

Nosemosis Prevention and Control

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Abstract: Nosemosis is a serious microsporidian disease of adult European honey bees caused by the spore-forming unicellular fungi *Nosema apis* and *Nosema ceranae*. In this paper we describe the currently known techniques for nosemosis prevention and control including Good Beekeeping Practices (GBPs) and biosecurity measures (BMBs). Topics such as queen renewal, nosema-resistant bees and hygienic and control methods are described. Strong efforts are currently provided to find more a sustainable solution than the use of antibiotics. So far, it seems that the best way to approach nosemosis is given by an “integrated pest management strategy”, which foresees the contemporary application of different, specific GBPs and BMBs.

Keywords: nosemosis; prevention; control; Good Beekeeping Practices; biosecurity measures



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1. Introduction

Nosema spp. are microsporidia responsible for nosemosis of honey bees (*Apis mellifera* L. and *Apis cerana* F.). These pathogens are obligate parasites of the epithelial cells of the ventriculus of adult honey bees [1,2] and are spread throughout the world [3,4]. Infection of the bees occurs by the oral ingestion of spore-contaminated honey, pollen or water [5–8]. Other ways of transmission of the spores may be by trophallaxis [9,10], foraging activities on the flowers or cleaning tasks in the colonies [11]. The spores are released in large quantities with the faeces of diseased bees and can be infective for more than one year [12]. So far, three *Nosema* species have been identified to infect honey bees: *N. apis* [13], *N. ceranae* [1] and *N. neumannii* [14].

N. ceranae is a microsporidium firstly isolated in the Asian bee *Apis cerana*, and then found in *Apis mellifera* [3,15]. It is currently endemic worldwide [16], as well as the other well-known *Nosema* species: *N. apis* [13]. However, in some regions, usually with colder climates, *N. apis* is still prevalent. *N. apis* has a low prevalence in Southern Europe [16–19] while *N. ceranae* is widely present in Southern Europe, specifically in areas with a temperate-warm climate [3,20]. *N. ceranae* infection has important differences with *N. apis* control due to the microsporidium biology and because *N. ceranae* tends to persist throughout the year [3].

N. apis is responsible for clinical signs of nosemosis type A. Such signs include a high mortality of adult bees and the presence of fecal spotting at the hive entrance due to digestive disorders in adult bees. *N. ceranae* is responsible for clinical signs of nosemosis type C

that are represented by evident loss of adult population, decrease in honey production and final collapse [7,21]. The absence of a clear symptomatology of *N. ceranae* has complicated its distinction from *N. apis* in Europe [22].

Nosema ceranae has been frequently related to colony losses [7,23,24], even with the possible contribution of trypanosomatids such as *Crithidia mellificae* and *Lotmaria passim* [25–28]. *N. ceranae* presence in association with other pathogens such as viruses [29–31] or varroa [32] and contaminants [33,34] has been demonstrated to increase honey bee colony losses.

Recently another *Nosema* species was identified in Uganda (Africa): *N. neumannii* [35]. The distribution and pathogenicity of this microsporidium is still under investigation.

This paper aims to give an overview of the main strategies for the prevention and control of nosemosis in order to prevent the previously described damages.

2. Prevention Methods: Good Beekeeping Practices and Biosecurity Measures

Good Beekeeping Practices (GBPs) are those integrative activities that beekeepers apply for on-apiary production to attain optimal health for humans, honey bees and the environment [36]. Biosecurity Measures in Beekeeping (BMBs) are all those operational activities implemented by the beekeepers to reduce the risk of introduction and spread of specific honey bee disease agents [37].

The application of the GBPs together with the BMBs, therefore, have a positive effect on colony health and on society in general and at the same time guarantee high production standards [38]. These specific actions should be implemented by beekeepers at the apiary level, with the support of veterinarians or other specialized practitioners, when needed.

