Advances in *Nosema* Research

Ben Jones, Fera Science Limited, summarises current understanding

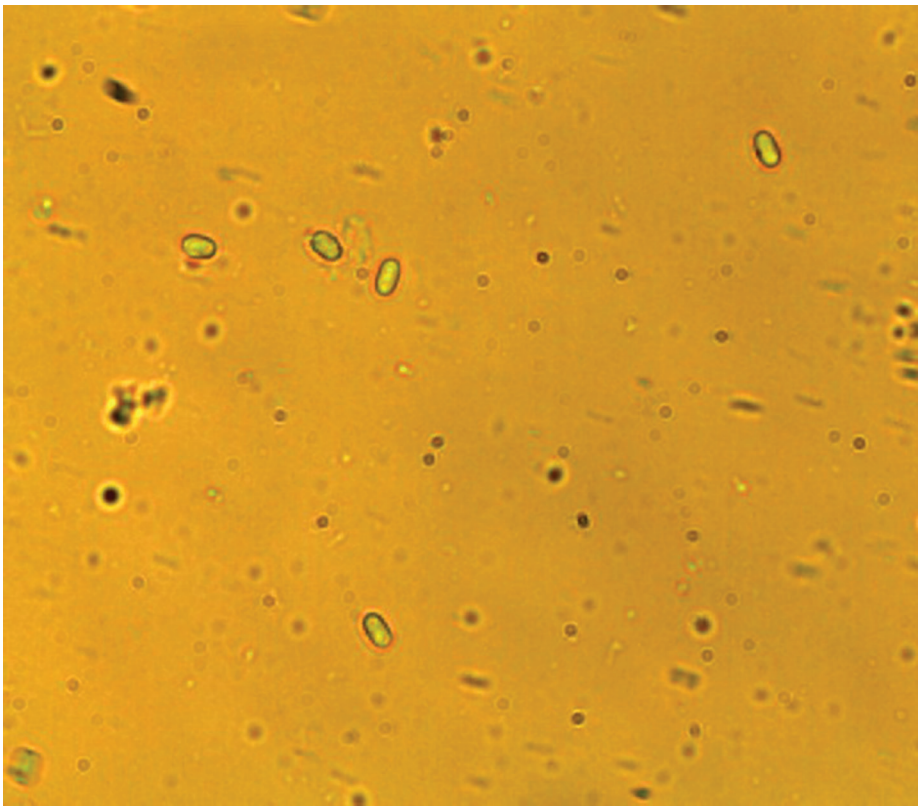
Nosema belong to a group called microsporidia. Microsporidia are unicellular parasitic fungi that infect the cells of their hosts. The *Nosema* spores that you may have seen via a microscope are how *Nosema* moves between individual honey bees and represent its extracellular and infective stage (Figure 1). Spores of microsporidia are very resistant, being able to survive in the environment for long periods (Han, et al, 2020). For example, *Nosema* spores can remain viable in frozen honey for up to one year (MacInnis et al, 2020).

Once spores are ingested with food, the spores germinate and infect the epithelial cells of the honey bee mid-gut (Bailey and Ball, 1991; Fries et al, 1996). A filament is protruded from the spore and 'injected' into the host cell. This filament acts as a tube for the sporoplasm to be transferred from the interior of the spore into the honey bee cell. Once the sporoplasm has been transferred, it develops into new spores that eventually become so numerous that they overwhelm the host cell. The infected cells then burst and release the tightly packed mature spores back into the gut where they can re-infect honey bee cells or are voided into the colony with faeces and potentially re-ingested by nest mates (Fries et al, 1996; Han et al, 2020). In addition, spores can be transmitted sexually from infected drones to queens (Roberts et al, 2015).

Species of *Nosema* and Distribution

The distribution of *Nosema* has altered over time with the trading of bees around the planet. There are two species of *Nosema* that cause disease in honey bees in the United Kingdom (UK): *Nosema apis* and *Nosema ceranae*. *N. apis* was initially reported in North America, Australia and Europe but has since been reported on every continent. *N. apis* infections reduce in the summer and increase in winter and spring when foraging is limited, whereas *N. ceranae* infections seem to lack this consistent seasonality. *N. ceranae* traditionally occupied South East Asia but has been replacing *N. apis* as the dominant species in some regions, although co-infections of both *N. apis* and *N. ceranae* have been detected in bees in the USA as early as 1975 (Traver and Fell, 2015). Consequently, both species no longer occupy distinct geographical regions (reviewed in Gorblirsch, 2017; Grupe and Quandt, 2020; Martín-Hernández et al, 2018).

