Nosema Testing

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Introduction

Nosema disease (sometimes called Nosemosis) is a fecal-oral transmitted disease of adult honey bees caused by either the microsporidia *Nosema apis* or *Nosema ceranae*. Note that these pathogens have been classified as *Varimorpha*, but most beekeepers and researchers still refer to them as nosema. This gut pathogen can affect the physiology, behavior, and survival of adult bees.

Indications

Nosema does not cause obvious visual signs at the colony or individual bee level. A beekeeper may note multiple bees exhibiting k-wing (Figure 1) or note fecal spotting on the hive (Figure 2). However, both of these signs also are seen in association with other diseases and hive conditions, and it is common to have high levels of nosema infection with no visual or apparent signs.

Figure 1: Honey bee exhibiting k-wing, where the wings on one or both sides are held out at an angle indicating discomfort caused by nosema disease or other disease. Photo by UF/IFAS Honey Bee Research and Extension Laboratory.

SUPPLIES NEEDED

Labeled sample containers that will hold 100 adult bees (approximately ¼ cup) such as a plastic baggie or small jar (One per colony to be tested)

Hemocytometer

Cover slips (One per sample)

Light microscope with x400 capability

Isopropyl alcohol

Forceps or tweezers to separate the abdomen from bee

Mortar and pestle or something that can work as a rolling pin

Syringe, graduated cylinder, or other equivalent way of measuring 100mL water

Deionized water

Single use drop pipette or pipette with disposable tips

Fecal spotting (feces on the woodenware of the hive) in the early spring is a sign commonly associated with infection with *N. apis*. However, this is not a clear indication of nosema disease as *N. ceranae* does not cause fecal spotting and many other conditions may result in fecal spotting including viruses and poor food. In the case of a nosema infection, the beekeeper may notice that the colony is dwindling in size, that the colony is not growing as expected for that time of year, or higher than average winter losses, although these observations can also be caused by other stressors.

Molecular tests or microscopic examination are the only methods of definitive diagnosis of the presence of nosema. The relationship between pathogen presence and colony survival, however, remains unclear, as colonies with nosema may not necessarily experience mortality or noticeable adverse outcomes. Nosema load testing can be used to help a beekeeper understand the cause of colony dwindling or high losses and to determine if antibiotic treatment would be appropriate.

Figure 2: Fecal spotting on the front of a hive caused by a high infection with *Nosema apis*. Photo by Meghan Milbrath.

Sampling Bees

Label a sample container for each colony that you plan to test. From each colony, collect at least 100 worker bees in the colony's corresponding sample container. Nosema loads increase over time, so older bees will have more spores, and newly emerged bees will not have any spores. Older bees can be most easily collected using an insect collecting vacuum. If a bee collection vacuum is not available, bees can be collected into a container directly from the frames. Bees can be euthanized by putting the collection container on dry ice or by putting the bees in a jar with ethanol.

Preparing the Sample

1. Count out 100 worker bees onto a clean paper towel. The nosema spores are found in the midgut of the bee. The entire abdomen or whole bee can be used, but there will be a lot of extra debris and pollen that can make the spores difficult to visualize. To improve visibility, the midguts can be removed from the bee by holding the thorax firmly and using forceps to gently pull away the last abdominal segment of the bee (tip including the stinger), until the gut contents are removed onto the towel. The midgut (ventriculus) can be cut away from the rectum and crop. Alternatively, you can remove the entire abdomen of the bee, discarding the heads, legs, and thorax.

2. Add 100 mid guts or whole abdomens to a mortar, homogenizing them with a pestle (Figure 3).

3. Add 1 mililiter of water per bee (100 mL for 100 bees) and mix until a suspension is created (Figure 3). If a mortar or pestle is not available, bees can be mashed in a plastic bag, using a rolling pin or other suitable implement.

Figure 3: Water being added to the bee abdomens in the mortar. Photo by Geena Hill, UF/IFAS Honey Bee Research and Extension Laboratory.

Quantifying Nosema

1. Use a pipette to draw a small amount of the liquid bee mix and add one drop to each side of the counting chamber of the hemocytometer. Place a cover slip over the hemocytometer (Figure 4).

2. View the hemocytometer with a 10x objective to locate the counting chamber grid (Figure 5). After you locate the grids, change to 20x or 40x objective.

