

Distribution, epidemiological characteristics and control methods of the pathogen *Nosema ceranae* Fries in honey bees *Apis mellifera* L. (Hymenoptera, Apidae)

Distribución, características epidemiológicas y métodos de control del patógeno *Nosema ceranae* Fries en abejas *Apis mellifera* L. (Hymenoptera, Apidae)

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RESUMEN

El parásito microsporidio *Nosema ceranae*, hasta hace algunos años fue considerado como patógeno de *Apis cerana* solamente, sin embargo en el último tiempo se ha demostrado que puede afectar con gran virulencia a *Apis mellifera*. Por esta razón, ha sido denunciado como un agente patógeno activo en la desaparición de las colonias de abejas en el mundo, infectando a todos los miembros de la colonia. Es importante mencionar que las abejas son ampliamente utilizadas para la polinización y la producción de miel, de ahí su importancia en la agricultura, además de desempeñar un papel ecológico importante en la polinización de las plantas donde un tercio de los cultivos de alimentos son polinizados por abejas, al igual que muchas plantas consumidas por animales. En este contexto, esta revisión pretende resumir la información generada por diferentes autores con relación a distribución geográfica, características morfológicas y genéticas, sintomatología y métodos de control que se realizan en aquellos países donde está presente *N. ceranae*, de manera de tener mayores herramientas para enfrentar la lucha contra esta nueva enfermedad apícola.

Palabras clave: parásito, microsporidio, *Apis mellifera*, *Nosema ceranae*.

SUMMARY

Up until a few years ago, the microsporidian parasite *Nosema ceranae* was considered to be a pathogen of *Apis cerana* exclusively; however, only recently it has shown to be very virulent to *Apis mellifera*. Therefore, it has been named as a pathogenic agent active in the disappearance of honey bee colonies globally, infecting all members of the colony. Honey bees are widely used for pollination and honey production, hence their importance in agriculture. They also play an important ecological role in plant pollination: a third of human food crops are pollinated by bees as well as many plants consumed by other animals. In this context, the object of this review is to summarise the information published by different authors on the geographical distribution and the morphological and genetic characteristics of this parasite, the symptomatology of the disease and the control methods used in those countries where *N. ceranae* is present, in order to identify better tools to confront this new bee disease.

Key words: parasite, microsporidian, *Apis mellifera*, *Nosema ceranae*.

INTRODUCTION

Honey production has become an important feature of world agriculture, since the bees that produce it play a role in pollination and food production (Bradbeer 2009). In recent years there has been a reduction in populations of *Apis mellifera* L. (Hymenoptera, Apidae) in Northern Hemisphere countries (Potts *et al* 2010, vanEngelsdorp *et al* 2010).

According to Ellis *et al* (2010), the cause of this depopulation and the eventual disappearance of colonies is not yet clear. Honey bee populations have been declining steadily since 1940 and the consensus of researchers indicates that parasites are an important factor in this disappearance. One such parasite is *Nosema ceranae* Fries, and perhaps the most recent and virulent (Martin-Hernández *et al* 2007,

Gisder *et al* 2010). The credibility of this theory is based on the fact that all parasites are dependent on the energy from their hosts to reproduce, causing them significant nutritional stress and eventually, death (Mayack and Naug 2009).

Nosema spp. are single-celled parasites belonging to the phylum Microspora. They are characterised by the production of a resistant spore containing a polar filament, which transmits genetic material into the host cell (Wittner and Weiss 1999). Infection ranges from the most common chronic form to the less frequent acute form of the disease. The genus *Nosema*, which belongs to the class Microsporidia, contains more than 150 species, these can infect invertebrate hosts including at least 12 orders of insects (Higes *et al* 2007). The parasite *N. ceranae* is one species of the genus, infecting the Asian bee *Apis cerana* Fabricius and the European bee *A. mellifera* (Fries *et al* 1996, Higes *et al* 2006). In addition *Nosema* spp. are commonly found in Lepidoptera and Hymenoptera,

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causing them diseases such as silkworm pebrine, caused by *Nosema bombycis* Nägeli and bee dysentery, caused by *Nosema apis* Zander (Higes *et al* 2007).

