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Review Article

Current Insight into Nosema Disease of Honeybees and Their Future Prospective

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ABSTRACT

Honeybees (*Apis mellifera*) play a crucial role in global agriculture and ecosystem stability through pollination services. However, their populations have faced numerous threats in recent years, with Nosema disease emerging as a significant concern. Nosema is caused by microsporidian parasites of the genus *Nosema*, affects the digestive tracts of honeybees, leading to compromised health and reduced foraging efficiency. This literature review comprehensively examines the current knowledge regarding Nosema disease in honeybee populations, encompassing research from diverse fields, including entomology, microbiology, and ecology. The review begins with an overview of honeybee importance and Nosema disease's ecological and economic consequences. It subsequently delves into the etiology of *Nosema* species highlighting the various factors contributing to infection dynamics. By synthesizing a wide range of research studies, we explore the diagnostic methods, and management strategies currently employed in combating Nosema disease. Furthermore, we discuss the implications of Nosema infection on honeybee colony health, emphasizing its role in colony collapse and the intricate interactions between Nosema and other stressors, such as pesticides and pathogens. As we peer into the future, this review contemplates the potential consequences of climate change and environmental stressors on Nosema disease prevalence and distribution. We also consider the advancements in molecular techniques and genetic research that hold promise for more targeted and effective interventions. Additionally, we discuss the importance of sustaining honeybee populations for agriculture and biodiversity conservation and highlight the need for further research into Nosema disease. In conclusion, this literature review underscores the importance of understanding Nosema disease in honeybee populations. It highlights the multifaceted nature of this threat, its current status, and the promising directions for future research and management strategies. As we strive to safeguard honeybee health and ecosystem services, a comprehensive understanding of Nosema disease remains pivotal for ensuring the sustainability of both honeybees and the ecosystems they support.

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INTRODUCTION

The Western honeybee holds immense economic, agricultural, and environmental significance. Over the past decade, certain parts of the world have experienced a notable decline in honeybee colonies (Hristov *et al*., 2020). Which may be due to the Nosema disease one of the key factor associated with decline in honeybee populations worldwide (Hristov *et al*., 2020), which has recently garnered increasing attention from scientists, beekeepers, and policymakers (Chantawannakul *et al*., 2016). This parasitic infection, caused by microsporidian pathogens of the Nosema (Burnham, 2019), has posed significant challenges to beekeeping and agricultural ecosystems, leading to colony losses and disruptions in pollination services critical for food production (Hong *et al*., 2011; Alaux *et al*., 2010). In this era of heightened environmental awareness and concerns about pollinator health (Maderson, 2023), a comprehensive understanding of Nosema disease and its impact on honeybee colonies

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has become paramount (Galajda *et al*., 2021). It is attributed to two variants of Microsporidia: *Nosema apis* and *Nosema ceranae*. This intestinal condition, stemming from these microorganisms, significantly contributes to the decline and depletion of bee colonies (Galajda *et al*., 2021). Infection by *N. ceranae* proved highly harmful to honeybee colonies, leading to substantial declines in colony population, brood development, and honey yield (Botías *et al*., 2013). These adverse impacts on the colony scale could impact the economic viability of beekeeping and pose significant threats to pollination efforts (Botías *et al*., 2013). Furthermore, it offers a glimpse into the future prospects of mitigating this threat, emphasizing the importance of continued scientific inquiry, innovative solutions, and collaborative efforts to safeguard honeybees' well-being and their vital role in sustaining our ecosystems and food security (Goblirsch, 2018).

Understanding the current state of knowledge regarding Nosema disease, its causes, transmission, and management, as well as exploring potential prospects such as novel treatment strategies, ecological impacts, and the role of climate change, is essential for informing conservation efforts, sustainable beekeeping practices, and the development of strategies to mitigate the ongoing decline of honeybee populations and ensure the stability of our ecosystems. Thus, this study was designed to comprehensively explore the current understanding of Nosema disease in honeybee populations and to outline prospects for research, management, and mitigation of this significant threat to bee health and pollination ecosystems.