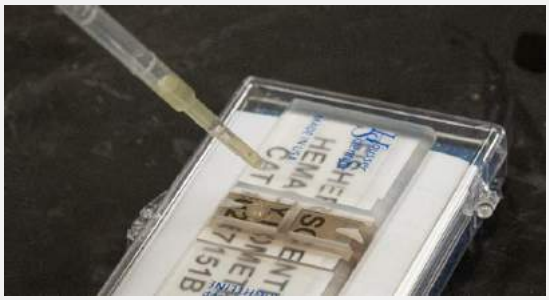


Figure 4: A drop of the bee mixture being added to the counting chamber. Photo by Geena Hill, UF/IFAS Honey Bee Research and Extension Laboratory.

3. Nosema spores are small and oval-shaped and exhibit Brownian motion (i.e., random motion of suspended particles). Count the spores in the five squares shown in Figure 6. Be sure you count on both the top and bottom counting chambers of the hemocytometer and then use the average for your calculations [(Chamber 1 + Chamber 2)/2]. Note that each side of the hemocytometer has an identical grid system consisting of 25 large squares in which each large square is divided into 16 smaller squares.

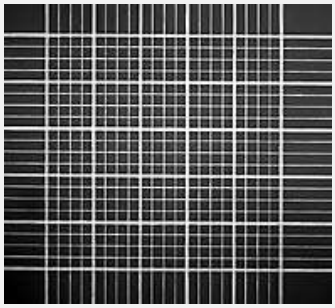


Figure 5: A low magnification view of the hemocytometer grid system. The hemocytometer is 0.1 mm deep, and the 25 large squares represent an area of 1 square mm. The volume of the above 25 large squares shown is 0.1 µl.

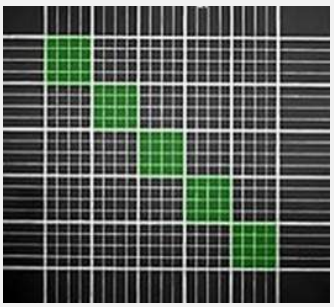


Figure 6: Count the spores in these five squares.

4. For spores that are touching the lines (regardless of whether it is 5% touching or 95% touching) (Figure 7), only count the ones that are touching the left and bottom lines. This will prevent spores from being counted multiple times.

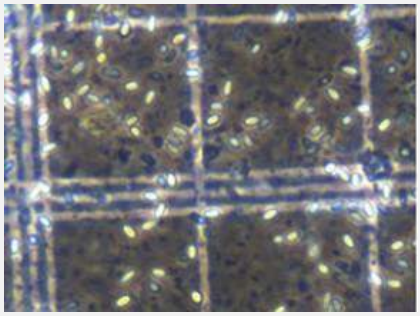


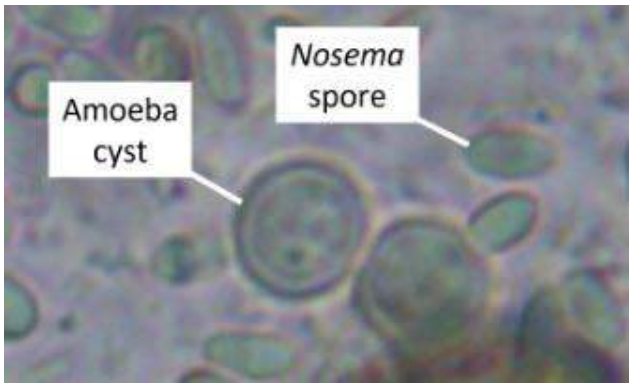
Figure 7: Nosema spores touching the gridlines within a hemocytometer. Photo by Zachary Huang.

5. Calculate the number of spores per bee using this equation:
   Spore counts in 5 squares x 50,000 = number of spores per bee

   Note: This equation only works for counts that follow the above method, using 1mL of water when homogenizing the samples and counting only the 5 larger squares.

Interpreting the Results

Quantifying nosema spores in a sample of bees can be highly positively correlated with the proportion of bees infected in a colony, but this is not always the case. Additionally, the number of spores per bee in a colony can fluctuate depending on season as well as the age and nutrition of the bees sampled. Finally, spore count per bee does not necessarily correlate with individual bee mortality. As such, there is debate over the appropriate threshold above which should be considered a harmful level of nosema spores per bee. However, many consider a spore count of 1 million or more spores per bee to require management action by the beekeeper.



Amoeba Disease

Using methods similar to those described above, it is also possible to identify *Malpighamoeba mellificae*, an internal parasite that causes amoeba disease in honey bees. In contrast to nosema spores, *M. mellificae* cysts are considerably larger and more circular, while still being somewhat oblong in shape (Figure 8).

Figure 8: Nosema spores and *M. mellificae* under magnification. Photo by Etienne Tardiff, Western Apicultural Society.

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