Figure 1. *Nosema apis* spores seen through a compound microscope (x 400 resolution)



Individual and Colony Effects

The effects of *Nosema* infection in individual bees and in the colonies are well documented and comprehensively reviewed in Gorblirsch (2017) and Martín-Hernández et al (2018).

Nosema can infect workers, drones and queens, and both *N. apis* and *N. ceranae* can co-infect colonies. Infection causes physical damage to the honey bee gut and reduces the lifespan of individual bees. *Nosema* infection can also cause a broad range of subclinical responses including: a reduction in hypopharyngeal gland size, nutritional and energetic stress through a reduced ability to metabolise carbohydrates, inhibition of cell signalling, suppression of natural defences, such as apoptosis of infected cells, and disruption of age-related task partitioning.

Some laboratory studies suggest that *N. ceranae* is more virulent and can produce more spores, although results are inconsistent (eg, Forsgren and Fries, 2010) and although these individual effects can reduce colony health, reports of colony losses specifically attributed to

N. ceranae are again inconsistent across geographical regions.

At the colony level, dysentery, winter losses, poor spring build up, reduced honey yield and reductions in colony size have been traditionally reported after infection with *N. apis*. Reduced colony size and low honey yield have also been reported with *N. ceranae* (Botías et al, 2013).

Research for the Control of *Nosema*

Nosema infections were previously treated with fumagillin, an antibiotic first isolated from *Aspergillus fumigatus* in 1941 (Van den Heever et al, 2014). The production of fumagillin was discontinued in 2018 due to concerns about contamination in honey and wax, prompting investigations into the efficacy of alternative treatments. Many compounds have been investigated in recent years and those found to have beneficial effects, such as increased longevity of infected bees, reduced spore loads, or reduced spore viability, are reviewed and summarised in Table 1 from Burnham (2019).

Research has focused on the identification of candidate drugs, organic extracts, RNA interference (RNAi), microbial dietary supplements and the repurposing of existing veterinary medicines. Recent examples include the oxalic acid-containing varroa treatment Api-bioxal® and dietary supplement Api-herb® which have been shown to reduce the abundance of spores in bees infected with *N. ceranae* (Cilia et al, 2020). The extract of the mushroom *Agaricus blazei* has also been shown to reduce spore loads (Glavinic et al, 2021) and treatments with certain pre and probiotics have reduced *Nosema* spore loads in both laboratory and field studies (Borges et al, 2020; Klassen et al, 2021; Valizadeh et al, 2020).

Prebiotics consist of food ingredients that are not digested but stimulate or inhibit specific gut microbes and probiotics are live micro-organisms that alter the community of gut microbes after being ingested. These promising results are perhaps unsurprising given that certain prebiotics like chitosan and naringenin can stimulate the immune system, are antimicrobial and are anti-oxidants. Likewise, *Enterococcus faecium* is a probiotic that causes the peritrophic membrane to thicken in honey bees, which *Nosema* spores must pass through to infect epithelial cells.

Studies that experimentally infect honey bees with *Nosema* can help

Table 1. A summary and comparison of anti-*N. ceranae* treatments that have displayed efficacy in previous works. Taken from Burnham (2019). Copyright © 2019 Burnham

