Guide to Nosema Biology, Monitoring, and Control

Biology:

Nosema disease is caused by two different species of microsporidian, spore-forming, fungal parasites: *Nosema apis* and *Nosema ceranae.* While *N. apis* has long been known as a parasite of honey bees (*Apis mellifera*), the more recently discovered *N. ceranae* is now be the more dominant strain found in many parts of the world, including Canada.

Both species have similar lifecycles and only infect adult honey bees. All castes are susceptible, although infection is more common in workers. Nosema infection begins when bees ingest Nosema spores that are passed through honey bee fecal matter. Workers most commonly ingest spores as they clean fecal material from cells. Thus, the transfer of spores occurs more rapid when workers have few opportunities for cleansing flights (e.g. winter months or long periods of inclement weather) and are forced to defecate inside the hive or near the entrance. Bees may also ingest spores after being fed by an infected worker, or from a shared food or water source. Residual spores on the comb are resistant to temperature extremes and desiccation, and therefore remain viable for long periods of time (more than a year), continuing to infect bees long after cleansing flights resume.

Once a spore is ingested, it travels to the bee mid-gut, where it uncoils a long, harpoon-like polar filament which penetrates the outer epithelial cells of the bee's digestive tract. The spore then injects infective sporoplasm which hijacks the host's resources to grow and multiply, ultimately producing more spores. Once the epithelial cells become densely packed with spores, they bursts open, releasing more spores back into the midgut where they may reinfect other parts of the gut or be shed in bee feces.

Older bees (e.g. guards and foragers) normally harbour greater numbers of spores as infections have had more time to develop. Nosema infections typically increase over the winter, peaking in the spring or late summer.

Impact and Symptomology:

Although dysentery is one of the classic symptoms of *N. apis* infection, dysentery may also occur from other factors (e.g. poor quality feed, amitraz), and dysentery is not associated with *N. ceranae* infection. Thus, *N. ceranae* is sometimes referred to as dry Nosema. Both species, however, decrease the ability of the gut lining to absorb nutrients which leads to increased hunger (food consumption) and degeneration of hypopharyngeal glands, impacting the production of royal jelly and the ability to perform nursing duties. Nosema impacts the bee's immune system, making it more vulnerable to secondary pathogens, and a link has been found between Nosema infections and the prevalence of Black Queen Cell Virus (BQCV). Overall, Nosema reduces a bee's lifespan. Queens infected with Nosema may have degenerated ovaries, impacting her laying potential, and Nosema infected queens are often superseded. Colonies infected with Nosema are often slow to build up in the spring the colony may gradually depopulate.

Monitoring and Diagnosis:

Diagnosing Nosema disease is done by examining the gut contents of adult bees under a microscope to estimate the average number of spores per bee. A sample of 50-100 bees should be collected from a hive and may be preserved in alcohol or frozen (it is important to collect older bees either from the hive entrance or from the lid or outer frames as younger bees will not have had time to become infected).

Follow the steps below to prepare a sample for microscopic analysis and to estimate the number of spores per bee.

1. **Equipment needed:** 400x compound microscope, mortar & pestle, clean water, 25-100ml syringe or other measuring device, mesh or screen filter (approx. 40-70 mesh; not essential), eyedropper, haemocytometer & cover slip, paper towel or soft cloth