Starting from a previous study of the BPRACTICES consortium, a GBPs and BMBs list for Nosemosis prevention has been identified, taking into consideration the OIE and FAO documentation [38] and similar previous works [39].

The list of the good practices and measures to adopt for *Nosema* spp. can be found in Table 1 and in Boxes 1–3.

Table 1. Good Beekeeping Practices (GBPs) and Biosafety Measures in Beekeeping (BMBs) for *Nosema* spp.

Buy queens from breeders with stocks free of <i>Nosema</i> spp.
Prevent pollution of artificial water sources with faeces or drowned or dead bees.
Remove and destroy combs with signs of dysentery.
Take samples of forager honey bees (or powder sugar or hive debris) early in autumn or spring for laboratory analysis to diagnose Nosemosis (microscopic examination and PCR).
Adopt a proper pathogen (e.g., <i>V. destructor</i>) control, to ensure a proper balance in the composition of the bee colony (equilibrium of nurse-forager bees).
Treat the colony if percentages of infected bees are higher than 40%, if there are any registered/permitted products in your country against Nosema.
Strengthen and stimulate the colonies in autumn and spring with the administration of stimulant integrators or feed supplements.
Do not reuse combs originating from depopulated or collapsed hives,
Select and breed Nosema-resistant honey bees
Strengthen and stimulate the colonies in autumn and spring with the administration of stimulant integrators composed by vegetal substances/molasses or vitamin integrators.
Disinfect beekeeping tools and equipment between uses: torching (<i>Nosema ceranae</i> spores are inactivated over 60 °C); gamma irradiation; fumigation of combs with glacial acetic acid, sodium hydroxide 5% (caustic soda); sodium hypochlorite 0.5% (bleach) and ammonium hydroxide 1.65% (ammonia solution).

Table 1. *Cont.*

Do not feed extracted honey or combs with stores (honey or pollen) from <i>Nosema</i> -infested colonies to healthy colonies.
Replace the queens every two years, minimum, except those of high genetic value.
Have only healthy, strong colonies in the apiary,
Keep colonies of new introduction separate from the existing stock for an appropriate period (at least 1 month) to monitor them for diseases and infestations in order to prevent transmission of diseases.
Wintering; perform beehive box maintenance (replace parts or painting and verify the integrity of hive boxes, if needed).
Clean or disinfect (in case of infectious diseases) the hive box before installing new colonies.
Remove beehives with dead colonies as soon as possible.
Renew 30% of the hive combs every year.
Keep all documents/certificates about the commercial feed used.
Ensure the bees access to safe water sources.
Control varroa infestation levels.
Reduce bee stress (e.g., avoiding unnecessary winter inspections of the hives; limiting the use of the smoker; feeding properly the bees, etc.).

Box 1. Queen renewal.

A very effective method to control *N. ceranae* in field conditions is the queen's replacement, as demonstrated by [40]. Indeed, this biotechnical management is a basic apicultural practice because of the crucial role of the queen in the renewal of the bee population and consequently to replace the bees lost due to the infection, keeping the colony homeostasis. Young queens have a greater egg laying potential, and they produce a higher proportion of uninfected newly hatched bees to compensate for adult bee losses.

To prove it, the authors developed a field study to determine the effect of induced queen replacement on *Nosema* infections in honey bee colonies, focusing on the measurement of colony strength and honey production. Queen replacement resulted in a remarkable decrease in the rates of *Nosema* infection, comparable with that induced by fumagillin treatment. Similar observations were made also by [41], who observed that the infection consequences worsen when the age of the queen increases

Box 2. *Nosema*-resistant bees.

In Denmark, honey bee colonies have been selected for the absence of *Nosema* infections for decades [42]. This breeding scheme resulted in a honey bee strain in which *Nosema* infections are rarely found, and individual adult bees showed a high tolerance towards experimental *N. ceranae* infections [43]. More recently, regions that seem to be related with resistance to *Nosema* were identified. That resistance has been observed due to the capacity of reducing the energetic stress and the better capacity of tissue regeneration in resistant bees and avoiding the reduction of apoptosis caused by *Nosema* [42,44,45].

