



Nosema and Honey Bee Colony Health

Brenna E. Traver

Richard D. Fell

What is *Nosema*?

Members of the *Nosema* genus are microsporidians, which are now classified as a fungus. *Nosema* species (spp.) are obligate, intracellular parasites. This means that they require a host (in this case a honey bee) to complete their lifecycle. There are two species from the genus that infect our honey bees (*Apis mellifera*) – *Nosema apis* and *Nosema ceranae*. *Nosema apis* is a well studied species that has been known to infect honey bees for over 100 years. A thorough review is given by Fries [1]. *Nosema ceranae* is a closely related species and was first found in *Apis cerana*, the Asian honey bee in 1996 [2] and then in the European honey bee in 2006 [3-4].

Life cycle

Both *N. apis* and *N. ceranae* are spore producing organisms. The spore is the infectious life stage. Honey bees become infected when they ingest *Nosema* spores. Once ingested, spores pass through the esophagus and honey stomach and into the honey bee midgut. In the midgut, spores germinate and inject their sporoplasm (their cellular content) into the epithelial cells that line their gut. The sporoplasm contains *Nosema* spp. genetic material and allows *Nosema* spp. to successfully replicate and reproduce within the honey bee midgut. One reason *Nosema* can be so devastating is the resilience of the spores. Spores are very resistant to changes in temperature, to desiccation, and can survive outside of the bee in the hive environment for extended periods of time.

What's the story in Virginia?

The recent discovery and widespread prevalence of *N. ceranae* in the United States raised concerns as to whether this new pathogen is involved with increased colony losses [5-6]. In order to determine if *N. ceranae* is present in Virginia, we initiated a statewide survey in March 2009 to determine whether *N. ceranae* is present and if so, at what levels [7].

We detected *N. ceranae* in over 70% of colonies in Virginia, whereas *N. apis* was found in less than 3% of colonies, always at low levels and as a co-infection with *N. ceranae*. Of the infected hives analyzed, 11% of hives were classified as having a heavy infection, 16% as a moderate infection, and 73% as a low level of infection. We also found no significant difference between: infection levels and geographic location within the state, infection prevalence and location, and infection levels and colony strength. Interestingly, some of the colonies with the highest levels of *N. ceranae* were ranked as the strongest colonies.

Which honey bee caste does *Nosema ceranae* affect?

Nosema spp. is typically spread in an oral-fecal manner. Healthy bees do not defecate in the hive, but sick, heavily infected bees may do so. Other healthy workers become exposed when they clean up after the sick bees. Transmission of *Nosema* spp. can also occur during food exchange (trophallaxis) and with the consumption of contaminated food and/or water. The rapid spread of *N. ceranae* suggests that there

are additional mechanisms for transmission. We examined whether bees in other castes (i.e. queens and drones) are naturally infected with *N. ceranae*. In our study, drones of different ages (pupae, in-hive drones, and flying drones) were analyzed and found to be infected with low levels of *N. ceranae* [8]. We also examined queens to determine if they were naturally infected with *N. ceranae*. We examined queen larvae and the corresponding royal jelly from the cell, newly emerged virgin queens, and older mated queens. Queens of all ages were found to be infected at low levels [9] suggesting that re-queening colonies could have helped in the initial spread of *N. ceranae*. Low-level infections were also found in the reproductive organs of drones, suggesting that they could play a role in the infection of queens through mating. Infection of the spermatheca and ovaries suggest the possibility of vertical transmission (parent to offspring), but additional studies are needed to determine if such a transmission mechanism is possible.

***Nosema* diagnosis**

The traditional method for diagnosing *Nosema* infections has been to crush adult worker abdomens in a small amount of water and examine the lysate (fluid plus cellular debris from crushed bees) for spores under a microscope. Typically the more spores observed, the higher the level of infection present. However, this is not always the case. *Nosema apis* is a prolific spore producer, but *N. ceranae* does not produce as many spores and favors reproduction of vegetative stages (non-spore life stages). For this reason molecular analytical techniques provide a more accurate diagnosis of *N. ceranae* infections. However, this approach is not practical for beekeepers who wish to sample their hives. The use of the traditional spore analysis method can provide the beekeeper with a good indication of whether heavy *Nosema* infections are present in his or her hives and we recommend their use for beekeepers who want to monitor their hives for a *Nosema* infection.