Treatment type	Bee spore load[1]	Bee survival[1]	Hive spore load[2]	Other effects
Small Molecules				
Metronidazole (in vitro only**)	N/A	N/A	N/A	↓ spore viability
Tinidazole (in vitro only**)	N/A	N/A	N/A	↓ spore viability
Porphyrin: $PP(Asp)_2$	↓	↑	N/A	↓ spore viability
Porphyrin: TMePyP	↓	N/A	N/A	↓ spore viability
Fumagillin analogs*[a]	↓	↑	N/A	N/A
Fumagillol*	↓	↑	N/A	N/A
Semisynthetic aspirin*	↓	↑	N/A	N/A
Enilconazole*	↓	↑	N/A	N/A
Piperonyl analog*	↓	↑	N/A	N/A
Thymol*	↓	↑	N/A	N/A
Formic acid (fumigation)	N/A	N/A	↓	N/A
Oxalic acid	↓	N/A	N/A	N/A
Oxalic acid (topical field trial)	N/A	N/A	↓	↑ colony survival
Thymol	↓	↑	N/A	N/A
Resveratrol	No effect	↑	N/A	N/A
Thymol	↓	No effect	N/A	N/A
Resveratrol	↓	↑	N/A	N/A
RNA Interference				
ADP/ATP transporter RNAi	↓	N/A	N/A	↑ response to sucrose
ptp3 RNAi	↓	↑	N/A	↑ immune expression
nkd RNAi	↓	↑	N/A	↑ immune expression
Extracts and Supplements				
Polysaccharide extracts*	↓	↑	N/A	N/A
Pentadecapeptide BPC 157	N/A	N/A	↓	↓ bee midgut lesions; ↑ colony strength
EtOH *L nobilis* extract	↓	No effect	N/A	N/A
C. Alba EO extract**	↓	↑	N/A	N/A
Compounds detected in *C. Alba* EO extract+*	↓	↑	N/A	N/A
MeOH *A. chilensis* extract	↓	No effect	N/A	N/A
MeOH *U. molinae* extract	↓	↑	N/A	N/A
MeOH *G. avellana* extract	↓	No effect	N/A	N/A
MeOH propolis extract	↓	↑	N/A	N/A
MeOH propolis extract[b]	↓	↑	N/A	N/A
MeOH propolis extract[c]	↓	↑	N/A	N/A
BEEWELL AminoPlus	↓	No effect	N/A	↑ immune expression
Nozevit[d]	N/A	N/A	↓	↑ colony strength
HiveAlive	N/A	N/A	↓	↑ colony strength
Microbial Supplements				
Bacterial surfactin	↓	↑	N/A	↓ spore viability
L. johnsonii metabolites	N/A	N/A	↓	↑ fat bodies per bee; ↑ colony strength
Bifidobacteria	↓	N/A	N/A	N/A
Lactobacilli	↓	N/A	N/A	N/A
P. apium	No effect	↑	N/A	N/A
Bacillus sp.	No effect	↑	N/A	N/A
Bactocell	No effect	↑	N/A	N/A
Levucell SB	No effect	↑	N/A	N/A

*Treatments not delivered orally are labelled as such. An increase is marked by '↑' and a decrease by '↓'. Metrics that were not measured are labelled non-applicable (N/A). [1]Measured in cage/inoculation experiments; [2]Measured in full colonies; **As effective as fumagillin according to authors; *Less effective than fumagillin according to authors; [a]Four in-house synthetic fumagillin analogues were tested; [b]Tested only in* Apis cerana*; [c]Tested only in* Apis florea*; [d]van den Heever et al (46) found no effect; [1]Bee mortality varied between treatments and compound concentrations; +β-phellandrene, eucalyptol and α-terpineol*

identify beneficial compounds for potential new treatments. However, these in vivo studies are time consuming and costly. High throughput screening for novel drugs can be enhanced by infecting in vitro cell cultures with *Nosema*. Cell cultures have the advantages of rapid screening for multiple candidate drugs, standardisation and allowing screening all year round. A previous study demonstrated the value of using cell cultures by identifying two compounds that reduced *N. ceranae* spore viability – metronidazole and tinidazole (Gisder and Genersch, 2015).

RNA interference (RNAi) is the biological process by which small interfering RNA (siRNA) molecules turn off specific genes. RNAi is a natural component of the honey bee immune system (Brutscher and Flenniken, 2015) and represents a powerful tool for potential new treatments of diseases in many organisms, including humans (Dana et al, 2017). Previous work has investigated the use of synthetic siRNA to target and silence specific genes in *Nosema*. When researchers treated bees with siRNA, they found reduced spore loads, increased lifespans and a partial reversal of changes in gene expression in honey bees after infection with *N. ceranae,* presumably using its own RNAi response to increase its own proliferation (Huang et al, 2016, 2019; Kim et al, 2020). Although research has demonstrated promising results using siRNA, many non-target gene expression changes are reported. Further work is therefore needed to understand the non-specific responses in honey bees and downstream effects of silencing specific genes.

Practical Management

While many compounds have been demonstrated to reduce the impact of *Nosema* infections, most are not registered veterinary medicines, may have detrimental effects on honey bees and lack commercial development and regulatory safety testing. For now, the best advice remains to keep strong, healthy colonies and practice good apiary hygiene. Scorching or washing hive tools, using disposable (non-leather) gloves, regular comb replacement or fumigation with acetic acid can limit the spread of *Nosema* within and between colonies. □

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