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NOSEMOSIS AND ITS EFFECT ON PERFORMANCE OF HONEY BEES- A REVIEW

MUDASAR MANZOOR*, V. MATHIVANAN, GH. NABI SHAH, G M MIR AND SELVISABHANAYAKAM

[*]Department of Zoology, Annamalai university, Annamalai Nagar 60 8002, Tamil Nadu, india
Division of Entomology, Sher-e Kashmir university of Agricultural Science and technology Kashmir -191121

ABSTRACT

Nosemosis is one of the most widespread disease of honey bees with potentially serious effects on beekeeping. The transmission of nosema disease to the adult honey bees infecting epithelial cells, lining the midgut after spores are ingested causes various clinical symptoms ,including digestive disorders ,shortened life span ,decreased population size and negative effects on honey production capacity. In this review, the historical and recent data on Nosema ,covering the tissue tropism, pathology ,diagnoses, multiplication, phylogeny and genetics, virulence, clinical symptoms, control, and transmission of this important honey bee parasite and discuss these within the wider theoretical concepts, have been summarized.

KEYWORDS:Nosema *cerana*, Nosema *apis*, , phylogeny, pathology, virulence, control

MUDASAR MANZOOR
Department of Zoology, Annamalai university, Annamalai Nagar 60 8002, Tamil Nadu, india

***Corresponding author**

INTRODUCTION

Nosemosis (*Nosema* disease) is one of the most serious and prevalent adult honey bee diseases worldwide (Bailey, 1981; Matheson, 1993; and Fries, 2010) and is caused by intracellular microsporidian parasites from a genus of *Nosema*. For decades, *Nosema* disease was exclusively attributed to a single species of *Nosema*, *N. apis*, which was first described in European honey bees, *Apis mellifera* (Zander, 1909). In 1996, a new species of *Nosema* was first discovered in the Asian honey bee, *Apis cerana*, thus named *Nosema ceranae* (Fries *et al*., 1996). In 2005, a natural infection of *N. ceranae* was reported in *A. mellifera* colonies from Taiwan (Huang *et al*., 2005) Shortly thereafter, the infection of *N. ceranae* to *A. mellifera* was reported in Europe (Higes *et al*., 2006; Paxton *et al*., 2007), United States (Chen *et al*., 2007), China (Liu *et al*., 2008), Vietnam and worldwide (Klee ., 2007). Since its emergence as a potentially virulent pathogen of *A. mellifera*, *N. ceranae* has been associated with colony collapse of honey bees (Higes, *et al*., 2008; Paxton, 2010). A recent study showed that *N. ceranae* expanded its host range to South American native bumblebees (Plischuk *et al*., 2009) causing a new epidemiological concern for this pathogen. The present review summarizes recent findings on *Nosema ceranae* infection of *A. mellifera* in the USA and Asia, with particular emphasis on the comparative epidemiological, morphological, pathological, and genomic analysis of two *Nosema* species.

Whilst *N. apis* infection causes a fast acting, short duration syndrome, this has not been the case for N. ceranae, which instead has been observed in association with non-specific symptoms, such as a gradual depopulation, higher autumn/winter colony deaths or low honey production (Fries *et al*., 2006). It has also recently been shown that *N. ceranae* does not display the seasonality that is seen with *N. apis*. In a study of bee samples collected in Spain from 1999 to 2005 (Martin-Hernandez *et al*., 2007), the typical Nosema seasonality was observed between 1999 and 2002, as characterised by an increase in infection levels especially in spring. However, from 2003 to 2005, this seasonality diminished and consistently high numbers of samples infected with N. ceranae were detected throughout 2005 (Martin-Hernandez *et al*., 2007). *N. ceranae* has also been demonstrated to cause significantly higher mortalities in laboratory experiments indicating that it may be more virulent than *N. apis* (Paxton *et al* 2007).