Sampling procedures

We recommend that 25-30 bees be used per colony in diagnosing colonies for *Nosema*. A single pooled sample of bees can be used (25-30 bees from a colony) or several sets of 10 bees can be used instead. Examining a large pooled sample of bees will give a good colony overview of a *Nosema* infection. However, using a single sample of 25-30 bees may be misleading if only one bee is highly infected. A better approach is to examine multiple samples of five bees for spores to reduce the likelihood of overestimating the infection level. Once you obtain a bee sample from your colony, you can store the bees in 70% ethanol or in rubbing alcohol (isopropyl alcohol). Place the bees into a small ziplock bag and using a rolling pin or similar tool (like a tall thin wine bottle), crush the bees, while being careful not to puncture the bag (Figure 1). Two teaspoons of water (approximately 10 milliliters) per 10 bees should be added prior to crushing. Alternatively, a large mortar and pestle can be used to crush the bees (Figure 2). Once crushed, a small drop of the crushed liquid, the lysate, can be put on a microscope slide with a cover slip and examined for *Nosema* spores (Figure 3).

Nosema infections can be expressed as a spore count per number of bees examined. Scientists typically use a special slide called a hemocytometer with grids to more accurately estimate the spore count in a given sample. But you do not need a hemocytometer to examine samples for spores. When looking under the microscope, what you see is called a field of view. If you only see one to several spores, then you probably have a very low level infection. The more spores you see, the greater the infection present. If you observe 5 spores in a field of view at 400X, then there are approximately 1 million spores per bee [10]. While *N. ceranae* is a less prolific spore producer than *N. apis*, estimating 1 million spores per bee sample is a good threshold value to use when considering the need for colony treatment.

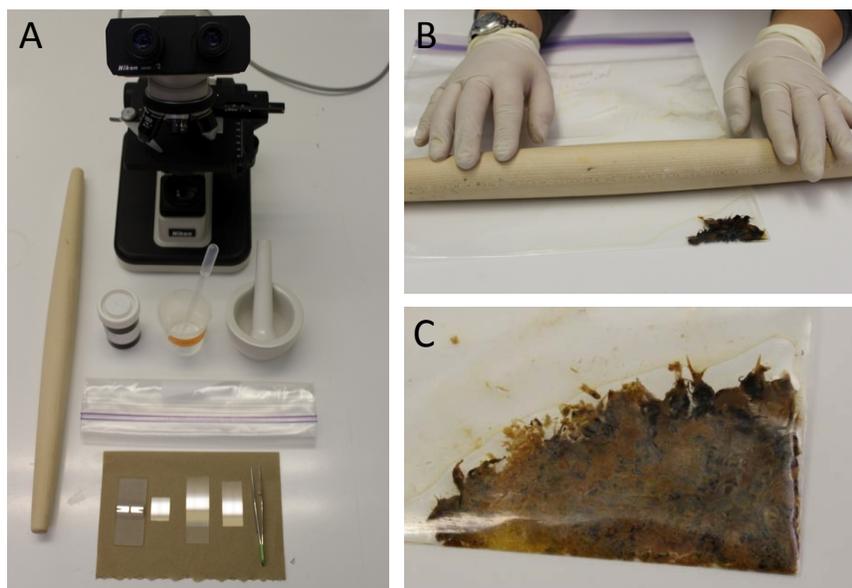


Figure 1. Crushing bees for spore counting. (A) All materials needed for spore counting using a plastic bag or a mortar and pestle. (B) Ten bees were put into a plastic bag and then crushed using a rolling pin. (C) Bees were mashed until all major body parts were crushed. Photo credit: Neema Syovata.



Figure 2. Crushing bees using a mortar and pestle. Ten bees were placed in a mortar and crushed into a paste using the pestle. Water is then added to the bees to form the lysate. Photo credit: Neema Syovata.