Until recently *N. apis* was thought to be the only cause of nosemosis in the european bee *A. mellifera*, whilst *N. ceranae* had only been detected in oriental bees *A. cerana*. However, recent studies have shown that *N. ceranae* has changed its feeding habits and is no longer unique to the eastern bee, but has also become a very efficient parasite of european bees (Higes *et al* 2007, Chen *et al* 2008, Chen *et al* 2009^a, Chen *et al* 2009^b) and several species of bumblebees (Plischuk *et al* 2009, Li *et al* 2012, Graystock *et al* 2013). The resulting disease is known as type C nosemosis (Higes *et al* 2010^b).

Initial investigations were focused on finding the differences and similarities that exist between these microsporidia, the first conclusion being that both directed their attack at the epithelium of the ventricle (Higes *et al* 2007). The disease can infect all members of the colony including worker bees, drones and queens. Traver and Fell (2011) point out that drones could be important agents in the spread of this disease between apiaries. According to Alaux *et al* (2011), the effect of *N. ceranae* on queens could result in changes to the production of pheromones, which may explain their replacement. Another similarity is that *Nosema* infection occurs mainly by the ingestion of spores through food and water. They breed in the mid-intestine of the honey bee and the spores are finally eliminated in the faeces, providing new sources of infection through cleaning and feeding activities within the colony (Chen *et al* 2009^a).

The symptoms of honey bee nosemosis caused by *N. apis* are easier to observe; there are large numbers of dead bees in the colony and diarrhoea stains at hive entrances, indicating digestive system disorders (Bourgeois *et al* 2010). However, this is regarded as a fairly benign pathogen that rarely causes colony losses (Bailey 1967, Bailey and Ball 1991). By contrast, the symptoms of infection by *N. ceranae* are less obvious; colonies grow weaker (Bourgeois *et al* 2010) and it is possible to detect the disease throughout the entire year (Higes *et al* 2010^b). Moreover, it has been identified as the main causative agent for the loss of colonies in many geographical regions (Martin-Hernandez *et al* 2007, Higes *et al* 2008), suggesting that *N. ceranae* is more virulent than *N. apis* (Higes *et al* 2007). It may be added that infestation by any type of *Nosema* will result in a decline in honey production and foraging activity, and therefore a decline in pollination (Bourgeois *et al* 2010).

According to Paxton (2010), *N. ceranae* is an emerging, potentially virulent pathogen which has spread throughout the world in the past 10 years, which explains why researchers have detected it in both healthy and weak honey bee colonies (Martin-Hernández *et al* 2007, Chen *et al* 2008, Higes *et al* 2008).

The object of this review is to summarise the information available in various scientific publications to document biological characteristics of the pathogen: its geographical

distribution, the symptomatology of the disease, and control strategies, in order to systematize new background material in the fight against this dangerous pathogen of the european honey bee *A. mellifera*.

DISCOVERY AND GEOGRAPHICAL DISTRIBUTION

In 1994 *N. ceranae* was detected for the first time in Beijing (China) in Asian bees (*A. cerana*) (Fries *et al* 1996). Later Higes *et al* (2006) reported the presence of *N. ceranae* infecting *A. mellifera* in Europe for the first time.

Chen *et al* (2008) collected samples of honey bees between the years 1995 to 2007 and found the pathogen in 12 states in the USA; Liu *et al* (2008) reported it in China, while other reports over the years include Canada (Williams *et al* 2008a); Greece (Bacandritsos *et al* 2010); Thailand (Chaimanee *et al* 2010); different prefectures in Japan (Yoshiyama and Kimura 2011); several Balkan countries (Stevanovic *et al* 2011); and Turkey (Whitaker *et al* 2011). Moreover, Klee *et al* (2007) reported that *N. ceranae* is a disease of global proportions detected in many parts of the world, including Europe, Africa, Asia, America and Oceania (Higes *et al* 2006, Calderón *et al* 2008, Giersch *et al* 2009, Higes *et al* 2009^b, Chen and Huang 2010). In South America, studies have confirmed the presence of *N. ceranae* in Brazil (Texeira *et al* 2013), Uruguay (Invernizzi *et al* 2009), Argentina (Plischuk *et al* 2009, Medici *et al* 2012) and Chile (Martínez *et al* 2012, Rodríguez *et al* 2012).