For *N. apis*, the lifespan of infected bees is reduced and infected colonies suffer increased winter mortality or poor spring buildup and reduced honey yield (Fries *et al*., 1984; Anderson and Giacon, 1992). Though *N. apis* infected colonies may not exhibit overt infection symptoms, this disease organism is nevertheless considered to be a major scourge of beekeeping in temperate climates (Fries, 1993; and Fries *et al*., 2003). For *N. ceranae*, an emergent pathogen of *A. mellifera*, there is less information available. It has been thought to be a factor in the increased mortality of colonies detected across the year in central and southern Europe (Higes *et al*., 2005, 2006 and Imdorf *et al*., 2006). In support of this view, a recent infection experiment with *A. mellifera* comparing the effects of *N. ceranae* versus uninfected control bees revealed high mortality of infected bees (Higes *et al*., 2007). However, the lack of comparison in these experiments of the virulence of *N. ceranae* with *N. apis* are under identical laboratory conditions.

History of discovery of Nosema ceranae

In 1994, Fries *et al* (1996) discovered and described a new microsporidium, *Nosema ceranae*, infecting adults of the eastern honey bee, *Apis cerana*, around Beijing, China. Differences between the two microsporidia, *N. apis* and *N. ceranae*, lie in their ultrastructure and small subunit (16S) rRNA gene sequence (Fries *et al.,* 2006), allowing ready identification

by transmission electron microscopy and DNA sequencing respectively. Indeed, the rRNA gene sequence seems to be an excellent DNA barcode to differentiate among these and other microsporidian species (Klee *et al*., 2006), but not for intraspecific characterisation of variants (O'Mahony *et al*., 2007). Though cross-infection experiments demonstrated that *N. ceranae* was infective for the western honey bee (Fries and Feng, 1995), little more was made of the observation until *N. ceranae* was detected in *A. mellifera* in the spring of 2005 in Taiwan (Huang *et al*., 2007), within the natural range of *A. cerana*. In summer 2005 the first confirmed record of *N. ceranae* in *A. mellifera* was made within the western honey bee's natural range, in Spain (Higes *et al*., 2006), and outside the range of *A. cerana*. Its spread has undoubtedly come about through increased international trade, as has that of many other infectious microorganisms whose human mediated dispersal is occurring at unprecedented levels through the global transport network (Wilson *et al*., 2009).

Source of Nosema spp. Spores

Nosema Apis isolates

N. apis infects and replicates in the epithelial cells of the honeybee midgut (Fries, 1993). Nine isolates of *N. apis* were used in this study . Distinct, geographical isolates were chosen to increase the probability that genetic variation would be detected. Except for the Java isolate, all were obtained from the European honeybee*, Apis mellifera* (Apoidea: Apidae). The isolate from Java was obtained from the Asian honeybee *Apis cerana* (Apoidea: Apidae). The isolates from Canada, New Zealand, and Sweden were provided as purified spores, while the remainder of the isolates were obtained as infections in whole bees from which the spores were recovered .Samples of 35 honeybees, potentially infected with *N. apis,* were collected from the entrances ofbee hives. Five of the 35 honeybees were chosen at random. Crushing the thorax between the fingers, grasping the sting and terminal sclerites with tweezers, and gently pulling the alimentary tract away from the abdomen removed their alimentary tracts. A small piece of the midgut was removed from each honey bee, crushed between a microscope slide and a cover slip and microscopically examined at 400x magnification for the presence of *N. apis* spores. On confirmation of a *N. apis* infection within the beehive, the alimentary tracts of the remaining 30 honeybees were removed and stored at 4°C awaiting spore recovery and purification.

