mites (*Acarapis woodi*) in any sample. Honey bee tracheal mites are known to exist in the country and our failure to find them may be the result of our sampling procedure. Honey bee tracheal mites are most abundant in overwintering colonies and all samples were taken from colonies actively rearing brood. Varroa mites continued to be observed in all states with the exception of the Hawaiian Islands of Maui, Kauai and Molokai.

This survey was designed to be representative of the managed honey bees across the broad geography of the United States. We chose states as the units to determine the distribution of samples taken as funds were insufficient to allow for a more comprehensive representation based on colony abundance. We targeted key beekeeping states as a primary selection criterion and then secondarily, we chose states to fill in geographic voids to insure a degree of coverage across the U.S. When choosing states, attempts were made to include a distribution of states that represented queen production, honey production and had stationary and migratory practices. We also focused on high risk states that have key ports, long growing seasons and diverse agricultural crops. The results can thus be interpreted as representative of the pests and pathogens present in the U.S.

Introduction

This 13 state USDA survey of honey bee pests and pathogens began in 2010 and was completed in 2011. The survey encompasses all states sampled in the 2009/2010 pilot study plus 10 additional states. Funding was provided by the USDA Animal and Plant Health Inspection Service (APHIS) and the survey was conducted in collaboration with the USDA Agricultural Research Service (ARS), Pennsylvania State University (PSU) and the Florida Department of Agriculture and Consumer Services (FDACS). A total of 349 samples were collected from 50 apiaries in California (17 from migratory beekeepers who were in that state for pollination contracts and 33 from beekeepers originating from there), 24 from Hawaii and 25 from the remaining states (Alabama, California, Florida, Georgia, Hawaii, Indiana, Michigan, New York, Pennsylvania, South Dakota, Tennessee, Texas and Washington).

Survey Description

Survey kits were distributed to most of the participating states' Department of Agriculture offices in the spring of 2010. Apiary inspectors and agents conducted an aggregate sampling from previously identified commercial, migratory, and sideliner 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 US Honey Bee Survey Sampling Protocol). In each state, apiaries were chosen on a case by case basis 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 (move out of state and return prior to sampling) and 1/2 were from stationary operations (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 US Honey Bee Survey Project Plan).

Three distinct collection methods were used to sample each apiary. The first sample was a collection of live adult bees composed of ¼ cup of bees (¼ cup = ~ 150 bees) that were knocked off of brood frames from each of the 8 sampled colonies. The live bees were deposited in a live bee shipping box containing a water source and hard sugar candy. This box was shipped the same day to the USDA/ARS in Beltsville, MD where it was immediately frozen at -80C until molecular testing could be performed. The molecular tests were performed with quantitative-PCR techniques outlined by Dr. Jay Evans at the USDA/ARS Bee Research Laboratory to look for genetic markers in widely known and recognized viruses and other pests (2006 and Honey Bee PCR Diagnostics). The molecular tests were designed to detect the presence of the following:

1. Acute Bee Paralysis Virus (ABPV)
2. *A. cerana* mitotype
3. Deformed Wing Virus (DWV)
4. Israeli Acute Paralysis Virus (IAPV)
5. Kashmir Bee Virus (KBV)
6. *Nosema ceranae*
7. *Nosema apis*
8. Slow Paralysis Virus (SPV)
9. *Crithidia* spp.

The second sample of bees, consisting of ¼ cup of bees from each of the 8 sampled colonies, originated from the same brood frames as the live bee sample. These bees were put into a bottle of alcohol for preservation. This alcohol sample was shipped to PSU for microscopic analysis to quantify the following:

1. *Nosema* spp. spores
2. Tracheal Mite loads
3. Varroa Mite loads

Finally, the third sample was taken from anything dislodged from 'bumping' sampled brood frames over a collection pan. This technique was developed by Dr. Jeff Pettis and Dr. Dennis vanEngelsdorp and funded by APHIS as a quick and cost effective way to detect for the *Tropilaelaps* mite. The sample, also preserved in alcohol, included any mites, beetles and other hive debris filtered from bumping the brood frames. This sample was shipped to USDA/ARS Beltsville, MD and analyzed for the presence of the *Tropilaelaps* mite.

All participating beekeepers, as well as State Apiarist/Inspectors, received two reports for each sample taken. The first report, sent within 3 months of collection, details the analysis results for Varroa mite load, *Nosema* load, detection of *Tropilaelaps* and tracheal mites. The second report, a molecular report sent within 6 months of sample collection, summarizes the presence or absence of any of the five viruses, identifies the *Nosema* species, and notes the presence or absence of *A. cerana* and Trypanosomes.

Using the U.S. Postal Service, live bee shipments were made to USDA/ARS and percent survivability was tracked for all live bee shipments. The results of this analysis, previously proven to be robust and a suitable alternative for shipping bees on dry ice by the pilot study, continued to work well and the survivability analysis can be seen by Figure 1 in the Appendix. In some states, a small number of live bee samples were degraded badly enough that no molecular data could be retrieved from the samples. This occurred in the states of FL, HI, PA and TN.