Box 3. Nosema spore disinfection.

Different disinfection strategies have been evaluated and applied to reduce the viability and potential infectivity of microsporidian parasites, as spores can remain viable for long time periods. In general, microsporidians can be killed by boiling spores for at least 5 min or applying disinfectants including quaternary ammonium, 70% ethanol, formaldehyde, phenolic derivatives, hydrogen peroxide, chloramines, sodium hydroxide or amphoteric surfactants [44]. In relation to honey bee microsporidian pathogens, differences on thermotolerance between species have been reported. While *N. ceranae* spores can survive at 60 °C for 6 h [45–47], the same temperature kills the spores of *N. apis* in 5 min [48].

Surfaces contaminated with *N. ceranae* spores can be effectively disinfected using any of the following products: (i) an ammonia solution, (ii) a sodium hypochlorite solution or (iii) a ready-to-use commercial alcohol-based solution (Mycoplasma OFFTM); therefore, these products are proposed for the disinfection of beekeeping equipment. The usefulness of other products, such as acetic acid, which causes spore destruction, requires further research.

3. Control Methods

The availability of control methods specific for nosemosis and the awareness about them may prevent a reduction in vitality or losses of affected colonies [21,24,49–52].

So far, there is a lack of knowledge regarding control methods for nosemosis. Here we classify the *Nosema* control strategies in antibiotic treatments, organic control methods, (phytotherapeutics, essential oils, bacteria metabolite and organic acids) and RNA-based technologies.

3.1. Antibiotic Treatments

Given that honey bees are food-producing animals [53,54], the use of antibiotic treatments in beekeeping should consider the impact on the hive products, mainly in terms of residues [54].

Nosemosis control with antibiotics has been mainly based on fumagillin administration. Depending on the geographical location and colony conditions (e.g., weather, stress, strength, etc.), it is advised to treat infested colonies from once (in autumn during feeding) to twice a year (in autumn and in spring, in the case of severe infections) [55–58]. While the autumn treatment aims to keep the colony alive during the cold season, the spring treatment is focused on improving the health status of adult bees that will be able to properly take care of the next generation of bees that are raised in spring.

While efficacy of fumagillin against *N. apis* has long since been proven [59], recent evidence indicates that fumagillin is effective against *N. ceranae* in western honey bees too [60]. Nevertheless, the use of fumagillin in heavily infected colonies with *N. ceranae* did not improve the size or increase the survival rate of colonies during winter, regardless of the dose or administration strategy adopted [61,62]. Moreover, the study carried out by Li et al. (2017) suggested that the elimination of gut bacteria by an antibiotic treatment weakened the immune function and made honey bees more susceptible to *Nosema* infection.

Treating colonies with 120 mg/colony of fumagillin in four applications (total amount of syrup 250 mL, each application 62.5 mL) was effective against depopulation and colony death, although relapses were detectable 4 months after treatment ended [7]. The total amount of active fumagillin ingested by a bee is the key to effectiveness. Administration of fumagillin with sucrose syrup gave better results than with medicated patties, which were not entirely consumed by the bees during field trials [7].

The use of fumagillin is not authorized in Europe due to the lack of a maximum residue limit definition in honey and of a registered veterinary medicine with this active substance. Its use in the EU may be admitted only under exceptional circumstances. Fumagillin is registered for use in Canada to treat *Nosema* disease. It is available in the commercial product as a salt, dicyclohexylamine (DCH). This chemical contaminant is a potential hazard for human health, as it is five times more toxic than fumagillin according to studies conducted on rats, and it is a genotoxic and oncogenic compound [61]. DCH is significantly more resistant to degradation in honey than fumagillin. Observed half-lives for DCH

ranged from a minimum of 368 days, when stored at 34 °C in darkness, to a maximum of 852 days, when stored at 21 °C in darkness. A maximum half-life of 246 days was observed for fumagillin in samples kept in darkness at a temperature of 21 °C, while the observed half-life of fumagillin was estimated to be 3 days when exposed to light at 21 °C, and complete degradation was observed after 30 days under the same conditions. The stability of DCH, combined with its toxicity, make it an important hazard to be considered regarding hive products for human consumption safety [62].