Date of spread of Nosema ceranae

When originally discovered in Europe in 2005, it was assumed that *N. ceranae* was a recent arrival (Higes *et al*., 2006). Sampling of historical material has demonstrated that it was present in European *A. mellifera* from 1998 (Klee *et al*., 2007) and perhaps from the mid-1990s in the USA (Chen *et al*., 2008) and possibly elsewhere (e.g. Invernizzi *et al*., 2009). Older records of Nosema from A. mellifera are, however, with one exception (Invernizzi *et al.,* 2009), all of *N. apis*. We can therefore be fairly confident in assuming that *A. mellifera* was not an original host of *N. ceranae*, and that *N. ceranae*, or a particularly virulent strain of *N. ceranae*, has recently jumped the species barrier into *A. mellifera* to become an EID.

Detection (Diagnosis)

Nosema could aptly be called "no-see-um" disease because infected colonies show few characteristic symptoms other than retarded colony development and disappearance of infected queens. Crawling bees are the only characteristic of the disease during the first few days of a heavy honey flow—apparently they are too weak to handle heavy loads of nectar. If the gut is carefully removed from crawling bees by pulling the last abdominal segment and gently drawing out the gut, the brown, fecesladen hindgut is seen first and then the midgut. In a healthy bee, the midgut is amber and translucent; in nosema-infected bees, the midgut is often swollen and milky. It later becomes chalky white and returns to normal size fig. . When chalky or milky guts are macerated with a tweezer in a droplet of water on a microscope slide and viewed at about 440X, almost a pure culture of nosema spores

(as many as 10^ spores per bee) can be seen. *Nosema apis* spores can be readily seen without staining by means of a compound microscope at 440X. Spotting or dysentery, not a symptom, may or may not characterize a nosema-infected colony. Bees of weak colonies that are dying from whatever cause—nosema, starvation, or queenlessness—may defecate. Conversely, grossly infected nosema bees may not void noticeable amounts of feces.

There is no specific outward sign of disease in bees infected with *N. apis*, although the ventriculus of heavily infected bees may appear whitish and swollen (Fries, 1997). Similarly, there are no outward symptoms reported for *N. ceranae*. Thus, diagnosis requires light microscopy, or more sophisticated molecular methods. The spores of *N. ceranae* are slightly smaller than in *N. apis*, but the two species are nevertheless difficult to tell apart with certainty under a light microscope (Fries *et al.,* 2006a). Queens can become infected while in mating nuclei, in transit with package bees, or after package bees are installed. Most beekeepers would not take time to look for a dead queen, but if they found one they probably would not be equipped to examine her for nosema. The loss be called "supersedure." Roberts 1967 described a method of detection of nosema spores in living queens by inducing them to defecate, thus enabling coprological examination without injury to the queen. In northern latitudes, the annual cycle of natural nosema infections in honey bee colonies has been shown to reach the highest levels in March or sometimes later in April, May, or June . Müssen *et al* 1975 sampled apiaries instead of individual colonies to g a survey of nosema incidence across the country. They also used an alternative sampling method based on hemocytometer counts giving an average number of spores per bee. This method was also used by Cantwell when the disease is acute, colonies may become depleted in population and eventually will dwindle to a handful of bees and a queen. They defecate in the hive and look dirty and sluggish. "Oldtimers" called this stage "spring dwindling." Eventually some colonies can outgrow the disease, as foraging becomes possible, but they are usually nonproductive and develop queen problems. In colonies not so severely affected, brood emergence eventually allows the colony to recover and produce a normal honey crop. How much honey is annually lost because of such subacute or endemic nosema infection is impossible to estimate, but the loss must be substantial.

Current distribution of Nosema ceranae

Klee *et al*., (2007) analysis of Nosema isolates from *A. mellifera* from across the world, interrogation of DNA databank entries and published records (based on rRNA sequence data) indicated that, post-2003, *N. ceranae* was widespread, and already found in North and South America, across Europe and Asia.