Results

The results of all molecular and microscopic analysis can be found in the Appendix. An average taken over all 349 collected samples yielded a mean *Nosema* spore load of 454,000 spores per bee, about half the threshold of 1 million spores per bee thought to cause damage from infection with *N. apis* (Figure 2). Of those samples that tested positive for *Nosema* (removing all those samples that had no *Nosema*) the average spore count was 918,000 spores per bee, right at the threshold for potential damage. These samples accounted for 179 out of 349 (51%) of all samples. Of the samples that tested positive for *Nosema*, 36 samples (20%) exceeded the threshold to cause damage (> 1 million spores per bee).

While the economic threshold for Varroa mites is seasonally and regionally specific, the average load of almost 4 mites per 100 bees is of concern, as this rate of infestation is almost certainly an indication of mite populations which, left unchecked, would cause damage (Figure 3). The detection of Varroa in the range of 3-10 mites/100 bees are thought to cause damage. Of the 349 samples received, 323 (93%) had at least 1 Varroa mite detected. About 43% of the samples that tested positive for Varroa (138 out of 323) exceeded the lower threshold for possible damage to a colony from Varroa.

Figures 4 and 5 illustrate the dynamic nature of *Nosema* and Varroa mite populations over the course of the year. *Nosema* levels typically appeared highest in late fall and spring months and spring 2011 samples showed higher loads than last year. Varroa mite levels were highest in the late summer and fall months. It should be noted; however, that the majority of the states sampled apiaries in those months (summer and fall) where Varroa mites may occur at a higher level. It is unknown whether the sampled apiaries treated for Varroa and/or *Nosema*.

The percentage of colonies (n= 25 unless otherwise noted) testing positive for DWV (Figure 6), IAPV (Figure7), KBV (Figure 8) and ABPV (Figure 9) showed that viral profiles did differ between states. Both *Nosema ceranae* (Figure 10) and *Trypansome* sp. (Figure 11) were found in all states. *N. ceranae* was found in 44% of samples using the PCR technique and Trypanosomes were found in 50% of all samples. Because of the DNA extraction methods employed, our molecular identification methods would only confidently find actively reproducing *Nosema* (vegetative stage) but not detect dormant (spore stage) *Nosema*. For this reason it is possible that examined samples had detectable levels of *Nosema* as determined with one detection method while not having detectable methods using another method. This accounts for the difference in the PCR and microscopic detection of *Nosema* in these samples. Although *N. ceranae* and Trypanosomes are also very common, they, like most viruses, display slight seasonality but further data are required to confirm this (Figure 13).

The ubiquitous nature of DWV is further demonstrated in Figure 12 as it remains fairly constant over the months while other viruses demonstrate some seasonality. DWV was, in fact, found in 90% of all samples. As 8 colonies were combined for each apiary sample, any direct link between Varroa mite prevalence contributing as a virus vector cannot be distinguished.

Finally, this study found no evidence of *Tropilaelaps*, SPV, or honey bee tracheal mites. Visual analysis of samples collected in alcohol, in addition to a screening process using *A. cerana* DNA, did not detect a presence of this exotic *Apis* species.

It should also be noted that no detection was made of *N. apis* from any of the composite molecular samples. This agrees with previous findings (Chen *et al*, 2008) that *N. ceranae* has largely replaced *N. apis* in the European honey bee (*Apis mellifera*) after migrating from its original host, *A. cerana* to *A. mellifera*.

Conclusions

The increased samples from 13 states allow for the expansion of our database of pests and pathogens and place the collected data into a temporal context. Using this increased data base we can draw broader conclusions but there are still insufficient data to formulate comprehensive statements about invasive mites or exotic *Apis* species. As the survey continues in coming years and by gathering yearly, sequential samples from a growing number of states, we may be able to see trends and patterns that relate to colony health. The survey does provide strong evidence that *Tropilaelaps*, Slow Paralysis Virus and *Apis cerana* are not present in the U.S.