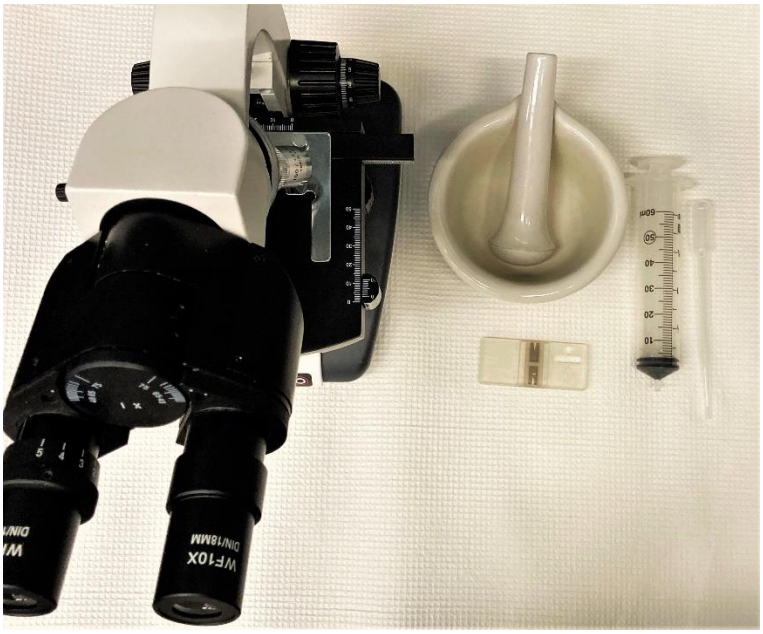

Figure 1. Nosema diagnosis equipment (mesh filter, water, and paper towel not shown)

2. **Prepare the sample:** Count 100 bees (OK to use less but 100 is a good number) and place in mortar. Note: a cleaner sample may bee prepared by removing bee heads and using only the abdomen, or by further removing and using only the digestive tracts from the abdomen. However, full bees will also do the job.

3. Using the pestle, grind bees, abdomens, or digestive tracts into a paste.

4. Measure and add 1ml of water for each bee in the sample (e.g. 100ml of water for 100 bees).

5. Grind bee and water mixture some more to create a well-mixed solution

6. If a mesh filter is available, filter the solution through a fine mesh to remove bee parts.

7. **Prepare the slide:** Using the eye-dropper, place a drop of solution on each chamber of the haemocytometer (2 chambers).

8. Place the cover slip on the haemocytometer. The solution will distribute evenly. Allow the sample to settle for 2 minutes.

9. Starting on the lowest setting, place the slide on the microscope, then focus and centre the grid of the haemocytometer chamber. The grid consists of 25 (5x5) larger squares, which each contain 16 (4x4) smaller squares. You will be counting the Nosema spores present within 5 of the larger squares. A good option is to count the 4 corner squares as well as the middle square.

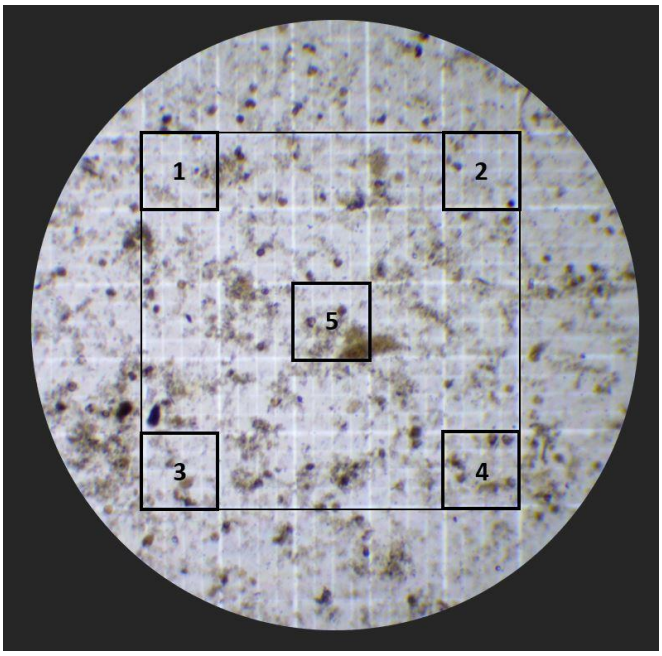

Figure 2. Haemocytometer counting chamber

10. Switch to high-power (400x), then use the fine focus adjustment knob to avoid breaking the cover slip or slide.

11. Count the Nosema spores present within 5 of the larger squares. Nosema spores are ovoid, bright white, and darkly outlined. Be aware of round shaped pollen grains which are often larger than nosema spores.