Phylogeny and genetics

The first genetic analysis of *N. ceranae* based on the 16S small sub-unit rRNA gene suggested that *N. apis* was not as phylogenetically close to N. ceranae as one may have suspected (Fries *et al*., 1996). Later analysis, based on the same gene and from GenBank entries have given some conflicting results. Three analyses found *N. ceranae* to be closer to *N. bombi* than to *N. apis* (Fries *et al*., 2001; Wang *et al.,* 2006; and Chen *et al*., 2009), whereas, the analysis of Slamovits *et al*., (2004) placed N. apis closer to *N. ceranae*. In contrast, the analysis of Vossbrinck and Debrunner-Vossbrinck (2005) put *N. apis* closer to N. bombi than to *N. ceranae*. In the most recent attempt to compile a phylogeny of microsporidians infecting bees, Shafer *et al*. (2009) used multiple sequence data sets, rather than sequences for a single gene, and concluded that *N. ceranae* is a sister species to *N. bombi* and that *N. apis* is the basal member of the clade. Based on their analysis, they (Shafer *et al.,* 2009) suggest that either an ancestral N. bombi switched host from a Bombus lineage to *A. cerana*, or an ancestral *N. ceranae* switched host to Bombus

Chen *et al.,* (2009) sequenced the DNA of the rRNA gene from *N. ceranae* and found the size to be 4475 bp, slightly larger than

reported by Huang *et al.,* (2007). The GC content of the 16S SSU-rRNA cistron is approximately 36% (Huang *et al*., 2007; and Chen *et al*., 2009). The internal transcribed spacer (ITS) region consists of a 39-bp sequence and is located between nucleotides 1260 and 1298 (Huang *et al.,* 2007; and Chen *et al.,* 2009).

The use of sequence similarities in the conserved rRNA gene is common for building phylogenies among eukaryotes. In the case of microsporidian parasites, this strategy may not be optimal. The presence of multiple copies of rRNA is common in Microsporidia (Gatehouse and Malone, 1998; Tek Tay *et al.,* 2005) possibly representing a case of concerted evolution, the duplication of entire loci within a genome. However, analyzing the rRNA gene from a single spore of *N. bombi*, O'Mahony *et al*., 2007 demonstrated multiple copies of rRNA which were not all homologous. Multiple nonhomologous copies of rRNA may be a common feature of Microsporidia. Thus, homologs cannot be compared between isolates, which reduces the utility of rRNA genes of microsporidians for phylogenetic analysis (O'Mahony *et al*., 2007). For future attempts to study *N. ceranae* phylogeny, there is a need to develop single-locus polymorphic markers (O'Mahony *et al*., 2007). Based on pyrosequencing data, a draft assembly of the *N. ceranae* genome (7.86 MB) has recently been presented (Cornman *et al*., 2009). The genome of *N. ceranae* is extremely reduced and strongly AT-biased (74% A + T) (Cornman *et al.,* 2009). Polymorphism among rRNA loci, as reported for N. bombi (O'Mahony *et al*., 2007) is likely to occur also in N. ceranae, which complicates the genome assembly of this operon (Cornman *et al.,* 2009). The genome analysis predicts 2614 protein-coding sequences, arguably an underestimate, since, a fraction of the genome likely did not assemble in this draft project (ca. 5–10%). About 50% of the predicted protein-coding sequences in the *N. ceranae* genome share significant similarity with the microsporidian Encephalitozoon cuniculi, so far the most closely related published genome sequence Cornman *et al.,* 2009). Interestingly, both parasites appear to differ from yeast and other fungi by using a larger fraction of the genome for growth related gene categories and a reduced fraction to transport and to chemical stimuli (Cornman *et al.,* 2009).