Appendix

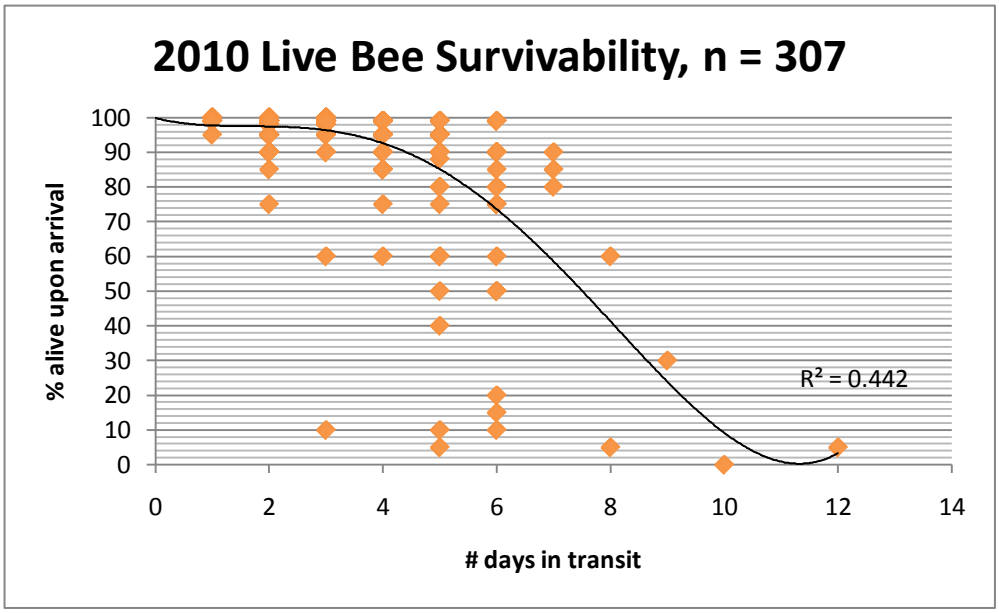

Figure 1: Live Bee Shipping Survivability

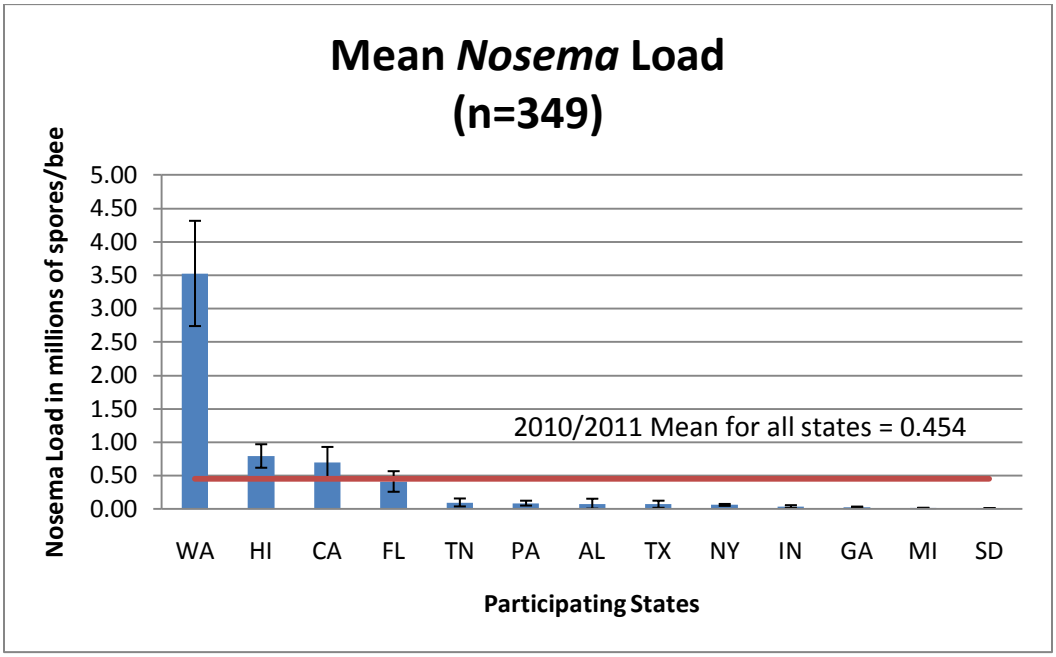

Figure 2: Mean *N. ceranae* Load Ranking by State
(Standard Error bars are reported)

