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Monitoring for Amoebic Disease (*Malpighamoeba mellificae*) in Honey Bee Colonies[1]

Hannah N. Gurland, James C. Fulton, Marley E. Iredale, Amy Vu, and Julia St. Amant[2]

Introduction

Digestive health is an overlooked but vital component of the success of western honey bee (*Apis mellifera*) colonies. Viral, bacterial, fungal, and protozoal infections can harm bees' digestive tracts and make it difficult for them to absorb nutrients and eliminate waste. Active management requires monitoring the health of individual honey bees as well as the health of the colony, providing adequate nutrition, and controlling for pests and diseases.

This publication supports beekeepers' management by detailing how apiarists can detect and quantify infection by the organism *Malpighamoeba mellificae*, an amoeba that can cause disease in severely afflicted colonies.

This amoeba infects the Malpighian tubules, organs comparable to human kidneys. Malpighian tubules are responsible for removing waste from insects' hemolymph (blood). The Malpighian tubules branch off the gut at the junction between the midgut and the hindgut.

Due to the difficulty of detecting and diagnosing *M. mellificae*, its distribution and impact on honey bee health have been understudied. The current distribution of this organism is thought to be worldwide, occurring wherever its host, *Apis mellifera*, is present. Historically, the prevalence of *M. mellificae* is low in the regions where it has been found. However, new diagnostic techniques have emerged, among them nucleic acid-based molecular assays. These new techniques may be able to provide a more thorough evaluation of this parasite, allowing us to determine how common it is and giving us an idea of its significance for apiculture.

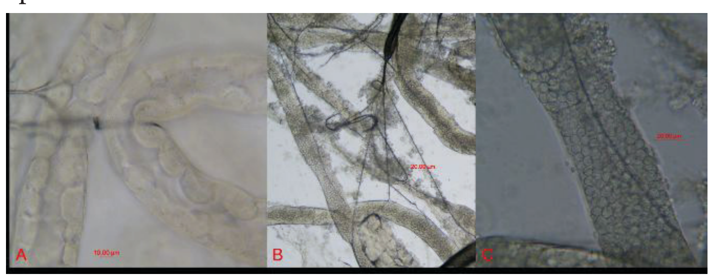

Figure 1. Left, healthy, uninfected Malpighian tubules of a honey bee. Center, multiple Malpighian tubules filled with *Malpighamoeba mellificae* cysts. Right, a single magnified Malpighian tubule filled with *Malpighamoeba mellificae* cysts.
Credits: Etienne Tardif

Worker honey bees are the most common caste to be infected with *M. mellificae*. This disease has not been found in honey bee drones and is seldom found in queens. Usually, queens become infected only when they are put in abnormal situations, such as caging. Transmission of the amoeba between bees likely occurs when bees are exposed

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2. Hannah Gurland, former research scientist, Florida Department of Agricultural and Consumer Services Division of Plant Industry; James Fulton, research scientist, Florida Department of Agricultural and Consumer Services Division of Plant Industry; Marley Iredale, former graduate student, Department of Entomology and Nematology, UF/IFAS Honey Bee Research and Extension Laboratory; Amy Vu, UF/IFAS state specialized Extension agent, apiculture; and Julia St. Amant, graduate student; Department of Entomology and Nematology, UF/IFAS Honey Bee Research and Extension Laboratory; UF/IFAS Extension, Gainesville, Florida 32611.

to comb contaminated with feces from infected bees. This route of spread is called "fecal–oral." Similarly, consumption of water contaminated with infected feces and trophallaxis (mouth-to-mouth food exchange) could be routes of exposure.

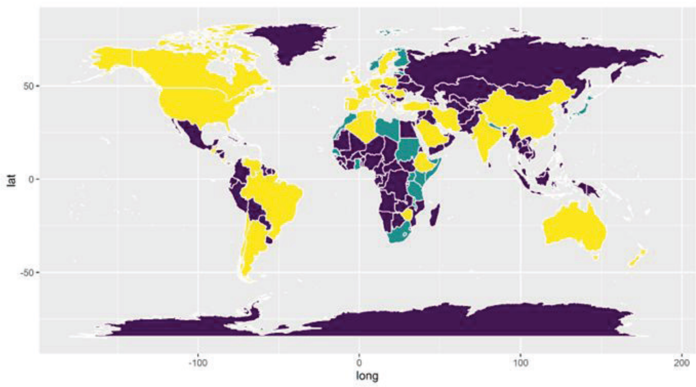

Figure 2. Global occurrence of *Malpighamoeba mellificae*. This world map distinguishes countries with known *M. mellificae* presence (yellow), suspected presence (teal), and no presence or unreported (purple).
Credits: James Fulton, Florida Department of Agriculture and Consumer Services

Figure 3. An *Apis mellifera* colony displaying signs of dysentery. Note the excretions on the hive entrance.
Credits: Etienne Tardif

Based on beekeepers' observations, the prevalence of disease associated with *M. mellificae* (amoeba or amoebic disease) may be seasonal, with greater prevalence occurring during the winter or early spring. Although colony losses correlated to *M. mellificae* have been reported, it is common that cysts become undetectable in the previously infected colonies by late spring. Clinical signs that a honey bee colony may be infected with *M. mellificae* have been observed to resemble signs of a *Nosema* sp. infection. *Nosema* spp. are microsporidian parasites that, like *M. mellificae*, parasitize the honey bee gut. Clinical signs of both infections are similar: colony populations dwindle; bees experience dysentery (Figure 4); and bee lifespans are shortened. These similarities may come about because the two pathogens often occur together in a colony, and there may be additive or synergistic effects when both parasites are present.