This is likely to reflect the extreme parasitic life form represented by microsporidians. Many aspects of the Nosema-honey bee interactions remain enigmatic. Identification of genes with specific functions is a first step in resolving such host–parasite interactions at the gene level. Cornman *et al.* (2009) stress the 89 gene models encoding signal peptides as being of particular interest, because, these proteins are candidate secretory proteins that may interact with host tissue. Antúnez *et al.* (2009) attempted to measure gene responses following microsporidia infections. Their results suggest the differences in upregulation of genes encoding the antibacterial peptides abaecin, defensin and hymenoptaecin between infections with *N. ceranae* and *N. apis*. However, previous work based on the antibacterial properties of hemolymph from *N. apis* infected bees did not show any antibacterial effects from such hemolymph (Craig *et al*., 1989). The results of Antúnez et al. (2009) are interesting, because they also suggest that immunosuppression results from *N. ceranae* infections. Given that their study (Antúnez *et al*., 2009) includes time limited data only, it is premature to conclude that the gene expression data available are indicative of variations in virulence between *N. ceranae* and *N. apis*.

Tissue tropism and pathology

The tissue tropism (affinity to specific tissues) of a parasite is an important pathogenic factor. Infection of Nosema starts through ingestion of spores with food or water. Following ingestion, the spores develop at the site of the primary infection and multiplied parasites can spread to different tissues of the same host. A study conducted by Chen *et al.* (2009a) using PCR method showed that *N. ceranae* has a broad tissue tropism in the host of *A. mellifera*.The infection of *N. ceranae* was not restricted to the midgut tissue but spread to other tissues

including the malpighian tubules, hypopharyngeal glands, salivary glands, and fat bodies . Among bee tissues dissected and examined, *N. ceranae* was detected in 100% of alimentary canals, malpighian tubules, and hypopharyngeal glands, in 87% salivary glands, and in 20% of the fat bodies. No *N. ceranae*-specific PCR signal was detected in the muscle tissue. The infection of Nosema in European honey bees has often been reported to be associated with effects of reduced bee longevity, decreased population size, higher autumn/winter colony loss, reduced honey production and decreased brood production (Hassanein, 1953a, b; Rinderer and Sylvester, 1978; Goodwin *et al*., 1990., Anderson and Giacon, 1992 and Malone *et al.,* 1995).

However, none of the disease symptoms such as dysentery and/or crawling behavior and/or milky white coloration of gut that are usually related with *N. apis* infection has been found in *N. ceranae* infected bees (Fries *et al*., 2006). It was shown recently that *N. ceranae* exerts a significant energy cost to infected bees and changes their feeding behavior (Mayack and Naug, 2009; Naug and Gibbs, 2009). An early study by Bailey and Ball (1991) demonstrated that the infection of hypopharyngeal glands by *N. apis* could lead to worker bees losing the ability to produce brood food and digest food The absence of crawling behavior in *N. ceranae* infected bees might be the result of absence of *N. ceranae* infection in the muscles. Fat body is one of the primary sites of microsporidian infection in many insects. The infection of adipose tissue causes formation of whitish cysts and the infected gut becomes swollen and whitish as a result of impaired fat metabolism (Sokolova *et al*., 2006). The absence of milky white coloration of gut may reflect low infection of *N. ceranae* in the tissue of the fat body. Because all previous tissue tropism studies on *N. apis* were conducted using the presence of spores as a criterion (Hassanein, 1953a, b; Gilliam and Shimanuki, 1967; De Graaf and Jacobs, 1991); new efforts are under way as part of a recently funded USDA-CAP project to determine the tissue tropism of *N. apis* in the host of *A. mellifera* (Lee Solter, unpubl. data). While N. *apis* was known to cause earlier foraging in *A. mellifera* (Hassanein, 1953; Wang and Moeller, 1970), this behavioral change seems to be mediated by higher juvenile hormone titers in infected bees due to elevated juvenile hormone production (Huang, 2001), comparative data is lacking in *N. ceranae*.

Further studies on the pathogenesis of both parasites will shed light on why *N. ceranae* has different pathological effects on the host of *A. mellifera* compared to *N. apis*. In tissue tropism of *Nosema ceranae*, tissues such as hypopharyngeal gland, salivary gland, alimentary canal, malpighian tubules, muscle, and fat body were dissected and examined for the presence of *N. ceranae* by PCR method. For electrophoresis gel, numbers 1–6 indicate hypopharyngeal gland, salivarygland, alimentary canal, malpighian tubules, muscle, and fat body, respectively; N indicates negative control, and letter P indicates positive control. The size of PCR fragments is indicated on the right of the gel.