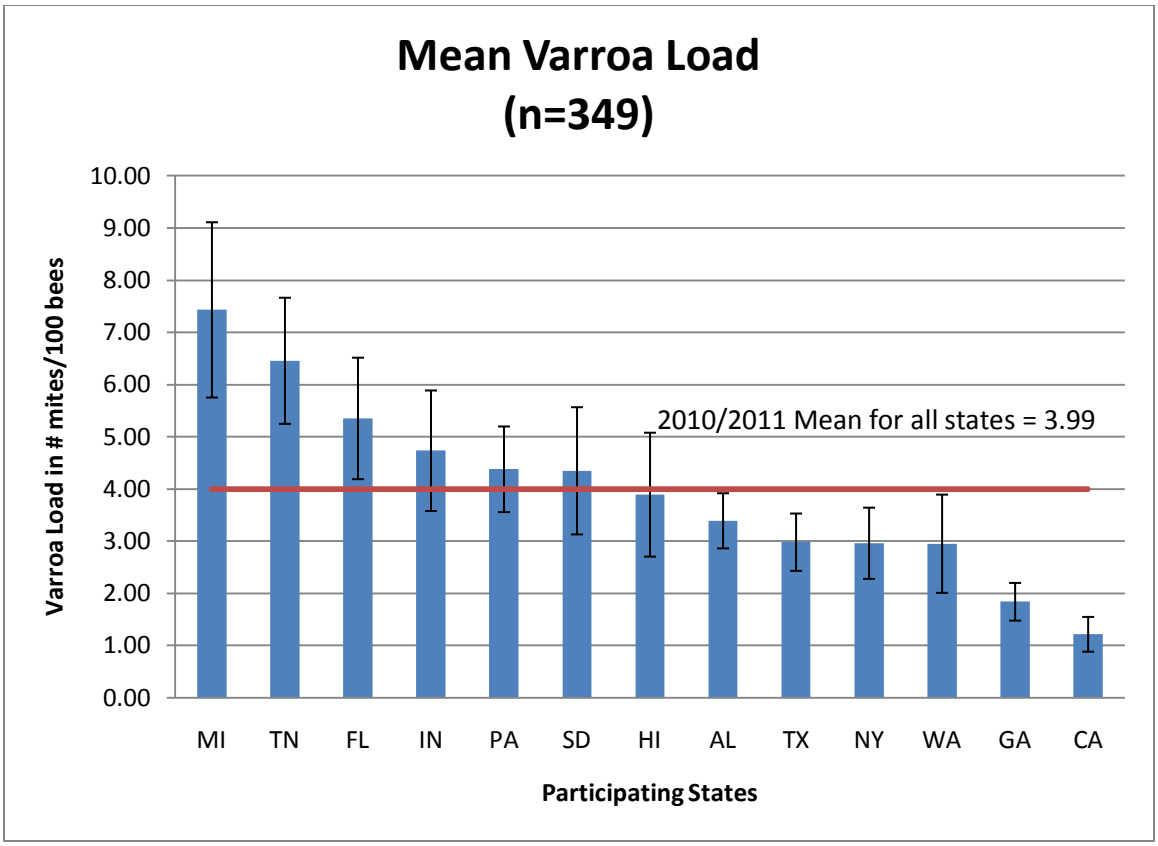

Figure 3: Mean Varroa Load Ranking by State
(Standard Error bars are reported)

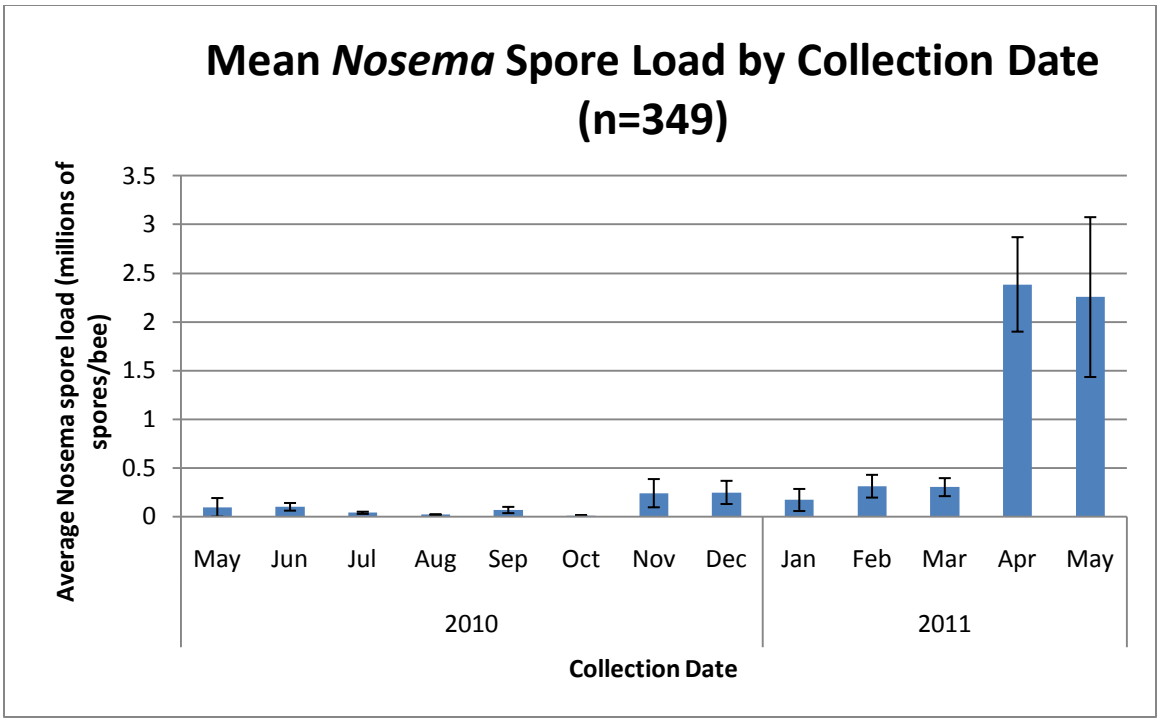

Figure 4: Mean *N. ceranae* Load
(Standard Error Bars are reported)