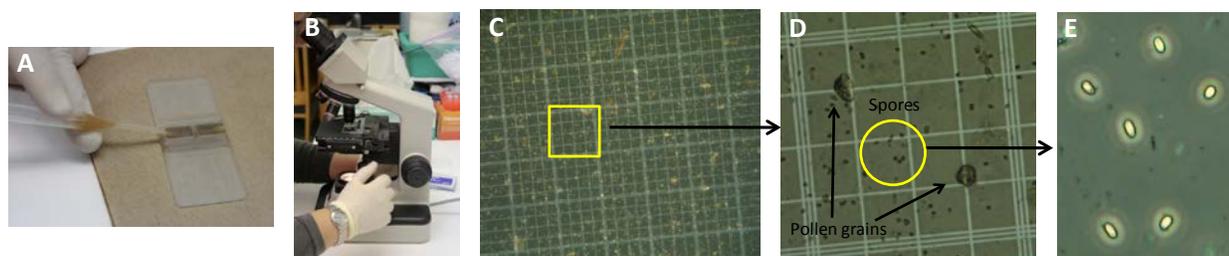


Figure 3. Spore counting process. After bees are crushed, the lysate is loaded onto a hemocytometer (a standard microscope slide can also be used). Images taken from a hemocytometer during *Nosema* spp. spore counting using a standard compound microscope. (A) Loading crushed bee lysate onto hemocytometer (B) Examining hemocytometer with bee lysate under a compound microscope (C) Picture of a hemocytometer 5x5 grid (from one chamber in hemocytometer) taken at 100X. Yellow box indicates one of the smaller 4x4 grids that are used for spore counting (D) One of the 4x4 squares highlighted in the former image. Image of spores and pollen grains taken at 200X. (E) Image of *Nosema*

spp. spores at 400X. Spores refract light making it difficult to focus but are oval-rod shaped. Photo credit for (A) and (B) Neema Syovata.

Sampling for *N. ceranae* has been controversial [11-14]. In order to provide beekeepers the best advice on how to sample, we examined whether there were different *N. ceranae* levels in bees sampled from different parts of the hive. When we compared bees sampled from different areas in the hive (i.e. brood nest, fringe of the brood nest, and honey supers), there was no significant difference in infection levels among the different groups of bees sampled [15]. For *N. apis* infections, it was thought that sampling foragers was the most accurate way to diagnose an infection. When we sampled foragers and in-hive bees, we did not find a significant difference in infection levels between the two groups of bees [15]. We now recommend that beekeepers take random in-hive samples for *N. ceranae* diagnosis. We also believe that sampling from in-hive bees provides a better representation of *Nosema* levels within a colony as foragers only represent about 25% of the colony population [16].

***Nosema* treatment**

Current recommendations for treating *N. ceranae* infections are based on knowledge of *N. apis* infections. Reliable threshold levels for *N. ceranae* treatment are still unknown. *Nosema apis* and now *N. ceranae* infections are treated with the antibiotic fumagillin, sold under the trade name Fumagilin-B. Fumagillin is an antimicrobial agent originally isolated from the mold *Aspergillus fumigatus* [17-18]. During a nosema infection, the parasite hijacks the host's cellular systems and suspends DNA replication of the honey bee host. Fumagillin inhibits *Nosema* development by inhibiting RNA synthesis and restores the host, honey bee, DNA replication.

Our studies on *N. ceranae* have helped determine the need and the most appropriate time for the treatment of *N. ceranae* infections. Our observations on seasonal variation of *N. ceranae* infections have shown significantly higher levels in April-June and lower levels in the fall and winter [15] (Figure 4). Treatment with chemicals during the spring, as the major nectar flow starts, is not advisable because of the potential for honey contamination. Disease levels are low during the winter, limiting the need for fall treatment, and the use of fumagillin is expensive and can be time consuming with large numbers of colonies. However, there are times when the treatment of colonies may be beneficial, particularly when dealing with weak, non-productive colonies in the early spring. Such colonies should be examined for *Nosema* and then treated if necessary, although studies have shown that fumagillin is only marginally effective at reducing *N. ceranae* levels. We no longer recommend treatment for *N. ceranae* in the fall since winter infection levels are low and do not appear to impact colony survival.