Martin-Hernández *et al* (2007) indicate that colonies exposed to *N. ceranae* have a depopulation risk almost six times greater than those without *N. ceranae*. In this context Higes *et al* (2007, 2008, 2010^c) and Chen *et al* (2008) suggest that *N. ceranae* can cause the sudden collapse of bee colonies, causing important diseases at an individual level (Chen and Huang 2010, Dussaubat *et al* 2012). *N. ceranae* also has an impact at the honey bee colony level (not only the collapse of the colony), for example the decrease in adult bee population and honey production (Higes *et al* 2008, Botias *et al* 2013). Subsequently, Paxton (2010) suggests that it may act not only as a single cause, but possibly also in combination with other factors such as loss of habitat and floral resources, which has clearly aroused the curiosity of beekeepers and the scientific world.

Nevertheless, although the presence of *N. ceranae* in Brazil was confirmed in 2006, it has been affecting Africanised bees for at least 34 years. Its presence was detected recently in Uruguay, although it has not yet caused particular damage in that area and its impact is still unclear (Invernizzi *et al* 2009, Texeira *et al* 2013), probably due to the presence of a less virulent strain of the parasite or a resistant feature in the bees (Higes *et al* 2008, Vandame and Palacio 2010). It is therefore suggested that the parasite is more pathogenic in Europe than in North America (Higes *et al* 2008, van Engelsdorp

et al 2009), making it difficult to assess the real mortality in western colonies (Williams *et al* 2010). Furthermore, tests carried out in Spain indicated that both *N. ceranae* and *N. apis* may be present in hives without causing symptoms of the disease, and that there is no evidence of the replacement of *N. apis* by *N. ceranae*, suggesting that nosemosis is not the principal cause of hive collapse and death (Fernández *et al* 2012).

Nosema ceranae IN CHILE

In Chile, nosemosis has been reported in hives in the Libertador General Bernardo O'Higgins District, caused mainly by *N. apis*, with greater prevalence during the spring due to high fecal contamination in colonies at the end of winter (Hinojosa and Gonzalez 2004). *Nosema* has been detected in 10% of colonies in the Azapa valley, Arica and Parinacota Districts (Huaiquil *et al* 2009). Although there is little information on the presence of *N. ceranae* in Chile, in 2009 the Agriculture and Livestock Service (SAG) officially confirmed the presence of *N. ceranae* in apiaries in the Biobio Region (Fuentelba and Linero 2010). During early spring 2010, beekeepers with apiaries in Curepto, Maule District (34°59.986'S, 71°53.377'W), reported mass deaths of bees in which 52% of the samples analyzed were infected with *N. ceranae* (Rodríguez *et al* 2012). During the 2010-2011 season Martínez *et al* (2012) examined 240 hives in the Biobio District in southern Chile and detected *N. ceranae* positive samples in 49% of these. The level of infection ranged from 200 to more than 100,000 spores per bee. Meanwhile, Rodríguez *et al* (2014) detected *N. ceranae* in 18% of hives analysed in the Biobío District out of a total of 180 colonies sampled. It is suggested that *N. ceranae* may be the real cause of nosemosis in *A. mellifera* in South America.

MORPHOLOGICAL FEATURES

Microsporidia are a group of single-celled fungi that infect a wide variety of animals including honey bees. They are also known for their simple genome and cell features, a result of their adaptation to obligate intracellular parasitism (Corradi and Slamovits 2010).

Chen *et al* (2009a) report that under the optical microscope fresh spores of *N. ceranae* are ovoid, oval or cylindrical in shape, straight or slightly curved, varying from 3.9 to 5.3 µm and from 2.0 to 2.5 µm width. Fries *et al* (1996) reported that they measure 4.7 x 2.7 µm fresh, and 3.6 x 2.7 µm when fixed and stained. The proteins observed in the vacuole and vesicle in *N. ceranae* are absent in *N. apis*, suggesting that species-specific genes are involved in the structure and function of cell components (Chen *et al* 2013).