ETIOLOGY OF NOSEMOSIS

The etiology of Nosemosis, a significant disease affecting honeybees, is a complex and multifaceted topic that has garnered substantial attention from researchers seeking to understand the underlying causes of this ailment (Crane, 2009). Nosemosis is primarily attributed to two microsporidian parasites: *N. apis* and *N. ceranae* (Ostroverkhova *et al*., 2020). These intracellular pathogens infiltrate the honeybee's midgut and disrupt its normal functioning, ultimately leading to detrimental effects on the individual bee and, by extension, the entire colony (Rouzé *et al*., 2019). Etiological investigations into Nosemosis have revealed a range of factors that contribute to the onset and severity of the disease, encompassing both the parasites themselves and the environmental conditions that facilitate their proliferation.

N. ceranae is recognized as the most aggressive species and holds a dominant position globally. Nevertheless, in certain areas, typically those characterized by colder climates, *N. apis* maintains its presence (Naudi *et al*., 2021). This shift in understanding has raised questions about the dynamics between these two pathogens and their respective contributions to the disease's prevalence and impact (Klee *et al*., 2007). Molecular studies have been instrumental in elucidating these microsporidians' genetic makeup and mechanisms, shedding light on their virulence factors and life cycles within the bee host (Chen *et al*., 2009).

Persistent infections can significantly impact an individual's physiology, behavior, overall health, and lifespan, and they could change the structure and population dynamics of social groups (Holt *et al*., 2013). Moreover, when honeybee colonies are exposed to suboptimal conditions, such as poor nutrition, pesticides, and habitat degradation, they are more susceptible to Nosemosis (Pettis *et al*., 2012; Branchiccela *et al*., 2019). Additionally, factors like temperature and humidity can influence the prevalence and intensity of Nosema infections within colonies (Punko *et al*., 2021; Chen *et al*., 2012). With its potential to alter environmental conditions, climate change has raised concerns about the future epidemiology of Nosemosis and its impact on honeybee populations (de Jongh *et al*., 2022).

Understanding the etiology of Nosemosis is further complicated by the intricate interplay between the parasites, honeybee physiology, and the bee's immune response (El-Khoury *et al*., 2018). Research has shown that *Nosema* spp. can suppress the bee's immune system, making it more susceptible to other pathogens and stressors (El-Seedi *et al*., 2022a). This immunosuppressive effect exacerbates infected bees' overall health challenges, often leading to a downward spiral in colony health.

Moreover, the transmission dynamics of Nosema within colonies and between colonies are areas of active investigation (Pinilla-Gallego *et al*., 2020). The mechanisms by which the parasites spread among individual bees and colonies have significant implications for disease management and control strategies (Formato *et al*., 2022). Nosema species are often linked to elevated defecation and transmission through a fecal-oral route. However, since *N. ceranae* does not trigger defecation, it could potentially be transmitted through an oral-oral route instead (Smith, 2012). Researchers also have explored various other aspects of transmission, including the role of contaminated pollen (Higes *et al*., 2008), and contact between infected and uninfected bees (Sulborska *et al*., 2019).

DETECTION AND DIAGNOSIS

Detection and diagnosis of Nosemosis, a prevalent and economically significant disease affecting honeybee populations, has garnered substantial attention in recent years (Mazur and Gajda, 2022). With honeybees playing a pivotal role in pollinating numerous crops and sustaining ecosystems, the timely and accurate identification of *Nosema* spp. infections are crucial for effective disease management and the preservation of bee health (Botías *et al*., 2013). This complex task involves a range of methodologies, each with advantages and limitations (Table I).

Microscopic examination

Traditionally, microscopy has been a fundamental tool for detecting Nosema infections. Using a hemocytometer or similar apparatus, spore counting allows for quantifying Nosema spores within bee gut tissue (Fries *et al*., 2013). Microscopic examination also identifies spore morphology and sporoplasm characteristics, aiding in distinguishing between *N. apis* and *N. ceranae*. However, this method can be labor-intensive, require skilled personnel, and may yield false negatives if spore loads are low or the spores are not evenly distributed within the bee. Moreover, it does not provide information on the viability of the spores (Gajger *et al*., 2010).