Prevalence

The typical pattern for N. apis infections in temperate climates are low prevalence or hardly detectable levels during the summer with a small peak in the fall. During the winter, there is a slight increased prevalence with a large peak in the spring before the winter bees are replaced by young bees (Bailey, 1955). The pattern is similar both in the southern and northern hemisphere (Doull and Cellier, 1961). Unfortunately, very few data exist for *N. apis* on the seasonal prevalence of tropical or subtropical conditions. The only published year round sampling under conditions where bees could fly all year round, revealed detectable levels of *N. apis* with no seasonal pattern of prevalence (Fries and Raina, 2003). Thus, a seasonal pattern of prevalence may be dependent on climatic conditions. However, from older Spanish records, *Nosema spp*. infections did have a seasonal pattern of prevalence, similar to descriptions from temperate climates. From 2003 onwards, a change in seasonality occurred with an increase

of *Nosema spp*. Positive samples throughout the year until 2005, when there was a total absence of seasonality in infection prevalence (Martín- Hernández *et al*., 2007). This strongly suggests that the fundamental epidemiological parameters, such as transmission rates and/or routes may be different between the two parasites.

Clinical Signs of N. ceranae infection in honeybee colonies

Probably the most controversial aspect of *N. ceranae* infection in beekeeping is its ability to depopulate or kill a colony. After *N. ceranae* parasitisation of honeybees was first detected and linked with colony collapse in Spain (Higes *et al*., 2006 and Martín- Hernández Goodwin *et al*., 1990., 2007), other authors ruled out its role in colony loss (Cox-Foster *et al.,* 2007 and Klee *et al.*, 2007). At this time, the little data available on the virulence of *N. ceranae* at the colony level was contradictory, probably due to a failure to properly identify the clinical sign of disease, the parameter with which to evaluate the impact of the illness and a poor understanding of the subclinical effects of parasitism. Moreover, at the time, the only common feature of colony collapse described all over the world was death.

Traditionally Koch's postulates have been used as criteria to determine whether a given microorganism causes a specific disease. Those postulates were initially developed for bacteria and despite their importance in microbiology; they have severe limitations particularly when applied to diseases caused by non-bacterial microorganisms. For example, for some microorganisms that cannot be grown in pure culture in the laboratory, including bee microsporidia, they can be used to infect the host, mimicking the disease. Additional limitations arise in cases where, the clinical signs of an infection have not been accurately described (or widely accepted), as is the case of nosemosis type C caused by *N. ceranae* infection.

Taking these limitations into account, Koch's postulates were demonstrated for honeybee colonies infected with N. *ceranae* (Higes *et al*., 2008), as previously confirmed in individual bees . *N. ceranae* was extracted from an affected colony and identified by PCR, and it was then transmitted to healthy colonies where it induced disease and colony collapse. Finally, the infective agent was isolated from these newly infected colonies. These findings were subsequently confirmed in later studies (Botías *et al*., 2010; 2012a) and among other pathogens, colony loss has been linked with the presence of *N. ceranae* in several reports (Higes *et al*., 2005, 2006 2008, 2009a; Borneck *et al*., 2010 and Hatjina *et al.,* 2011).

However, studies conducted in colder areas have revealed contradictory findings (Gisder *et al*., 2010; Stevanovic *et al*., 2011; Hedtke *et al.,* 2011 and Dainat *et a*l., 2012a,b). Thus, it is of great interest to determine whether these differential effects results are due to distinct behaviors of *N. ceranae* at different latitudes, or basic criteria are not the same such as the identificacion of clinical/subclinical signs of disease between researchers. In some works, definition of colony loss or collapsed colonies are the only description of a disease that seems not to present any other clinical sign. An expert is sometimes needed to detect signs as lower bee population, lower honey production, unexpected brood in cold months or younger bees starting to forage. Due to the fact that bees are social insects, a biological point of view must be properly differentiated from a veterinarian one, each one complementing the other.