In addition, *N. ceranae* spores contain a polar filament, diameter 96-102 nm, with between 18 and 21 coils (Fries *et al* 1996, Chen *et al* 2009^a). The parasite is characterised

by the expulsion of the polar filament from the spore in the presence of potential host cells in the surrounding area, using this mechanism to penetrate the cell membrane of the host. Subsequently, rapid swelling of the vacuole located in the back of the spore exerts strong pressure on the cell contents, pushing them through the polar tube. Successful propagation is followed by lysis of the host cell and spore release (Corradi and Slamovits 2010)

According to Gisder *et al* (2010), the intracellular life cycle is divided into two phases: the proliferation phase, called merogony, and the multiplication phase, which finishes with spore formation, called sporogony. Once the filament penetrates the cell, sporoplasm is injected and multiplies in the cytoplasm of the host cell. At this stage the spore injected from the mother spore develops through four distinguishable forms. These forms are schizonts, sporonts, sporoblasts and mature spores, which will eventually be released into the environment to attack another cell (Chen *et al* 2009^a, Gisder *et al* 2010).

GENETIC FEATURES

Chen *et al* (2009^a) conducted a parallel comparison of the rRNA gene sequences of *N. ceranae* and *N. apis*, and found that although they infect the same host and share similarities in their rRNA gene sequences *N. apis* is not the closest relative to *N. ceranae*; *N. ceranae* appears to be more closely related to *Nosema vespula*, a parasite in the same clade that infects wasps, while *N. apis* seems to have developed earlier and is more closely linked to *N. bombi*, a parasite infecting bumble bees.

However, Shafer *et al* (2009) used multiple data sequences to compile the phylogeny of microsporidia affecting bees and came to the conclusion that *N. ceranae* is a sister species of *Nosema bombi* Fantham & Porter while *N. apis* is the basal member of the clade. Nevertheless, it is suggested that future attempts to study the phylogeny of *N. ceranae* will require the development of markers with one polymorphic locus only (O'Mahony *et al* 2007). These will also be reliable, since Chen *et al* (2013), using specific primers for *N. apis*, successfully confirmed the specificity of the species by amplifying the fragment sequence using PCR, while the same primers did not generate any PCR product in *N. ceranae*. In view of this, Gisder and Genersch (2013) presented a reliable new protocol to discriminate between *N. apis* and *N. ceranae*, based on amplification of sequences of the largest subunit (RPB1) of RNA polymerase II, using species-specific primers. This proved more reliable than the widely used gene-based 16S rRNA protocols that have been used for the comparison. When both species are present in the same host, Carletto *et al* (2013) suggest that the PCR multiplex kit could detect small numbers of one species of *Nosema* when larger numbers of the other are also present. This confirms that the PCR technique in a single, multiplexed, real time reaction facilitates

the detection and quantification of both *N. apis* and *N. ceranae*, making it a useful technique for diagnosing diseases such as nosemosis (Bourgeois *et al* 2010).

Furthermore, high levels of genetic diversity have been observed in isolations, evidence of meiotic recombination in populations of *N. ceranae*, without signs of differentiation between populations from different locations (Van der Zee *et al* 2014).

Another important aspect of *N. ceranae* is the high level of intraspecific polymorphism (due to the high nucleotide diversity and allele content of all its genes), not only within the species, but also within individual samples, as well as the coexistence of a broad variety of haplotypes within each bee colony and the occurrence of genetic recombination in the RPB1 locus. The latter has led to the recent population expansion of the parasite, due to the presence of multiple haplotypes in individual isolations (Roudel *et al* 2013, Gómez-Moracho *et al* 2014). Differences in virulence have been reported in the different genes due to increased genetic diversity, resulting from variations generated by intragenomic and/or intergenomic recombination between the sequences (Ironsides 2013). This suggests a high degree of heterogeneity between the strains of *N. ceranae* infecting the individual colonies (Whitaker *et al* 2011, Van der Zee *et al* 2014), and this variation may be related to the entry of microsporidia at different points in the country, causing the variable symptomatology found in the field. It is therefore necessary to evaluate the current protocols for the recognition of this parasite as the principal agent causing nosemosis (Medici *et al* 2012), since proofs of gene recombination suggest that this species may effect hidden sexual reproduction. This possibility has profound implications for the evolution of the virulence of the species, the range of possible hosts, and its resistance to treatment (Ironsides 2013).