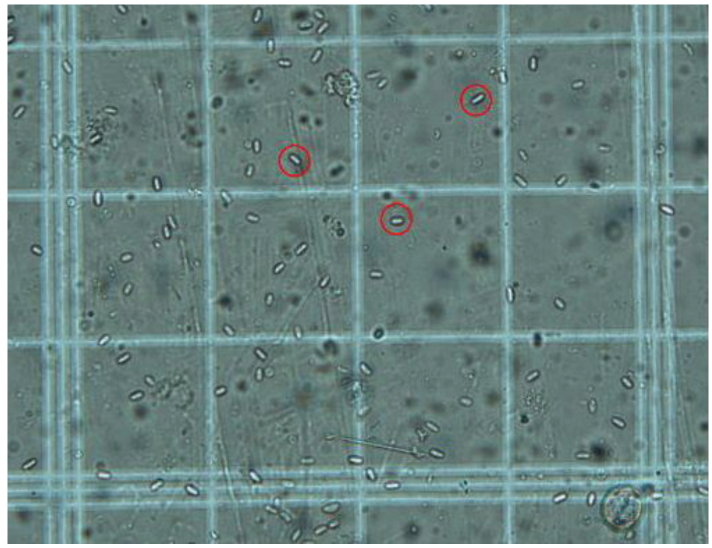

Figure 4. A hemocytometer under 400x magnification with three Nosema sp. spores circled in red.
Credits: James Fulton, Florida Department of Agriculture and Consumer Services

Detection

If you observe an unusual increase in honey bee mortality and/or dysentery (excessive, unusually colored fecal material), you may then investigate for *M. mellificae* infection by using a compound light microscope to examine honey bee frass (feces).

To perform a visual inspection of bee feces to detect amoeba or *Nosema* spp., you will need the following:

- Compound light microscope with a 40x objective (400x magnification)
- Improved Neubauer hemocytometer with corresponding cover slip (optional) (Figure 5.)
- Glass coverslips
- Pipette (eye dropper)
- Forceps
- Water
- Gloves

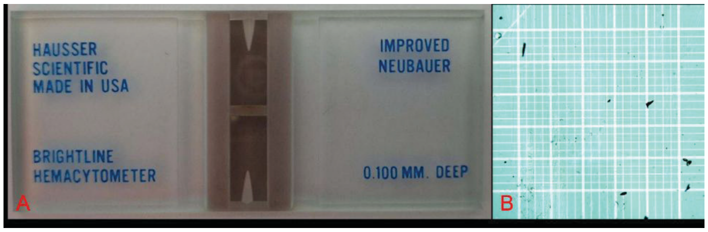

Figure 5. A: Neubauer hemocytometers are used to count microscopic objects by dividing the visual field into standardized areas. B: This image shows a hemocytometer under 50X magnification.
Credits: James Fulton, Florida Department of Agriculture and Consumer Services

Then perform the following protocol:

1. Collect ten live or dead bees from the front of each hive and place them into resealable bags labeled to identify the colony.

- Note: bees can be collected from anywhere in the hive, but selecting workers at the entrance may increase the likelihood of detecting the amoeba.

2. Add ten milliliters of distilled water to each bag, then crush to empty the bees' abdominal contents.

3. For simple detection (presence/absence), place a drop of liquid from the bag onto a microscope slide, apply a cover slip, and view with a light microscope. If you wish to quantify the contagion present to estimate the protozoal load, then you may use a hemocytometer to count *M. mellificae* spores in the same way you would use it to count *Nosema* spp. spores.

4. Spherical, colorless, translucent, 8–10 µm diameter cysts (Figure 6) will be visible with a light microscope at 400x magnification.

Figure 6. A compound slide prepared via the method described under "Conducting Visual Confirmation." The cyst stage of *Malpighamoeba mellificae* is identified with a white arrow. At 400x magnification, the cysts are approximately 8-10 µm in diameter and round, with a distinct cell wall. Note the background of *Nosema* sp. spores (bright ovals and spheres).
Credits: Marley Iredale, UF/IFAS Honey Bee Research and Extension Laboratory

Treatment

Currently, no standardized "economic threshold" is available for this amoeba. An economic threshold is the level of infection below which yields are unlikely to be affected and above which yield losses are likely. Additional research must be conducted to determine the number of amoebae necessary to cause disease in bees and what number indicates that management should be initiated. There are currently no commercially available treatments for amoebae. However, chemical treatments are continuously being developed, and other techniques for controlling amoebae are being tested. Management techniques that can be attempted if beekeepers suspect this infection in a colony include quarantining colonies and equipment, disinfecting equipment, and replacing contaminated comb. Common disinfection methods, such as dilute bleach, are likely effective in killing the amoeba in the environment; however, these methods have yet to be tested.

Final Remarks

Data useful for developing management solutions for *M. mellificae* are currently limited. Studies have shown how related amoeba species impair insects' ability to excrete waste and toxins. However, more research is necessary to understand the effect *M. mellificae* may have on the honey bee. Until then, management of other hive pests and pathogens is recommended to limit the burden of amoebic infection on bees. Performing regular hive inspections, conducting alcohol mite washes, actively managing for *V. destructor*, and cleaning equipment between uses may help control the spread of or disease associated with this amoeba. The Ask IFAS publication How to Quantify Nosema Spores Infection Rate in a Honey Bee Colony (Mortenson et al. 2022) provides information about alcohol mite wash techniques. The method described above for detecting amoeba in honey bee samples is technically straightforward, requires limited equipment, and can be performed by anyone with access to the materials. Therefore, it provides a means by which beekeepers can start to detect and quantify this organism in their hives. In this way, we can expand our understanding of *M. mellificae* as a pathogen of honey bees.

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