Recent Research on *Nosema*

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A NBU study in 2010 revealed that more than 40% of honey bee colonies were infected with Nosema. While there may be no overt signs of an infection, it could weaken a colony and increase its vulnerability to other pathogens. Kirsty Stainton updates us on Nosema research.

Figure 1. Dysentery in a colony suffering from *Nosema apis*. Images courtesy crown copyright.

What are *Nosema*?

Nosema are parasites which are referred to as 'microsporidians'. Microsporidians are a class of life-form related to fungi;[1] you could call them their parasitic cousins. They live inside the cells of other organisms and they need host cells to grow and divide, but they can exist outside of host cells in the form of spores. In the spore form they can move between hosts and spread, as the spores are quite resistant to harsh environments such as high temperatures or low humidity. When a spore encounters a host cell, the spore then 'germinates'; it injects its infectious 'sporoplasm' into the host cell where the parasite can replicate and produce many more spores.[2]

Nosema are gut parasites and when they enter the bee gut, they multiply and cause disease in the host. *Nosema* damage the gut cells and steal nutrients meant for the bee. Eventually, they will produce many millions of spores which will infect more cells or pass into the faeces where they can contaminate the colony and be ingested by other bees. Spores can remain viable for a year or more outside of the host, so combs contaminated with faeces from infected bees can re-infect a colony the following year if not properly disinfected.

Species of *Nosema*

In the UK there are two species of *Nosema* that infect honey bees; *Nosema apis*, which is native to Europe/UK, and *Nosema ceranae*, an invasive species which was found in the UK in 2008 but thought to originate from East Asia. *Nosema apis* causes dysentery (figure 1), an increase in winter mortality, poor spring build up and it reduces the honey yield;[3] although it may be present with no symptoms at all. *Nosema ceranae* is less well studied as it is a recent parasite in western honey bees but is thought to be a more virulent parasite based on laboratory experiments.[4] *Nosema ceranae* suppresses the honey bee immune system, while *Nosema apis* does not, and this could make bees more susceptible to other diseases, particularly viruses.[5] In some cases, it can also result in unexpected colony collapse as the colony does not exhibit obvious signs of disease prior to collapsing.[4]

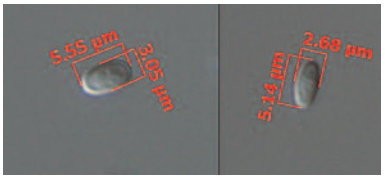

Figure 2. The native *Nosema* species, *Nosema apis*, (left) and the invasive *Nosema ceranae* (right) under the microscope at 400x. Images courtesy crown copyright.

A third species has recently been described which is closely related to *Nosema apis*, named *Nosema neumanni*. It was discovered in Uganda and found to be more common than *Nosema apis* or *Nosema ceranae*. Its spores are smaller than the other *Nosema* species, and it is 97% related to *Nosema apis*. It is early days so we do not really understand what sort of symptoms this strain causes, but it does not appear to be a virulent strain or to represent a threat to the Ugandan bees.[6] It is not known what threat this new strain could present to European subspecies of honey bee, but, hopefully, we will not get a chance to find out first-hand.

Probiotics for bees

How about a dose of 'friendly' bacteria for bees? A recent study reports that feeding bees with bacteria that occur naturally in bees improves the lifespan of *Nosema ceranae*-infected bees.[7] The study found there was no change in the number of *Nosema ceranae* spores by supplementing with probiotics, but the researchers did find that lifespan was significantly improved in bees fed with probiotics, suggesting that the supplements make the bees better able to withstand infection. This seems to make some sense as *Nosema* is a gut parasite and promoting a healthy gut might, therefore, be beneficial against gut parasites, but the study does not directly examine a link between gut bacteria and *Nosema*. This study also finds that bees fed with probiotics survive longer

than control (untreated) bees, even in the absence of *Nosema ceranae*. These data are presented from bees kept in a laboratory without access to a normal diet and laboratory rearing may have a negative effect on the bees' gut bacteria.

Novel technologies against *Nosema*

One technology that is being investigated by researchers for controlling pests, sometimes called RNA interference (or RNAi), uses double-stranded RNAs to specifically suppress selected genes within a cell. This technology involves switching off genes that are necessary to life for an organism, in order to kill it. When an essential gene is discovered, it can be switched off using a specially modified fragment of that gene. This technology is designed to be species-specific and researchers are careful to ensure that the gene they are trying to switch off does not exist in any other species in the environment, otherwise there could be terrible side-effects. Researchers in the USA experimented with switching off *Nosema ceranae* genes[8] by feeding honey bees that were infected with *Nosema ceranae* spores with double-stranded RNA which targeted two essential proteins in *Nosema ceranae*. They found that the honey bees fed with the double-stranded RNA had fewer *Nosema ceranae* spores than honey bees that had not been treated, but the treatment did not eradicate *Nosema* completely.