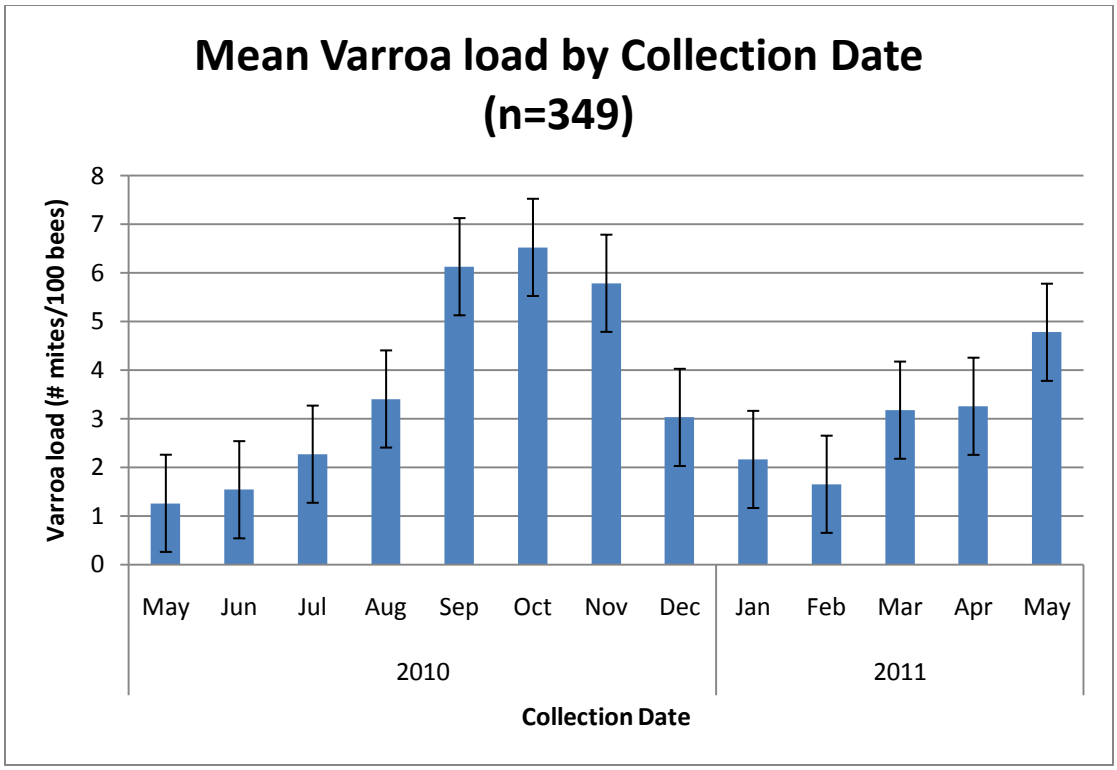

Figure 5: Mean Varroa Load
(Standard Error Bars are reported)