Effects by Nosema Apis

Effects on workers and queens

In 1990s, Liu (1992) in Canada conducted many studies, most of them at the ultra-structural level on the effects of *N. apis* on honey bees. His studies indicated that workers infected with *N. apis* showed ultrastructural changes in the cells from midgut epithelium, hypopharyngeal glands, and corpora allata (sources of juvenile hormone). Oöcytes in queens infected with *N. apis* for only 7 days were already degenerated. The ovariole sheath became wrinkled. In the oöplasm, yolk granules broke down into small spheres and granular substances and the

oöcytes became extensively autolysed. It was not clear whether the oocyte degeneration in infected queens due to a pathological process, a lack of protein nutrition, or to increased juvenile hormone production as a result of *Nosema* infection. Midgut (ventriculs) tissue of a bee infected by *N. apis* (top) and a healthy bee (bottom). Healthy bee midguts are straw colored, translucent and ring like structure can be seen, while infected midguts are milky and the structures are not as clear. It was said *N. ceranae* infection does not show this symptom, which is typical of *N. apis*.

Effects by N. Ceranae

Learning and homing behavior affected by N. ceranae

When Kralj and Fuchs (2010) studied the homing behavior of bees mainly infected with *N. ceranae*, some bees were co-infected with *N. apis*. They found that infected bees released 6 and 10 m away from the colony took longer times to return. The percentage of bees that did not make home was higher in the infected bees compared to the healthy bees when released 30 m away from the colony. They also found a lower rate of infected bees among the returning foragers compared to departing foragers, suggesting some infected bees did not return home successfully. It is not clear why infected bees did not return home as well. The study used bees of known ages, so this is not because infected bees were developing precociously. The alternative is that infected bees did not have proper protein nutrition which affected their brain development and capacity of learning. It is not clear whether *N. apis* causes the same effect in honey bee learning and homing behavior. We have tried to determine if *N. apis* infected bees drifted more to surrounding colonies but failed to find if this is the case (Huang w ,2007).

N. ceranae *causes immune suppression*

Antúnez *et al*. (2009) studied the immune response of honey bees after infection with either *N. apis* or *N. ceranae*. They measured gene expressions of several antibiotic peptides, abaecin, defensin and hymentoptaecin, produced inside honey bees after bacterial infection. In all three genes, *N. apis* infection caused an elevation of gene expression in either 4 or 7 days post infection, but *N. ceranae* did not show any difference in gene expression compared to the control (uninfected bees), or even significantly reduced it (abaecin at 7 days). These data suggest that *N. ceranae* actively suppresses the immune response in infected honey bees while *N. apis* does not.

Alaux *et al.* (2010) studied whether a neonicotinoid (imidacloprid) and *Nosema* (a mixture of both species) would show a synergistic interaction in affecting honey bees. They found that the combination of both agents caused the highest mortality and food consumption. They also found that the activity of glucose oxidase, an enzyme bees use to sterilize colony and brood food, was significantly decreased only by the combination of both factors compared with control, *Nosema* or imidacloprid only groups, suggesting a synergistic interaction between the two agents. Because the combined group showed similar *Nosema* spore counts to that of *Nosema* infected bees alone, it seems that the synergistic effect is due to the immune suppression of *N. ceranae*, causing bees to be more sensitive to the pesticide, rather than the pesticide reducing bee resistance to allow a more severe damage by *Nosema*.