The effectiveness of commercial product Fumagilin B® showed to be influenced by several factors, such as storage conditions (e.g., temperature and RH), dosage, dilution and UV exposure. High temperatures inside the hive can drastically reduce the initial concentration of fumagillin within a few hours.

Moreover, the effect of low fumagillin concentrations were tested. At lower fumagillin concentrations, significantly more pathogenic spores of *N. ceranae* were produced in treated bees than in untreated infected bees. Protein profiles of bees fed with fumagillin confirmed the hypothesis that fumagillin affects bee physiology when administered at concentrations lower than those that are effective against *N. ceranae*. In the case of mixed infections, the prevalence of *N. ceranae* may increase due to the use of fumagillin, replacing *N. apis*, which is more sensitive to the treatment [63].

A novel mass spectrometry allows the determination of traces of fumagillin and its degradation products in honey [7].

As an alternative to fumagillin, in vitro tests performed on the lepidopteran cell line showed that tinidazole and metronidazole can completely inhibit *N. ceranae* infection and were as effective as fumagillin. However, both substances cannot be used for the control of *Nosema* spp., as they belong to the active ingredients not allowed in the EU (Reg. 37/2010). The use of nitroimidazoles in dairy animals is prohibited in many countries [64].

3.2. Organic Control Methods

In Europe, we consider as organic methods the veterinary treatments allowed for organic beekeeping production [65]. A veterinary treatment is defined as “all courses of a curative or preventive treatment against one occurrence of a specific disease” [66].

Organic control methods can be identified with phytotherapeutics, organic acids, essential oils, polysaccharides, bacteria and metabolites. Their frequent common advantages are: their availability in many countries; the low risk for consumers to contaminate bee products; their low toxicity for the environment and the absence of demonstrated resistance of nosema. As a possible disadvantage, they could demonstrate high variability in reducing the infection levels of nosema in bees [21].

3.2.1. Phytotherapeutics

Other natural compounds have been tested with promising results in laboratory conditions. Among these are thymol showing a *Nosema*-inhibiting effect and thymol and resveratrol showing a positive impact in increasing bee longevity [67].

Herbal supplements (with or without C vitamin), have shown to reduce the *N. ceranae* infection levels in affected honey bee colonies enhancing their strength [68] and reducing the winter mortality [69]. Feeds containing *Brassica nigra* and *Eruca sativa*, with different amounts of glucosinolates (GSLs), reduced the *N. ceranae* infection [70]. Additionally, *Agaricus blazei* extract [71], *Andrographis paniculate*, Asteraceae (*Artemisia dubia*, *Aster scaber*, *Helianthus annuus*) and *Eleuthereococcus senticosus* may have a positive effect against nose-mosis [69].

Piperine (an alkaloid in the roots of the Piperaceae family) and curcumin (a natural phenol produced by *Curcuma longa*) are potential candidates regarding antinosemosis therapy too, being able to increase the activity of the antioxidant system in honey bees [69].

Another product derived from plants that have demonstrated activity against *N. ceranae* infection is propolis. This product administrated to bees before or after the *Nosema* infection reduced significantly mortality, infectivity and infection rates [72].