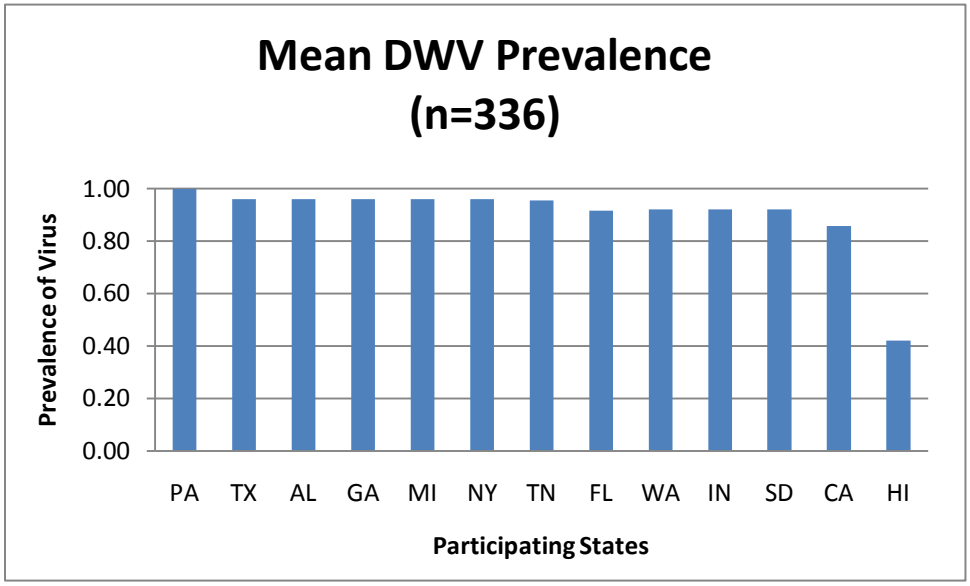

Figure 6: Prevalence of Deformed Wing Virus in sampled apiaries

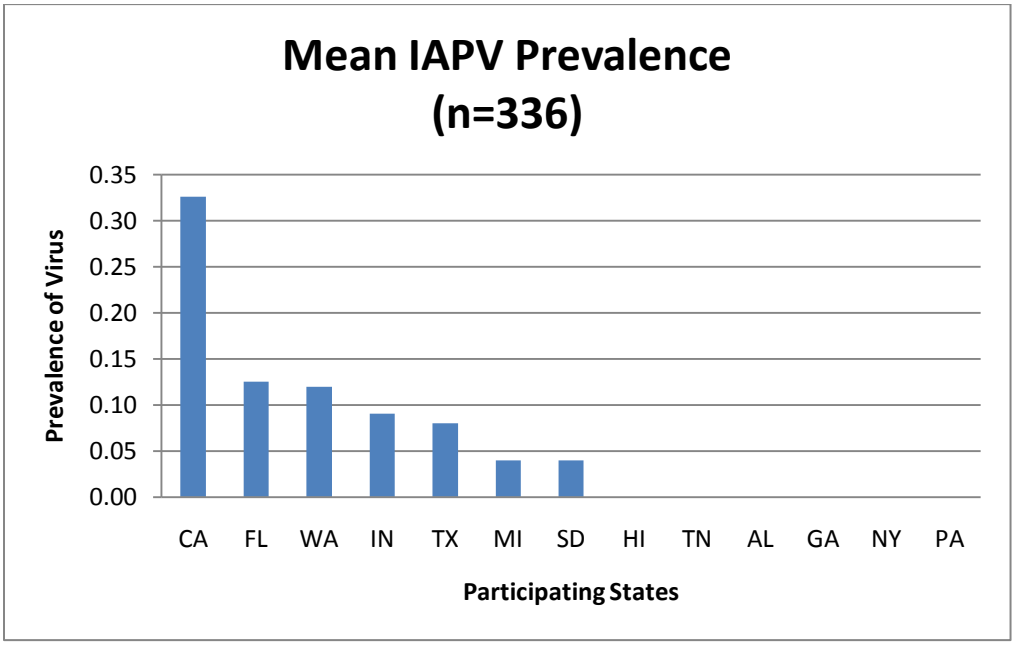

Figure 7: Prevalence of Israeli Acute Paralysis Virus in sampled apiaries

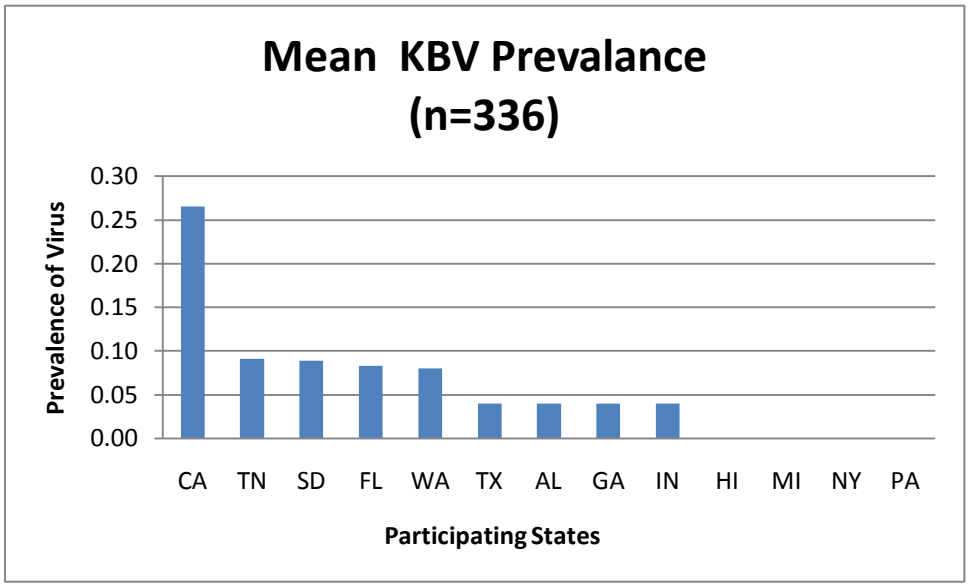