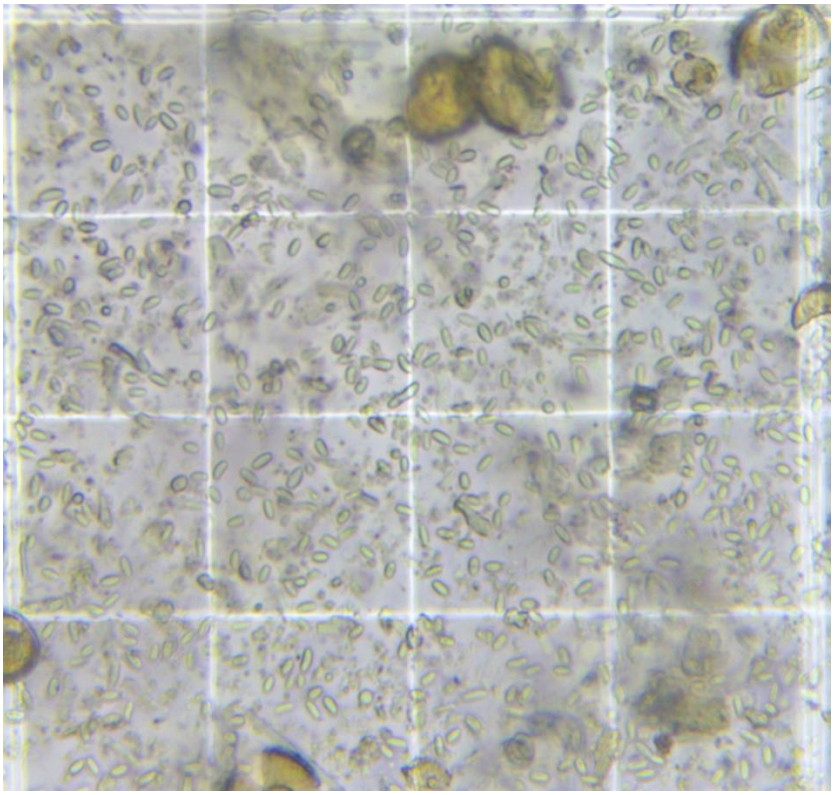

Figure 2. Slide showing a heavy Nosema infection

12. Count and record the number of spores in each of the 5 large squares for each of the two counting chamber grids on either side of the haemocytometer (5 x 2 = 10 large squares).

13. Calculate the average number of spores per large square by dividing the total number of spores by 10. Then multiply this number by 250,000 to calculate the average spore load per bee.

Example:

- Chamber 1: total = 25 spores
- Chamber 2: total = 35 spores
- 60 <u>total</u> spores counted in 10 large squares
- 60/10 = average of 6 spores per large square
- 6 x 250,000 = 1,500,000 spores/bee

<u>Control:</u>

<u>Integrated Pest Management:</u> Nosema can be prevented and controlled through a combination of cultural and chemical control strategies

Cultural control strategies:

Nosema disease is promoted during periods of stress. Colonies entering winter should have strong populations and a vigorous queen. Other stressors such as poor nutrition or the presence of other

diseases or parasites should be avoided. In the spring and fall, colonies should have access to adequate pollen and high-quality sugar syrup or honey. Outdoor overwintered colonies should be well-insulated, equipped with entrance reducers, and should face south to promote cleansing flights when weather permits.

Rotating out old combs is an important IPM strategy that can reduce the transmission of disease, since soiled combs can harbour Nosema spores and be a source of infection.

Irradiation or fumigation with acetic acid of stored combs is generally recommended as a disinfection technique. Heat treatment (49° for 24 hours) is also known to work on spores of *N. apis*, however, *N. ceranae* spores are significantly more heat tolerant so heat treatment is not recommended for *N. ceranae*.

Chemical control:

The use of chemical controls is justified when the **economic threshold of 1 million spores/bee** is exceed. Both *N. apis* and *N. ceranae* can be controlled by the antibiotic Fumagillin (Fumagilin-B®), mixed in sugar syrup. Treatment does not kill spores but suppresses the actively growing stages. Label directions for applying treatments differ in spring and fall and depends on the size of the colony. Please follow product label directions closely. Also see the Manitoba Honey Bee Health Treatment recommendations for more information (https://manitobabee.org/hive/category/bee-health-protection/).