# Developing Honeybee Diagnostics at the New Hampshire Veterinary Diagnostics Lab: Successes and Challenges

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## Introduction

The importance of honeybees
- Contribute to food security and economy
- 90% of commercial pollination is done by honeybees
  - $15 billion worth of crops in the US every year[7]

Challenges facing honeybees
- Known contributors to their decline include diseases, climate change, pesticides, crop monocultures
- The gut microbiome can be impacted by stress, diet change, antibiotic exposure, and immune response
  - Gut dysbiosis can lead to an increase in disease susceptibility and mortality
- Primary diseases
  - *Varroa destructor*
    - Causes the highest damage of all known bee pathologies[4]
    - Mite infestation causes a non-uniform disease called Varroosis
    - Feeds on the honeybee's fat stores and acts as a vector for viral infections[3]
  - *Nosema spp.*
    - Obligate, intracellular fungal parasite that decreases the gut integrity, weakens the immune system, and increases the energy demand of the host, a combination that consequently leads to a decreased life span[3]
    - Fumagillin is a long-term treatment, but prolonged use can lead to an upset in gut microbiome

Importance of diagnostic testing
- Potential high rate of disease spread due to the social nature of bees
- There has been an increase in veterinary interest and involvement in honeybee health due to the FDA's 2017 classification of honeybees as livestock.[6] This distinction has led to the requirement of a prescription or feed directive when using antibiotics in honeybee colonies

## Objectives

- Develop honeybee diagnostic testing at the NHVDL
- Explore infectious causes of honeybee population decline in NH

## Results

Quantitative *Nosema* spp. and *Varroa* spp. results

| Hive | *Varroa* count (%) | *Nosema* per honeybee |
|---|---|---|
| 1 | 14.4 | 50,000 |
| 2 | 3.9 | 350,000 |

**Table 1**: Quantitative *Nosema spp.* and *Varroa spp.* results from two NH hives

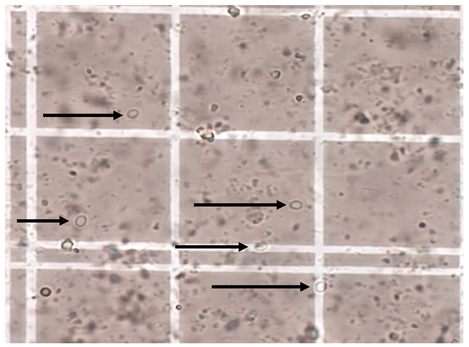

**Figure 1**: Photomicrograph of *Nosema* spp. spores (arrows) from a honeybee (400x)

**Figure 2**: Photomicrograph of *Varroa destructor* mite removed from honeybee host, 200x.

Histology

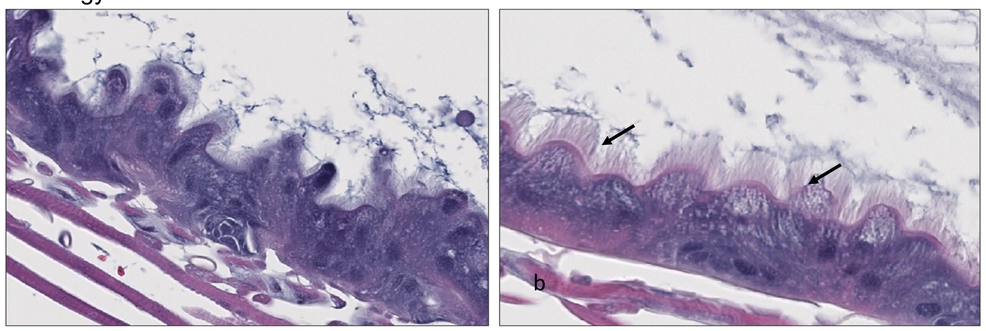


**Figure 3 (a and b)**: Photomicrograph of midgut (ventriculus) of a honeybee (Hematoxylin and eosin, 200x). **(a)** Unaffected portion of mucosa. **(b)** Infected portion of mucosa with numerous *Nosema* spp. spores (clear vacuoles) within the epithelium (arrows).

Abdominal Culture: No growth

## Discussion

### Successes

- *Varroa destructor* mite count
  - Sample 1 had an average of 0.14 mites per bee which is above the recommended values of 0.02-0.05 mites per bee[5] while sample 2 had an average of 0.04 mites per bee which is within the accepted range.
- *Nosema* spp. spore count
  - Both samples were found to have low levels of *Nosema* spores and neither had reached the 1 million spore per bee threshold where treatment is recommended. [1]
- Histologic processing and visualization of *Nosema* spp. in histological samples
  - *Nosema* spp. spores can be seen within the epithelium of the midgut in affected honeybees prior to their release into the lumen.

### Challenges

- Limited honeybee samples available for study
- Manual counts for *Varroa* mites and *Nosema* spores were time consuming and labor-intensive
- Potentially artifactually lower numbers of *Varroa* mites than expected due to them falling off after the honeybee dies (may not be representative of the true hive load)
- Potentially artifactually lower number of *Nosema* than expected due to time samples spent frozen [2]
- Shattering of the chitinous exoskeleton made histological processing difficult
- No bacteria were isolated from the abdominal contents to evaluate gut microbiome
  - Previous freeze/ thaw (for storage) is suspected to be a contributor to the lack of bacterial growth, so evaluation of the microbiome was not able to be evaluated in this group.

### Future Plans

- Collect and test more honeybee samples as part of routine testing over the summer
- Determine ways to decrease the effect of freezing temperatures on spore counts and postmortem gut microflora

## Materials and Methods

- Managed honeybees from NH beekeepers (Dover, NH) were analyzed for *Varroa destructor* and *Nosema spp.* at the NHVDL

### Quantitative Nosema procedure [8]
- Remove and grind 25 honeybee abdomens in a mortar, adding 25 mL of water until a well mixed solution is formed.
- Add sample to a hematocytometer and examine under a microscope, counting the spores.
- Multiply average number of spores found in the two chambers by 50,000 to calculate average *Nosema* spore count in each bee.

### Histology
- Whole bees were fixed in 10% neutral buffered formalin, trimmed sagittally or in cross section, embedded in paraffin wax, sectioned at 5 µm, mounted on frosted glass slides, routinely stained with hematoxylin and eosin (H&E), and examined by light microscopy.

### Varroa mite counts
- Examine each honeybee with naked eye for mites.
- Remove any Varroa mites found.
- Total mite numbers were counted and expressed as a percentage for each sample.

### Aerobic culture of abdominal contents to evaluate gut microbiome
- Cut open abdomen underneath a dissecting scope and use a sterile inoculating loop to mix the intestines inside the incised abdomen.
- Inoculate each of the following plates with the mixture; Blood agar, MacConkey agar, Phenylethyl Alcohol Blood agar, and Sabouraud agar.
- Incubate the plates at 34° C and check for growth at 24 and 48 hours.

## References and Acknowledgements

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