Molecular techniques

In recent years, molecular methods have gained prominence in detecting and diagnosing Nosemosis (Ansari *et al*., 2017). Polymerase chain reaction (PCR) assays targeting specific genetic markers of *Nosema* spp. offer high sensitivity and specificity. These assays can detect the presence of Nosema DNA in bee samples, even at low spore loads (Rivière *et al*., 2013). Moreover, quantitative PCR (qPCR) allows for the precise quantification of spore levels, aiding in monitoring and assessing infection severity (Vejnovic *et al*., 2018). Furthermore, UR-qPCR exhibited greater sensitivity in detecting two copies of *N. ceranae* DNA and 24 spores per bee than microscopic enumeration. Meanwhile, microscopy had a limit of detection of 2.40×10^4 spores per bee, with a consistent detection level of $\geq 2.40 \times 10^5$ spores per bee (Truong *et al*., 2021). Additionally, DNA sequencing techniques can provide valuable insights into the genetic diversity and strain-specific variations of *Nosema* spp. within bee populations (Maside *et al*., 2015). Molecular techniques are less dependent on observer subjectivity and can overcome the limitations of microscopy in terms of sensitivity.

Serological assays

Enzyme-linked immunosorbent assays (ELISAs) and other serological tests have also been developed for Nosema detection (Aronstein *et al*., 2013). These assays rely on binding specific antibodies to Nosema antigens, offering a rapid and less labor-intensive approach compared to microscopy. However, their sensitivity can vary, and they may need to be more accurate in distinguishing between different Nosema species or strains.

Metabolomics and biomarkers

Metabolomics, a relatively novel approach, involves the study of small-molecule metabolites in biological samples (Rinschen *et al*., 2019). This technique has shown promise in detecting Nosemosis by identifying specific metabolite changes associated with Nosema infection (Broadrup *et al*., 2019). Biomarkers, such as altered nutritional levels or metabolic pathways, can be indicative of disease presence (Grozinger and Robinson, 2015). While, the exploration of metabolomics-based biomarker discovery shows great promise in enhancing bee health monitoring and identifying stressors related to energy intake and various environmental challenges (Wang *et al*., 2019).

Table I. Diagnostic techniques for *Nosema* species.

Technique used	Advantages	Disadvantages	References
Microscopy	Gold standard method, simple, easy, and fast	No species determination	Dghim *et al*., 2021
Molecular techniques			
PCR	Sensitivity, species determination	Facility development, expensive	Fries *et al*., 2013
PCR-RFLP	Species determination, fast, precise	Expensive equipment needed	Tapaszti *et al*., 2009; Bourgeois *et al*., 2010
qPCR	Efficient, time-saving, sensitive	Expensive	Hamiduzzaman *et al*., 2010
UR-qPCR	Highly sensitive time-saving	Expensive, equipment needed	Truong *et al*., 2021
Serological techniques			
ELISA	Less expensive, rapid, faster	Labour intensive, expensive to prepare antibodies	Aronstein *et al*., 2013; Sakamoto *et al*., 2018

Imaging technologies

Advancements in imaging technologies, offer non-invasive and high-throughput methods for assessing bee health and detecting Nosema infections. The approach delves into deep learning and transfer learning techniques. Various methods were explored, including utilizing a convolutional neural network (CNN) classifier and applying transfer learning with models like AlexNet, VGG-16, and VGG-19 (Dghim *et al*., 2021). These models were fine-tuned and employed to discern Nosema images from other objects within sub-images. The highest level of accuracy, amounting to 96.25%, was achieved using the pre-trained VGG-16 neural network (Dghim *et al*., 2021). Moreover, the advantages and disadvantages are summarized in Table I.