Seasonal Infection Cycle for *Nosema ceranae* in Virginia

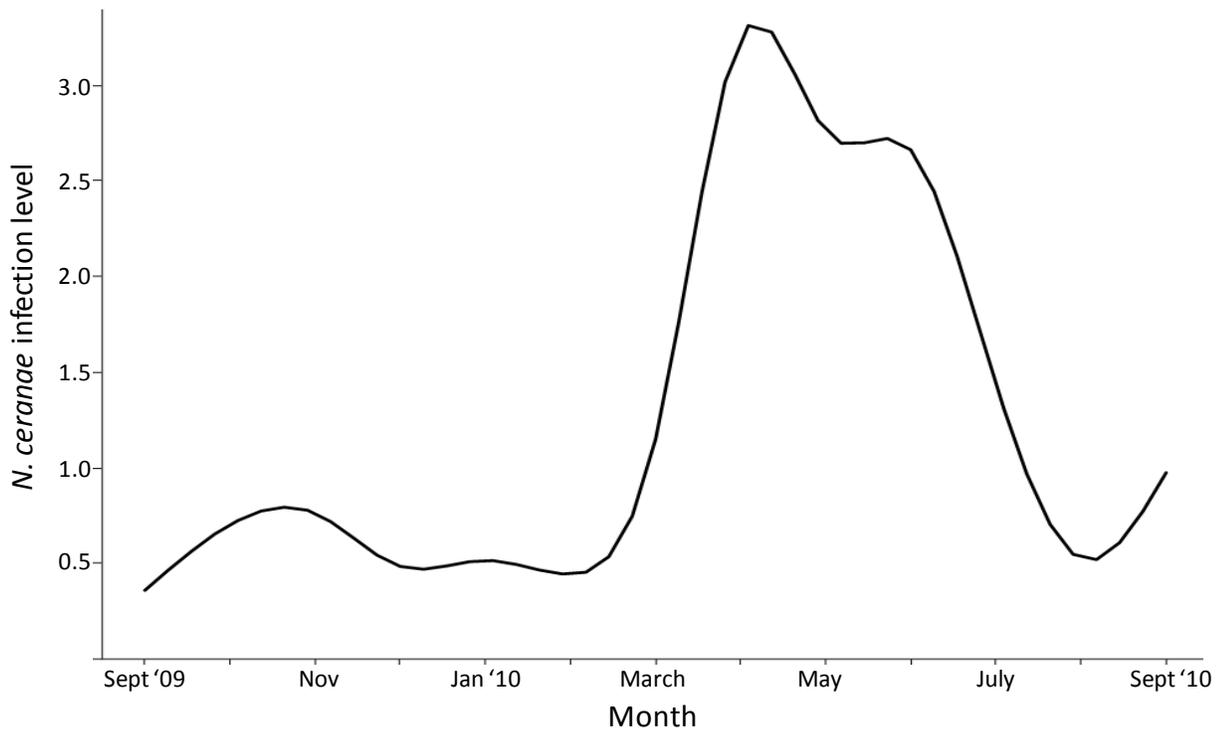


Figure 4. *Nosema ceranae* levels observed throughout 13 months of sampling. Sampling was initiated in September 2009 and ended in September 2010. The *N. ceranae* infection level is on the vertical axis while month is on the horizontal axis.

If planning to treat, we recommend using a one-gallon feeder pail with a 50% sugar solution containing fumagillin for each hive (a higher sugar concentration can be used for fall treatments if necessary; Figure 5). Five grams (approximately a teaspoon) of fumagillin should be used per gallon of syrup. Treatment should be administered twice, at two-week intervals.



Figure 5. Image of fumagillin, feeder pails, and how to apply a feeder pail to a colony. Holes are punched in the lids of the feeder pails so the bees can feed on the sugar syrup. A twig is put under the feeder pail to allow the bees better access to the syrup.

An alternate approach that we have investigated was stimulative pollen feeding during the winter to help improve overall colony health and spring build-up. However, pollen feeding in late January and early

February did not significantly reduce infection levels 3-months post treatment, nor did it significantly increase colony survival (we saw a small increase of 6%, but it was not statistically significant). On the other hand we do feel that feeding pollen supplements helps with colony build-up in the spring and utilize the practice in our apiaries.