Unfortunately, this is not a technology that we can use yet. Many experimenters have been finding that while these modified gene fragments can be useful for switching off genes in one species, they can also affect unrelated genes in others. For example, honey bees that are fed with gene fragments that should not switch off any of their genes, have been found to end up with the normal behaviour of hundreds of their own genes changed.[9] In one study, they found 1,400 honey bee genes were changed by feeding bees certain gene fragments.[10] Nobody knows why this happens, but it would be unwise to use this technology on honey bees without further in-depth understanding of how the technology affects the bees, not to mention other species in the environment that could also be affected.

What to do about *Nosema*

Nosema is difficult to detect in honey bees as there is no single definitive clinical symptom for its presence and there may be no obvious signs of disease. The only way to see for sure if *Nosema* is present, is through microscopy or using molecular biology. Under the microscope, at 400x, you can differentiate the species of *Nosema*. *Nosema apis* are shaped like barrels, while *Nosema ceranae* are shaped more like a grain of rice (shown in figure 2). *Nosema* used to be treated with an antibiotic called Fumagillin, but its use is no longer authorised in the UK. Treatment with thymol (at 100ppm) in syrup increases the lifespan of bees infected with *Nosema ceranae*, slightly reduces the number of *Nosema* spores and slows the rate of infection.[11]

Sterilising equipment

Spores can be killed by heating tools to 60°C for 15 minutes or combs to 49°C for 24 hours. Comb sterilisation is necessary if you think you have had *Nosema* infection, as this will prevent its further spread. Combs should be sterilised with 80% ethanoic acid (acetic acid). More information can be found in either the *Fumigating Comb* or the *Hive Cleaning and Sterilisation* fact sheets, both of which are available at http://www.nationalbeeunit.com.

Geographical variations in susceptibility to infection

The National Bee Unit conducted a random apiary survey in 2010/2011 and found that 46% of colonies studied contained *Nosema apis* and 40% contained *Nosema ceranae*. Importantly, there was no strong correlation between these parasites and poor colony health. Across the world, people are observing that in different locations, bees suffer more from certain conditions. For example, in Spain, *Nosema ceranae* causes colony losses while in the USA, the Israeli acute paralysis virus is involved in colony loss. Perhaps certain environmental conditions exacerbate some conditions and honey bee genetics surely influences susceptibility. For now, in the UK, we can stick to worrying about bad weather, varroa and Asian hornets.

References

1. Keeling P. Five questions about microsporidia. *PLOS Pathogens* 2009; 5(9): e1000489. https://doi.org/10.1371/journal.ppat.1000489.
2. Fries I. *Nosema ceranae* in European honey bees (*Apis mellifera*). *Journal of Invertebrate Pathology* 2010; 103: S73–S79.
3. Williams GR, Sampson MA, Shutler D, Rogers RE. Does fumagillin control the recently detected invasive parasite *Nosema ceranae* in western honey bees (*Apis mellifera*)? *Journal of Invertebrate Pathology* 2008; 99(3): 342–344.
4. Higes M, Martín-Hernández R, Botías C *et al*. How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environmental Microbiology* 2008; 10(10): 2659–2669.
5. Antúnez K, Martín-Hernández R, Prieto L *et al*. Immune suppression in the honey bee (*Apis mellifera*) following infection by *Nosema ceranae* (Microsporidia). *Environmental Microbiology* 2009; 11(9):2284–2290.
6. Chemurot M, De Smet L, Brunain M, Rycke RD, DC de Graaf. *Nosema neumanni* n.sp. (Microsporidia, Nosematidae), a new microsporidian parasite of honeybees, *Apis mellifera* in Uganda. *European Journal of Protistology* 2017; 6: 13–19.
7. El Khoury S, Rousseau A, Lecoeur A *et al*. Deleterious interaction between honeybees (*Apis mellifera*) and its microsporidian intracellular parasite *Nosema ceranae* was mitigated by administrating either endogenous or allochthonous gut microbiota strains. *Frontiers in Ecology and Evolution* 2018; 6(58): doi: 10.3389/fevo.2018.00058
8. Paldi N, Glick E, Oliva M *et al*. Effective gene silencing in a microsporidian parasite associated with Honeybee (*Apis mellifera*) colony declines. *Applied and Environmental Microbiology* 2010; 76(17): 5960–5964.
9. Brutscher LM, Daughenbaugh KF, Flenniken ML. Viruses and ds-RNA triggered transcriptional responses reveal key components of honey bee antiviral defence. *Scientific Reports* 2017; 7:6488.
10. Nunes FM, Aleixo AC, Barchuk AR *et al*. Non-target effects of green fluorescent protein (GFP)-derived double stranded RNA (dsRNA-GFP) used in honey bee RNA interference (RNAi) assays. *Insects* 2013; 4(1):90–103.
11. Costa C, Lodesani M, Maistrello L. Effect of thymol and resveratrol administered with candy or syrup on the development of *Nosema ceranae* and on the longevity of honeybees (*Apis mellifera* L.) in laboratory conditions. *Apidologie* 2010; 41:141–150.

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