Figure 8: Prevalence of Kashmir Bee Virus in sampled apiaries

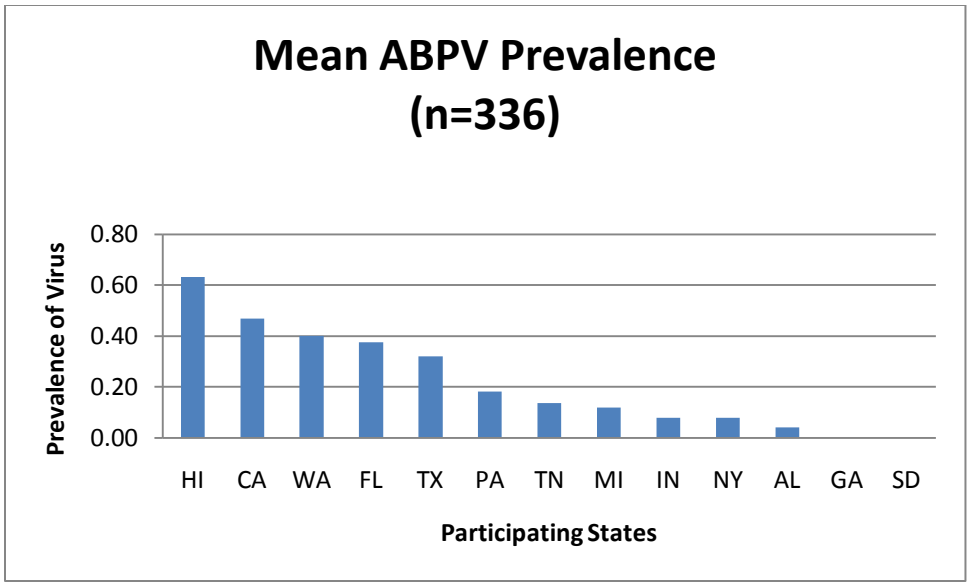

Figure 9: Prevalence of Acute Bee Paralysis Virus in sampled apiaries

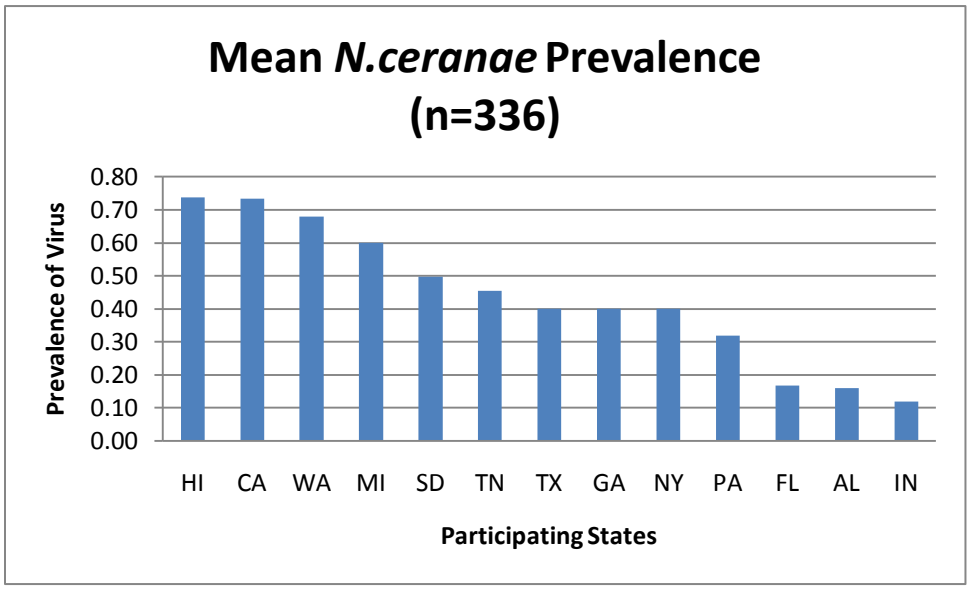

Figure 10: Prevalence of *N. ceranae* in sampled apiaries (PCR technique used)