DAMAGE FEATURES

The characteristics of this disease are the inexplicable disappearance of adult honey bees, lack of attention to the offspring, a reduction in the vigor of the colony, and high winter mortality without prior pathological changes (Higes *et al* 2009^a). Martín-Hernández *et al* (2007) say that it is a clear sign of weakness in a colony when the queen cannot replace the loss of infected bees. In fact, the risk of depopulation of a colony is six times higher in colonies infected with *N. ceranae* than in those not infected.

Higes *et al* (2010^a) studied the biotic potential of *N. apis* and *N. ceranae* at a temperature of 33 °C, recording a similar result in both cases; however, when measured at extreme values of between 25 °C and 37 °C, the biotic potential of *N. ceranae* was higher than that of *N. apis*, meaning that it will cause greater problems for beekeepers in summer months and in warm climates (Bourgeois *et al* 2010). This is due to the presence of proteins involved in the response to stress and endogenous stimuli that are more

representative in *N. ceranae* than *N. apis*, suggesting that *N. ceranae* could have a greater ability to survive under stress conditions (Chen *et al* 2013).

Chen *et al* (2009^b) studied the coexistence of *N. ceranae* with *N. apis* and found that bees captured in the United States and Asia were 31% positive for *N. apis* and 71% positive for *N. ceranae*, whilst 19% presented both infections. The authors therefore suggest that *N. ceranae* has better mechanisms for coping with honey bee immunity and reproduces faster than *N. apis*. However, the differences in response to bee infection are more likely to be related to the degree of tolerance to *N. ceranae* of each honey bee subspecies or of the local hybrids, or even to experimental conditions in the case of laboratory tests, than to differences between *N. ceranae* isolates (Dussaubat *et al* 2013^b). Furthermore, infection with *N. ceranae* weakens the chemical mechanisms that regulate behavior maturation, especially the balance between nurses and foragers, which in turn has an impact on colony homeostasis. This makes colonies more susceptible to other environmental stress factors (Dussaubat *et al* 2013^a), since the impact on colony homeostasis is a consequence of the former effects.

It is characteristic of the disease that the parasite attacks the epithelial cells of the ventricle, causing cell degeneration and subsequent rupture of cell membranes; this leaves the intestine edematous and friable (Higes *et al* 2007, 2009^a). Infected cells present an elongated appearance with apical displacement of the nucleus. The cytoplasm contains a greater number of mitochondria and shows evidence of degeneration such as the presence of vacuoles and lysosomes, the majority of which are secondary and irregular in shape with heterogeneous electron-dense areas (Dussaubat *et al* 2013^b). Chen *et al* (2009^a) performed PCR tests on specific nucleic acids, indicating that *N. ceranae* is detected in 100% of alimentary canals, malpighian tubes, and hypopharyngeal glands; 87% of salivary glands; and 20% of fat bodies. However, these results have not been confirmed by histopathological studies, and nor have different developmental stages of *Nosema* been observed in cell types other than the epithelial cells of the ventriculum. The latter are not a primary target for infection by *N. ceranae*, although body fat is one of the primary infection sites for these microsporidia which also infect muscle tissue. With regard to the mode of transmission, Smith (2012) suggests that the spores of *N. ceranae* can be transmitted orally, which may explain its rapid spread as the food produced by the colony is shared by its members.

Turning to the time which elapses from ingestion to infection, only a few epithelial cells of the ventricle are infected by day three, while the majority of cells are infected after day seven. It is at this point that evidence of degeneration appears, with a mortality rate of 66.7 % on the sixth day, 94.1 % on the seventh day and 100% on the eighth day (Higes *et al* 2007). Vidau *et al* (2011) on the other hand reported maximum mortality 20 days

after infection (47 %). Higes *et al* (2013) indicate that the cause of these mortalities is the fact that *N. ceranae* prevents apoptosis in the epithelial cells of the infected ventricle. This may be a mechanism designed to improve the development of the parasite.