The control of *Nosema* has also been focused on causing limitations to the gut cell invasion. That is the objective of the phyto-pharmacological preparation of Nozevit, a preparation that includes plant polyphenols, vitamins, minerals and amino acids. Histological studies showed that Nozevit induces the production of mucous from the epithelial layer of treated bees and provides an additional effect of coating the peritrophic membrane to form a firmer envelope that ensures protection from new invasions of *Nosema* spores [73]. Similar data had been previously observed in field experiments by the reduction of bee count spores [74] and by the reduction in the number of infected house and forager worker honey bees [52], although with lower efficacies than in the fumagillin control hives.

Other tested products such as Nosestat (a.i. Iodine 4 g.—Formic Acid 5 g. in 100 mL. of product), Phenyl salicylate or Vitafeed Gold (extract of *Beta vulgaris*) gave good results, highly related with low consumption of the different doses by bees in field conditions [21]. Concerning the essential oils, Porrini [75] and Damiani [76] observed significant antiparasitic activity of *Laurus nobilis* alcoholic extract, similarly, and found that different extracts of the same plant inhibited *N. ceranae* spore development, having the best results with ethanol extracts. More recently, *Origanum vulgare* and *Rosmarinus officinalis* alcoholic extracts (0.7% g/g, volatile oils) reduced the number of spores after three consecutive treatments without being related with bee mortality [77]. Bravo [78] found the essential oils of *Cryptocarya alba* to be effective in controlling *N. ceranae* development in vitro.

3.2.2. Bacteria and Their Metabolite

Acetobacteraceae are able to suppress the development of *N. ceranae* reducing the spore load [69].

Porrini [79] obtained good results with a bacterial metabolite and, specifically, surfactin, both in reduction of infectivity and in parasitosis development.

3.2.3. Organic Acids

Maggi [80] evaluated the effects of organic acids produced by *Lactobacillus johnsonii* CRL1647 (lactic acid, phenyl-lactic acid and acetic acid). A reduction on *Nosema* intensity was observed after two treatments, as well as the enhancement of fumagillin efficiency. No toxic effects were found in vitro, observing an increase of the beehive population and in the size of fat bodies in the bees. It is a clear example of the possibilities of natural new molecules on *Nosema* control.

Nanetti [81] tested the use of oxalic acid to control *N. ceranae* both in laboratory and in field conditions. They found that those oral applications interfered with the increase of artificial infections, and that two topical administrations in field conditions decreased the prevalence in the colony, finding a significant difference with untreated colonies and concluding that oxalic acid is a valid substance to be used to control *N. ceranae* infections.

3.2.4. Polysaccharides

The β -glucans (glucose homopolymers) are known for their immune-modulating impact on different species, including honey bees. The polysaccharide chitosan stimulates the bee immune system, leading to a decrease in the degree of infection with *N. apis* and to increased bee survival [69].

3.3. Other Control Methods: RNA Interference (RNAi)

The oral application of double-stranded RNA (dsRNA) in *N. ceranae* infected bees can activate the immune response, suppress the reproduction of *N. ceranae* and improve honey bees' health status [82]. The results obtained from the use of RNAi technology demonstrated the prospects of its applications in anti-nosemosis therapy, but more research is needed in order to be widely implemented in beekeeping practice [69].

4. Conclusions

Many different strategies exist to manage nosemosis in honey bees. Good Beekeeping Practices and biosecurity measures provide tools to prevent and control the disease, avoiding its spread among colonies in infected apiaries and also among different apiaries in the same region. Selection of resistant honey bee stock has a great potential among the sustainable solutions.

The use of antibiotic fumagillin is forbidden in some countries and should be passed in favour of more sustainable beekeeping. Its residues in honey bee products pose a serious threat to consumer's health. Many different plant extracts exhibit antinosemosis properties, but their efficacy is low. So far, it seems that the most effective and sustainable solution to prevent and control nosemosis is to adopt different measures (GBPs, BMBs), and combine them in a so called "integrated pest management" strategy. However, further studies must be conducted to determine which methods should be combined and also to determine how and when adopt them to reach the highest possible efficacy.

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