Colony mortality due to *N. ceranae* infections does not appear to be a major factor in colony losses throughout the mid-Atlantic region. We do not feel that *N. ceranae* is a major factor associated with colony decline. However, we do acknowledge that there could be different haplotypes or 'strains' of *N. ceranae* worldwide that could have different effects on colonies. Also, it is important to understand that *N. ceranae* may interact differently with different honey bee subspecies (*Apis mellifera mellifera* vs. *A. mellifera iberiensis*) which could also help explain some of the reported differences in the impacts of colony infections. While we do not think that *N. ceranae* is solely responsible for colony losses, interactions with other stress factors that affect the immune system, such as viruses, varroa mites and pesticide residues, could all increase the negative effects of a *N. ceranae* infection. Our understanding of *N. ceranae* is far from complete, but believe that continued research will give us a better understanding of how this parasite impacts the honey bee colony.

References

1. Fries, I., *Nosema apis*- A parasite in the honey bee colony. *Bee World*, 1993. **74**(1): p. 5-19.
2. Fries, I., et al., *Nosema ceranae* n. sp. (*Microspora*, *Nosematidae*), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, *Apidae*). *European Journal of Protistology*, 1996. **32**: p. 356-365.
3. Higes, M., R. Martín, and A. Meana, *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. *Journal of Invertebrate Pathology*, 2006. **92**(2): p. 93-95.
4. Huang, W.-F., et al., A *Nosema ceranae* isolate from the honeybee *Apis mellifera*. *Apidologie*, 2007. **38**(1): p. 30-37.
5. Cox-Foster, D.L., et al., A metagenomic survey of microbes in honey bee colony collapse disorder. *Science*, 2007. **318**(5848): p. 283-287.
6. Bromenshenk, J.J., et al., *Iridovirus* and microsporidian linked to honey bee colony decline. *PLoS ONE*, 2010. **5**(10): p. e13181.
7. Traver, B.E. and R.D. Fell, *Prevalence and infection intensity of Nosema in honey bee (Apis mellifera L.) colonies in Virginia*. *Journal of Invertebrate Pathology*, 2011. **107**(1): p. 43-49.
8. Traver, B.E. and R.D. Fell, *Nosema ceranae* in drone honey bees (*Apis mellifera*). *Journal of Invertebrate Pathology*, 2011. **107**(3): p. 234-236.
9. Traver, B.E. and R.D. Fell, *Low natural levels of Nosema ceranae in Apis mellifera queens*. *Journal of Invertebrate Pathology*, 2012. **110**(3): p. 408-410.
10. Oliver, R. *Scientific Beekeeping*. 2014; Available from: www.scientificbeekeeping.com.
11. Meana, A., R. Martín-Hernández, and M. Higes, *The reliability of spore counts to diagnose Nosema ceranae infections in honey bees*. *Journal of Apicultural Research*, 2010. **49**(2): p. 212-214.
12. El-Shemy, A.A.M. and R.S. Pickard, *Nosema apis* Zander infection levels in honeybees of known age. *Journal of Apicultural Research*, 1989. **28**(2): p. 101-106.
13. L'Arrivee, J.C.M., *The effects of sampling sites on Nosema determination* *J. Insect Pathol.*, 1963. **5**: p. 349-355.
14. Taber, S. and H. Lee, *Seasonal variation in levels of nosema infection in honey bees in Arizona*. *Glean. Bee Cult.*, 1973. **101**: p. 281-282, 299.
15. Traver, B.E., M.R. Williams, and R.D. Fell, *Comparison of within hive sampling and seasonal activity of Nosema ceranae in honey bee colonies*. *Journal of Invertebrate Pathology*, 2012. **109**(2): p. 187-193.
16. Seeley, T.D., *The wisdom of the hive: the social physiology of honey bee colonies*. 1995, Cambridge, Massachusetts: Harvard University Press. 295.
17. Eble, T.E. and F.R. Hanson, *Fumagillin, an antibiotic from Aspergillus fumigatus H-3*. *Antibiotics and Chemotherapy*, 1951. **58**(1): p. 54-58.
18. Hanson, F.R. and T.E. Eble, *An antiphage agent isolated from Aspergillus sp.* *Journal of Bacteriology*, 1949. **58**(4): p. 527-529.