One of the most serious problems with this disease arises from the fact that it destroys the intestine of the bee. As a consequence of the damage to their epithelial cells, the bees necessarily suffer energy stress (Alaux *et al* 2010), since the microsporidia use their remaining mitochondrial organelles to absorb the ATP of the host cell environment (Williams 2009). For this reason, honey bees affected by *N. ceranae* consume more food to meet their increased energy demand (Alaux *et al* 2010); because they are hungrier they are also more reluctant to share food, thus reducing the connectivity of the network within the colony (Naug and Gibbs 2009). In addition, there is significantly less trehalose in the hemolymph of foraging honey bees infected with *N. ceranae* than in uninfected foragers. This affects the bee's flying ability and may cause it to abandon its social conditioning in order to meet its individual food needs, despite the social signals imposed by hives, such as the demand for nectar (Mayack and Naug 2010). This results in some heavily infected honey bees (generally foragers) not returning to the hive (Higes *et al* 2009^a), due to the potential of increased flying activity by infected bees to reduce the transmission of the pathogen within the hive (Dussaubat *et al* 2013^a).

Furthermore Antunez *et al* (2009) and Genersch (2010) report that infection by *N. ceranae* seems to suppress the immune response, by reducing the transcription of some genes that encode antimicrobial peptides and other immunity-related enzymes, in contrast to infection by *N. apis* where the immune system is quickly activated. A dramatic effect of microsporidia infection is the inhibition of genes involved in homeostasis and the renewal of intestinal tissues, confirmed at a histological level, which would explain the early death of the bees due to tissue degeneration and prevention of the renewal of the intestinal epithelium (Dussaubat *et al* 2012).

Antunez *et al* (2009) indicated that the expression of the antibacterial peptides abaecin, denfessin and hymenoptaecin increases four days after infection with *N. apis*. On the other hand, the infection caused by *N. ceranae* did not affect the expression of antibacterial peptides, despite the fact that the pathogen had already invaded the ventricular epithelium (Higes *et al* 2007). This suggests that *N. ceranae* partially suppresses the humoral and cellular defense mechanisms, thus increasing susceptibility to other bee pathogens and also anticipating senescence (Antunez *et al* 2009).

Alaux *et al* (2010) indicate that the interaction between infection with *N. ceranae* and exposure to pesticides causes a decrease in the secretion of antiseptics for the young, which affects their survival.

In addition to the damage caused to the bee intestine, there is stress due to lack of energy and the reduction in

immunological capacity, the picture is further complicated if the disease affects the queen. The queen bee is susceptible to most of the diseases that affect the colony (Higes *et al* 2009^c). Alaux *et al* (2011) studied this change in the behaviour of the pheromones of the queen, indicating that it significantly reduces her level of vitellogenin, an indicator of longevity and fertility. In addition, the queen's total antioxidant capacity and production of mandibular pheromone decreases, indicating that she cannot withstand the physiological stress caused by *N. ceranae* in the long term. Death occurs usually in the winter months when there is more contact between the sick workers and the queen, as compared to spring when there is a greater presence of uninfected young workers and more movement in the colony. Death of the queen occurred within three weeks in an experiment in which nurse honey bees were collectively infected with approximately 5,000 viable spores per bee (Higes *et al* 2009^c). Production of *N. ceranae* spores presents a linear increase from 12 to 20 days after inoculation, with growth rates of 8×10^6 spores per day (Huang and Solter 2013).

It has been shown that sub-lethal doses of insecticides may increase mortality in bees previously infected by *N. ceranae*, making the bees more susceptible to the insecticides (Vidau *et al* 2011).

Regarding the application of insecticide treatments and the exposure of honey bees to *N. ceranae*, both these stress factors cause a significant reduction in survival when compared to healthy bees with no exposure to fipronil, for example (Aufauvre *et al* 2014). The interaction of parasites and insecticides added to other stress factors may have negative synergistic effect on bee survival, contributing to increased colony loss (Aufauvre *et al* 2012, Retschnig *et al* 2014). A synergetic effect has also been observed on the mortality of honey bees co-exposed to spores of *Nosema spp.* and to imidacloprid (Alaux *et al* 2010). Likewise the combination of *N. ceranae* and fipronil has led to a synergetic effect on mortality in honey bees, independent of the order of exposure to the stress factors (Vidau *et al* 2011, Aufauvre *et al* 2012).