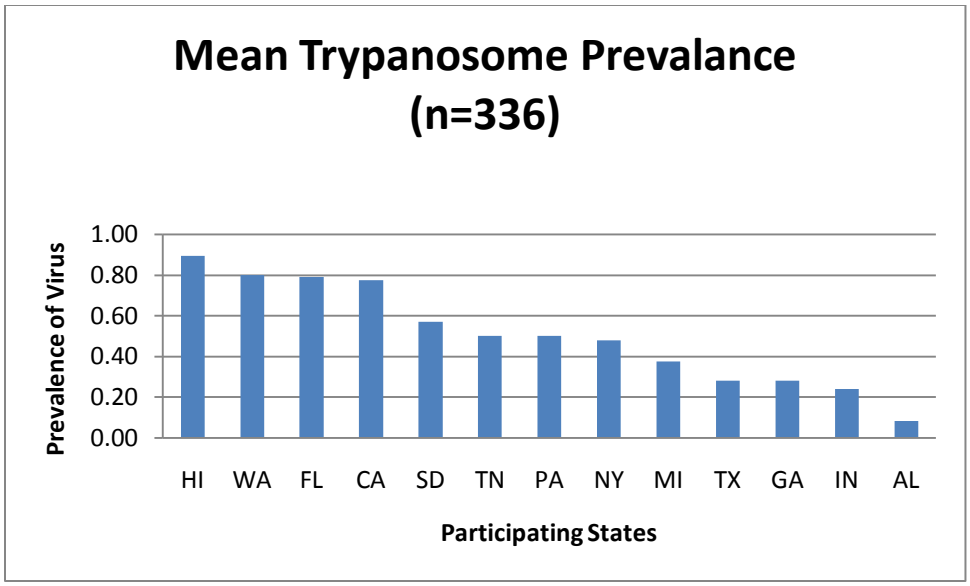

Figure 11: Prevalence of *Trypanosome* in sampled apiaries

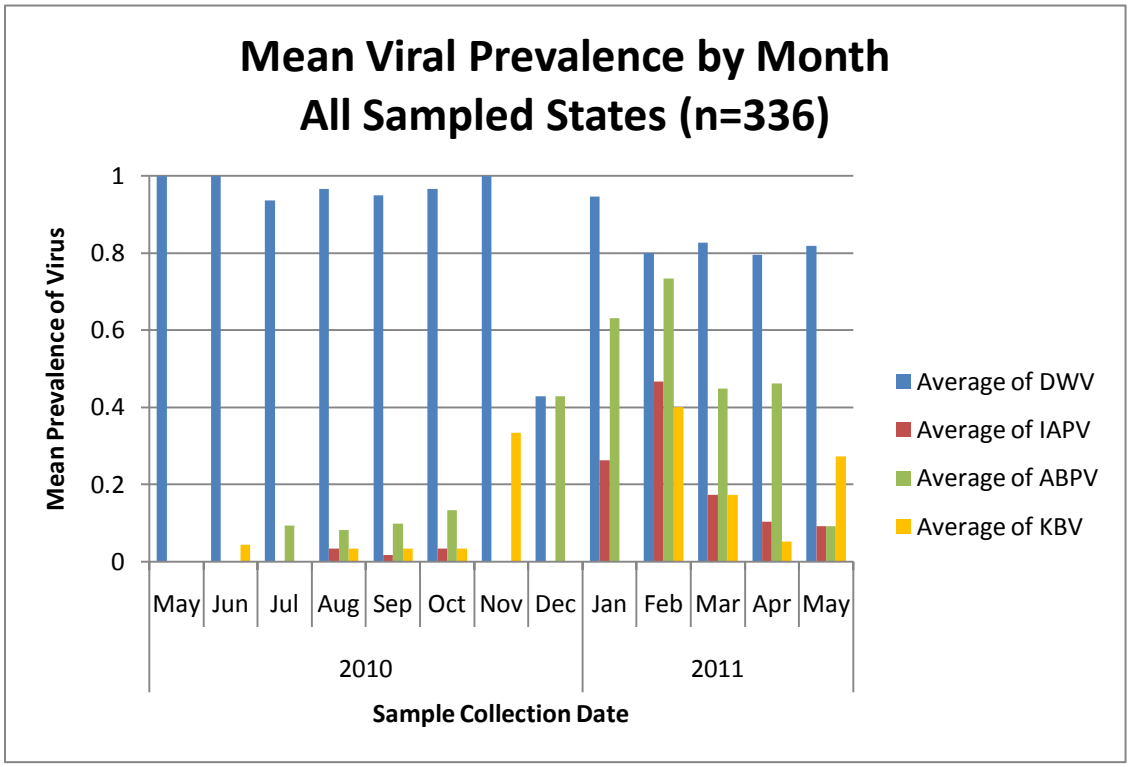

Figure 12: Mean Viral Prevalence by Month

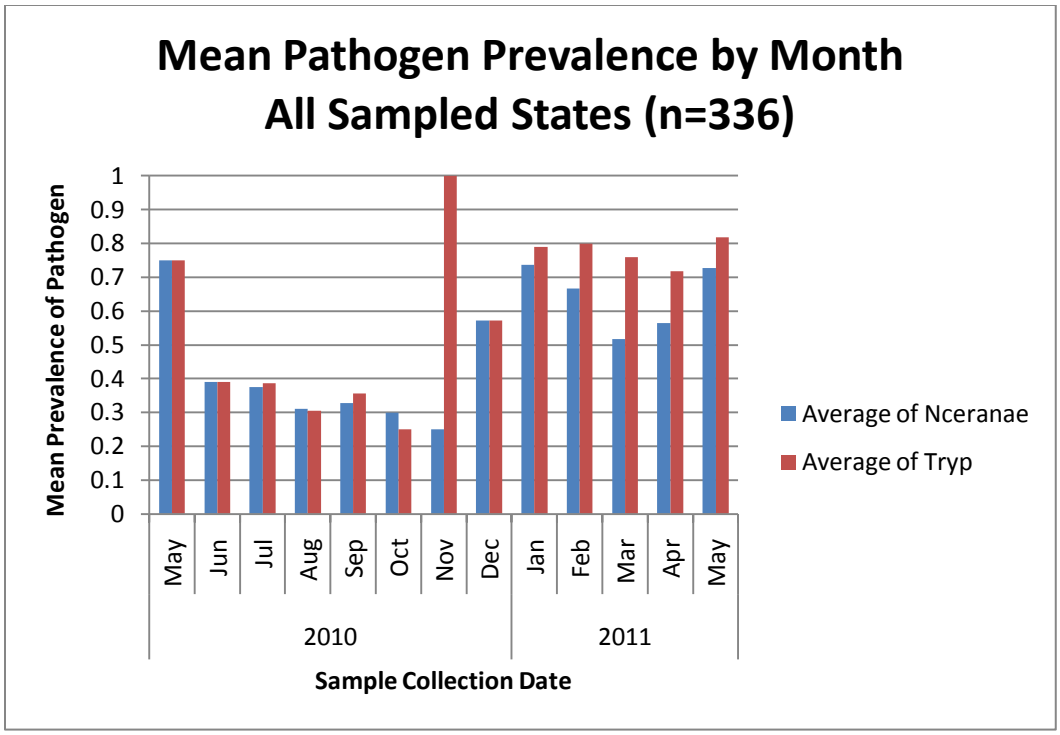

Figure 13: Mean *N. ceranae* and *Trypanosome* Prevalence by Month (PCR technique used)

References

Evans J.D. (2006). Beepath: An ordered quantitative-PCR array for exploring honey bee immunity and disease, Journal of Invertebrate Pathology, 93 (2), pp. 135-139.

Chen Yanping, J.D. Evans, I.B. Smith, and J.S. Pettis (2008). *Nosema ceranae* is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States, Journal of Invertebrate Pathology, 97, pp. 186-188.