In a more recent study, Vidau *et al*. (2011) found a similar synergistic effect between pesticides and *N. ceranae*. After being exposed to sublethal doses of fipronil or thiacloprid, *N. ceranae*-infected bees showed a higher mortality than in uninfected ones. The synergistic effect of *N. ceranae* and insecticide on honeybee mortality was not linked strongly to a decrease of the insect detoxification enzymes. This is because, *N. ceranae* infection induced an increase in glutathione-S-transferase activity in midgut and fat body but not in the 7-ethoxycoumarin-O-deethylase activity. It is not clear how tightly the insect detoxification system and the immune system are linked – they might well not be tightly linked since one is induced by pesticides and another by parasites.

N. ceranae *affects queen health*

Alaux *et al.* (2011)studied the effect of *N. ceranae* infection on 8 day old honey bee queens. They found that *N. ceranae* did not affect the fat body content, which is an indicator of energy stores, but changed the vitellogenin titer, which is an indicator of fertility and longevity, the total antioxidant capacity and the queen mandibular pheromones. The strange thing is that, these changes were contrary to the predicted direction that they were all increased in *Nosema*-infected queens. It is possible that these are only seen in 8 day old queens, perhaps due to accelerated development as seen in *N. apis* infected worker bees. It is not clear whether in older queens these changes will remain or become reversed.

Control

Until more research is available on the biology and transmission of *N. ceranae* it is difficult to say if general recommendations for *N. apis* (i.e. wax renewal, acetic acid fumigation of stored comb) are also relevant for *N. ceranae* control. The major commercial medication available, based on the antibiotic fumagillin, is effective on both parasites (Williams *et al*., 2008). However, in contrast to some other parts of the world where *N. ceranae* infections may be controlled by using fumagillin, shows the effect of Fumagilin dose on honey bee colonies.

Table 1

Effect of feeding Fumidil B on nosema disease in honey bee colonies.

			Colonies positives to *N. ceranae* or death				
Group	*N*	Intervention	30.10.06a	15.12.06	26.04.07	26.09.07	Nosemosis status 11.11.07
Positive treated	18	Fumagillin (120 mg)	18	0	5	18	18 in Phase 1
Positive treated	15	Syrup	15	13[b]	6	2	1 in Phase 2
				2 death	7 death[c]	4 death[c]	1 in Phase 4
	17	Syrup	0	15	17	14	4 in Phase 2
Negative untreated							5 death

(Mariano Higes et al 2006)

a. Pre-treatment.

b. Two colonies infected by N. ceranae and N. apis.

c. One colony infected by N. ceranae and N. apis.

CONCLUSION

Nosema is recognized as the most widespread and emergent pathogen of honey bees potentially causing serious effect on beekeeping since 2007,especially after appearance of colony collapse disorder (CCD) in our country. Under a scenario of global climate change, N. ceranae may exert an increasing impact on world beekeeping with A. mellifera, analogous to the impact of emergent fungal pathogens tied to global warming on amphibian populations (Pounds et al., 2006). The effects by two species of nosema are clearly identified. The infection levels of nosema in honey bees is higher in spring and winter than summer and autumn. However the nosema cerana pathology and epidemiology are still unknown facts ,despite of many studies . *N. ceranae* spores appear to be much more vulnerable than spores of *N. apis*, in particular to freezing, and the apparent replacement of *N. apis* for *N. ceranae* remains enigmatic. Experiments on caged worker bees have nevertheless revealed N. ceranae to be a potentially highly virulent pathogen (Higes et al., 2007), one that seems to be more pathogenic than Nosema apis (Paxton et al., 2007). Worryingly, Martín-Hernandéz et al. (2009) have recently suggested that N. ceranae may even have superior growth within its host than N. apis at a realistic range of environmental temperatures.

The impact of *N. ceranae* infections on the development of *A. cerana* colonies also needs to be investigated. An increased understanding of how *N. ceranae* invades its presumed original host, and how *A. cerana* resists or tolerates such invasions, will therefore be of interest for these species and, by inference, *A. mellifera*.

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