Pesticides can also act on the insects' immunological systems (Desneux *et al* 2007, Garrido *et al* 2013). For example, fungicides, acaricides, neonicotinoids and phenylpyrazoles alter the bee's immune response, affecting the regulation of immune genes and leading to significant over-expression of a chitinase-encoding gene in bees exposed to *N. ceranae* (Boncristiani *et al* 2012, Garrido *et al* 2013, Aufauvre *et al* 2014).

It has further been shown that co-infection with *Nosema* specifically alters the nutritional, metabolic and hormonal pathways, including the insulin signalling pathway. The latter is also linked to maturation behaviour in workers, affecting the foraging tasks of infected individuals, similar to the results found in workers fed with poor diets (Holt *et al* 2013). This shows the importance of nutrition in optimising immunity (Ponton *et al* 2011), since poor

nutritional states affect the immunological system (Holt *et al* 2013). For this reason early feeding of colonies infected with *Nosema* may help them to overcome the additional energy costs (Higes *et al* 2010^b).

CONTROL METHODS

When the bees cannot themselves control the attack of a parasite, it is necessary to find some alternative method to reduce the damage caused by the disease, such as pharmacological treatments. However, until more research on the biology and the transmission of *N. ceranae* becomes available, it is difficult to say whether the general recommendations for *N. apis* are relevant for the control of *N. ceranae* (e.g. wax replacement, fumigation of the comb with acetic acid) (Manzoor *et al* 2013). Currently, science is focused on discovering new drugs. Williams *et al* (2008^b) studied the use of Fumagillin for controlling *N. ceranae*, finding that the use of this drug in the autumn succeeds in reducing the intensity of the parasitic invasion in the following spring. Fumagillin treatment is therefore shown to be successful in temporarily reducing *N. ceranae* infection in colonies (Botias *et al* 2013). However, Williams *et al* (2008^b) raise the possibility that this treatment favors the replacement of *N. apis* by *N. ceranae*, since Fumagillin is more effective against the former.

Higes *et al* (2008) indicate that infection can be controlled by administering 120 mg of Fumagillin, but reinfection after 6 months cannot be avoided. It should further be noted that the use of Fumagillin is not yet permitted in the majority of the member states of the European Union due to the high concentration of drug residues in the honey (Nozal *et al* 2008, Porrini *et al* 2010). A medication needs to be found which does not contaminate the honey, and especially does not affect the health of consumers. In this situation, Porrini *et al* (2010) suggests the use of bacterial metabolites produced by bacteria such as *Bacillus* and *Enterococcus* isolated from the mid intestine of the honey bee and from its honey. One possibility is surfactin (a compound produced by *B. subtilis*), which significantly suppresses the parasite load and does not damage the health of the bee. There are also a number of metabolites produced by *Lactobacillus johnsonii* CRL 1647 (mainly organic acids), which have no toxic effects against honey bees, increase the hive population through the administration of lactic acids, and increase the amount of fatty bodies in the bee; this results in a reduction in pathogen intensity after the second application of organic acid treatment, as well as enhancing the effectiveness of Fumagillin (Maggi *et al* 2013). Nevertheless, further research is still required to determine the minimum and maximum concentrations to avoid leaving residues in the honey that are harmful to human health (Porrini *et al* 2010). Furthermore, the possibility of sexual reproduction by these microsporidia species has profound implications for the evolution of

their virulence, if they broaden their range of hosts and acquire resistance to the drugs through recombination of their genes (Ironsides 2013).

Another way of controlling disease is through hive management with the aim of creating unfavorable environmental conditions for the parasite. Following this principle Williams *et al* (2010) studied the effects of the environment on *N. ceranae* by hibernating a group of hives in controlled conditions as compared to a group in the open air. They concluded that treatment by hibernation does not have a significant effect on the attack intensity *N. ceranae* in the following spring. Huang *et al* (2012) suggest that it may be possible to select bees which are tolerant to *Nosema*, and in particular *N. ceranae*; this line of research might contribute to the management and control of this parasite. Furthermore, replacing the queen is vital for maintaining the homeostasis of a *Nosema* infected colony; it results in a notable reduction in *Nosema* infection rates, comparable with that induced by treatment with Fumagillin (Botias *et al* 2012). At present the only recorded treatment for *Nosema* disease is Fumagillin, which is banned for using in Europe since the maximum residue level has not yet been determined. As a result, prolonged use leads to resistance to the treatment of this disease (Huang *et al* 2013).