IMPACT ON COLONY HEALTH AND COLONY LOSSES

First and foremost, Nosemosis poses a direct and insidious threat to the health of individual honeybees (Mazur and Gajda, 2022). Infection typically occurs through ingesting Nosema spores, which subsequently invade the midgut epithelium of the bees (Goblirsch, 2018). Within the host, these pathogens disrupt the normal functioning of the midgut, impairing nutrient absorption and leading to a range of adverse effects (Macías-Macías *et al*., 2020), as indicated in Figure 1. Infected bees often exhibit reduced longevity, diminished foraging capabilities, and impaired immune responses (Macías-Macías *et al*., 2020). Consequently, weakened individual bees are more susceptible to other stressors, such as pesticides and pathogens, amplifying the overall vulnerability of the colony (Alaux *et al*., 2010).

The consequences of Nosemosis extend beyond individual bees, with colony-level impacts that are equally concerning (Oldroyd, 2007). One of the most striking manifestations of Nosema infection is a phenomenon known as spring dwindling, wherein colonies infected with Nosema experience a significant reduction in population size and foraging activity during the critical early spring period (Oldroyd, 2007). This reduced workforce can compromise the colony's ability to exploit available resources, particularly during the crucial brood-rearing period, ultimately leading to weaker colonies and lower honey production (Botías *et al*., 2013). Furthermore, Nosemosis can disrupt the division of labor within colonies, diminishing the efficiency of tasks such as nursing, foraging, and hive maintenance (Fig. 1), which are vital for the colony's survival and productivity (Goblirsch *et al*., 2013).

Colony losses resulting from Nosemosis are a growing concern for beekeepers and the agricultural sector (Formato *et al*., 2022). Overwintering colonies are particularly susceptible to Nosema infection, as the stressors associated with colder temperatures and limited forage exacerbate the adverse effects of the disease (Punko *et al*., 2021). The increased mortality and weakened colonies due to Nosemosis contribute to the overall colony losses observed in recent years, adding to the multifactorial challenges honeybee populations face, including habitat loss, pesticide exposure, and climate change (Hristov *et al*., 2020).

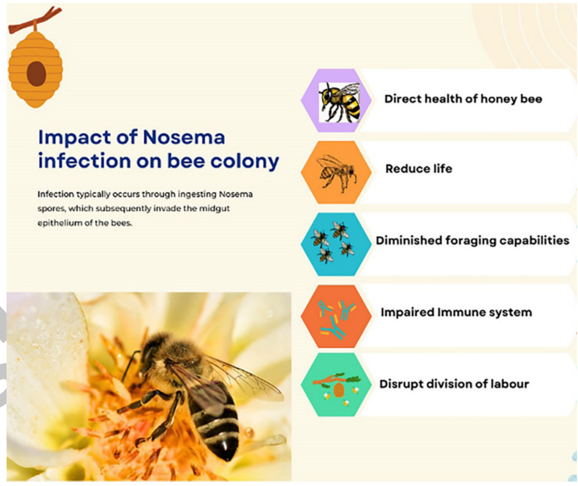

Fig. 1. Impact of Nosema infection on honeybee colony.

The impact of Nosemosis on colony health and losses is further exacerbated by its interaction with other stressors. For example, the synergistic effects of Nosema infection and exposure to neonicotinoid pesticides have been documented (Alaux *et al*., 2010), with the combination leading to more severe impairments in bee health than either stressor alone. This highlights the need for a holistic approach to bee health management, considering the complex interplay of factors that affect colonies.

EMERGING TREND OF PREVENTION AND NOSEMA TREATMENT

The emerging trends in preventing and treating Nosema disease in honeybees are paramount for safeguarding global bee populations and agricultural ecosystems (Grupe and Quandt, 2020; Emsen *et al*., 2020). As this devastating parasitic infection threatens honeybee colonies, innovative research and strategies are crucial to ensure their survival (Grupe and Quandt, 2020). By staying at the forefront of Nosema prevention and treatment methods, beekeepers and researchers can mitigate the disease's impact, improve bee health, and sustain pollination services vital for food production (Formato *et al*., 2022). These emerging trends encompass novel therapies, sustainable beekeeping practices, and genetic advancements. They hope for a resilient future where honeybees can thrive and fulfill their critical role in pollinating our crops and sustaining biodiversity. Moreover, Different approaches can be used for the prevention of *Nosema* spp. (Fig. 2).