It has been observed that vetiver oil, thymol, resveratrol and lysozyme oil have been considered suitable for treating nosemosis in bees, without causing toxic effects in adults or presenting anti-feeding properties. Bees fed with thymol candy show a progressive decrease in infection levels 19 days after infection, with 6% lower levels after 13 days and a fall of 68% between 19 and 25 days (Maistrello *et al* 2008). Therefore, thymol and resveratrol have potential for the development of alternative strategies for nosemosis control, resulting also in significantly longer-lived honey bees (Maistrello *et al* 2008, Costa *et al* 2010). In the case of thymol the longer survival may be related with the lower spore load, while in the case of resveratrol the increased survival may be explained by the antioxidant properties of the substance (Costa *et al* 2010).

Furthermore, because of the risk that the disease may develop chemical resistance to treatment, new, alternative control methods have been created such as the use of natural phytopharmacological preparations. One example of these is BeeCleanse, a natural preparation containing different herbs, vitamins, minerals and essential acids; its use did not cure the disease but caused a considerable reduction in the number of spores (Tlak *et al* 2013). Good results have also been observed with a preparation of natural herbs called Nozevit (a natural extract from a particular type of *Nothofagus* bark, recognised as a rich tannin source). It induces the production and secretion of mucus by the honey bees' epithelial layer, which covers the peritrophic membrane to form a more resistant coating ensuring protection and resistance against a new invasion by *Nosema* sp. spores. It also prevents spores from germinating (Tlak *et al* 2011). Other studies have assessed

the activity of plant extracts in the development of *N. ceranae*, such as ethanol extracts obtained from *Artemisia absinthium*, *Allium sativum*, *Laurus nobilis* and *Ilex paraguariensis*. They show that a 1% concentration of extract of *L. nobilis* significantly hindered the development of *N. ceranae*, suggesting that the use of natural substances may be put forward as alternative anti-parasite treatment (Porrini *et al* 2011).

Epidemiological information on *Nosema* will help to improve disease management practices, implementing new hygiene policies and reducing additional production costs (Razmaraii *et al* 2013). Since the treatment for *Nosema* is not available in many countries, there is an urgent need to develop possible treatments or beekeeping techniques to combat the rapid spread of this dangerous emerging disease (Botias *et al* 2013), helping to improve the natural defense behaviour of the colony through beekeeping practices and reducing the likelihood of colony loss (Dussaubat *et al* 2013^a).

CONCLUSIONS

N. ceranae is a disease that is already causing concern in the scientific world, and is considered to be a threat for the conservation of honey bee populations and the beekeeping industry. Studies have therefore focused on the recognition and description of this parasite, since changes in its feeding habits are affecting not only oriental bees, but also European honey bees and several species of bumblebee. Further studies are needed on the effects of infection in order to provide beekeepers with proper control strategies and combat the rapid spread of this dangerous disease.

The high virulence of this disease, coupled with the weak individual immunity of *A. mellifera*, makes it necessary to give more importance to genetic improvements, with the aim of improving features of collective behaviour. The parasite may very often be present in a hive without causing symptoms of the disease, probably because of the resistance of the bees or the presence of a less virulent strain of the parasite; the latter may result from its high levels of polymorphism and the wide variety of haplotypes in each colony. This would account for the differences reported in parasitism, the symptoms reported in the field and its resistance to treatment.

It must also be considered that when a colony is weakened by *N. ceranae*, it becomes susceptible to other stress factors such as pesticides, other pathogens and malnutrition. The negative synergic effects of these factors significantly reduce bee survival, affecting the hive's immunological system and contributing to the loss of more colonies.

Further investigation is needed on ways of controlling this disease to develop drugs that do not harm the health of either bees or consumers, and do not leave residues in the honey. Possible solutions include the use of bacterial metabolites or oils such as vetiver, thymol or resveratrol; and the use of natural phyto-pharmaceutical preparations

based on herbs, vitamins, tannins and essential fatty acids. These have been considered good alternatives for treating nosemosis.

Furthermore, *N. ceranae* is already present in all the world's continents, and particularly in Chile, implying that this disease is a serious threat. More information is required on its real impact and effect on our bees and commercial farms.

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