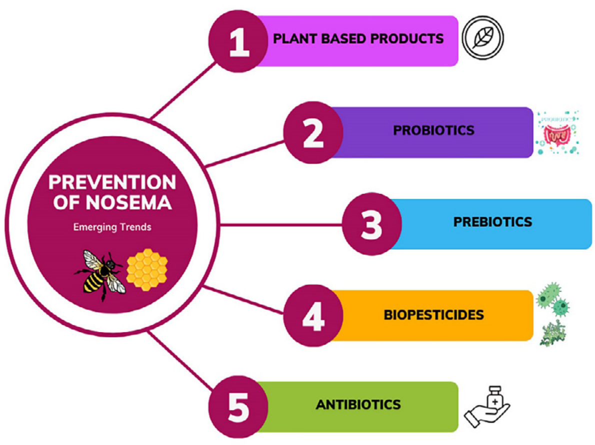

Fig. 2. Different prevention approaches for *Nosema* species.

Natural products controlling nosema diseases

The advancement of natural products in controlling Nosema diseases represents a promising avenue in honeybee health management (Iorizzo *et al*., 2022b). Traditional chemical treatments have raised concerns about their environmental impact and the development of resistance among the parasites (Marín-García *et al*., 2022). As a result, researchers and beekeepers have increasingly turned their attention to natural products and alternative strategies to combat this devastating disease. One of the most intriguing advancements in this field is using plant-based compounds, such as essential oils and plant extracts, as potential treatments for Nosema (Chaimanee *et al*., 2021). Several studies have demonstrated that essential oils, such as thyme, oregano, and lemon balm, inhibit the growth and reproduction of Nosema parasites (El-Seedi *et al*., 2022b; Kunat-Budzyńska *et al*., 2022; Özüiçli *et al*., 2023). These natural products can be administered to honeybee colonies through sugar syrup or as fumigants, providing a less toxic and environmentally friendly alternative to synthetic chemicals (Kunat-Budzyńska *et al*., 2022).

Another exciting development is the exploration of probiotics and prebiotics to manage Nosema infections (Borges *et al*., 2021). Probiotics, which consist of beneficial microorganisms, can help establish a healthy gut microbiome in honeybees, potentially reducing the susceptibility to Nosema infection (Motta *et al*., 2022). Moreover, probiotics may play a pivotal role in disease prevention by enhancing the bee's immune system and competitive exclusion of pathogens (Borges *et al*., 2021). Prebiotics, conversely, are compounds that stimulate the growth of beneficial gut bacteria. Research is ongoing to identify specific prebiotics that can improve honeybee health and resilience against Nosema (Borges *et al*., 2021; Iorizzo *et al*., 2022a). In addition to plant-based solutions and probiotics, honeybee products offer promising opportunities for Nosema control (Iorizzo *et al*., 2022a). Royal jelly, a substance secreted by worker bees to feed queen larvae, contains various bioactive compounds with potential antimicrobial properties (Nowak *et al*., 2021). Furthermore, genomics and molecular biology advancements have enabled researchers to explore the genetic basis of honeybee resistance to Nosema (Grozinger and Robinson, 2015; Chen *et al*., 2013). By identifying specific genes and mechanisms involved in bee immunity, scientists can develop targeted breeding programs to enhance the natural resistance of honeybee populations (Evans *et al*., 2006). This approach holds tremendous potential for the long-term management of Nosema diseases through selective breeding of resilient honeybee strains.

Although these advances in natural product-based Nosema control are promising, challenges remain. Standardizing treatment protocols, ensuring the safety of natural products for honeybees, and addressing issues of scalability and cost-effectiveness are essential considerations. Additionally, the efficacy of these treatments may vary depending on environmental conditions and Nosema strains. Therefore, ongoing research and collaboration between scientists, beekeepers, and policymakers are crucial to refining and implementing these innovative approaches.

Others methods

Various other methods and products have been explored for managing Nosema disease, and some have demonstrated greater efficacy. This comprehensive discussion will delve into the most effective methods and products for controlling Nosema diseases in honeybees.

Fumagillin has long been recognized as one of the most effective treatments for Nosema disease (Burnham, 2019). This antimicrobial compound specifically targets Nosema spores, inhibiting their growth and reproduction within the honeybee gut (Huang *et al*., 2013). Fumagillin-based treatments have successfully reduced Nosema infection levels and promoted colony recovery (Williams *et al*., 2008). Meanwhile, maintaining honeybee colonies' overall health and vitality is crucial in preventing and managing Nosema disease (Formato *et al*., 2022). Proper nutrition, including access to diverse forage sources and a balanced diet, can strengthen bee immune systems and resilience against diseases including Nosema (Ricigliano *et al*., 2022). Additionally, minimizing stress factors, such as exposure to pesticides and other environmental stressors, can help prevent outbreaks of Nosema (Almasri *et al*., 2021). Moreover, biopesticides and biological control agents offer environmentally friendly options for Nosema disease management (Garrido *et al*., 2023). Some microbial-based products, such as *Bacillus* spp. (Garrido *et al*., 2023) and beneficial fungi, fenbendazole, when combined with ornidazole, demonstrates promising antifungal properties that could be effective against *N. ceranae* under a controlled laboratory setting (Bahreini *et al*., 2022). These methods can be integrated into beekeeping practices as a sustainable disease management strategy.

RECOMMENDATIONS AND FUTURE DIRECTIONS

Recommendations

- Promote the adoption of Integrated Pest Management strategies for honeybee health that include monitoring and managing Nosema disease alongside other stressors like pesticides, habitat loss, and climate change.
- Encourage governments, institutions, and organizations to invest in research on Nosema disease to understand its dynamics and management better.
- Foster collaboration among researchers, beekeepers, and relevant stakeholders to share knowledge and resources.
- Develop and refine diagnostic tools for early and accurate detection of *Nosema* spp. in honeybee colonies.
- Create user-friendly monitoring systems that beekeepers can easily adopt to track Nosema infection levels.
- Support research into novel treatments and therapies for Nosema-infected honeybee colonies, focusing on chemical and non-chemical solutions.
- Investigate the potential of probiotics, prebiotics, and other alternative treatments to mitigate Nosema infections.
- Provide educational resources and training programs for beekeepers to raise awareness about Nosema disease and its management.
- Share best practices for hive management and hygiene that can reduce the risk of Nosema infection.

Future directions

- Explore the honeybee gut microbiome in greater detail to understand its role in Nosema infection dynamics.
- Investigate the potential of microbiome-based interventions for Nosema control.
- Identify and breed honeybee lines with resistance or tolerance to Nosema infections.
- Study the genetic basis of resistance and develop molecular tools for selective breeding.
- Assess the influence of climate change on the prevalence and distribution of *Nosema* spp. in honeybee populations.
- Develop adaptive strategies for beekeeping in changing climates.
- Investigate the broader ecological context of Nosema disease, including its impact on native pollinators, plants, and ecosystems.
- Understand how Nosema interacts with other pathogens and stressors in honeybee colonies.
- Establish long-term monitoring programs to track Nosema disease prevalence and its impact on honeybee populations over time.

CONCLUSIONS

In the face of declining honeybee populations and their critical role in pollinating crops and maintaining ecosystem diversity, understanding and mitigating Nosema disease has become increasingly vital. This literature review has provided a comprehensive overview of the current knowledge regarding Nosema disease in honeybees. It has shed light on the potential prospects in managing and preventing this threat. The insights gleaned from many studies indicate that Nosema disease is a complex condition influenced by various factors, including environmental stressors, Nosema species variation, and host-pathogen interactions. While substantial progress has been made in elucidating the disease's mechanisms and impacts, gaps in knowledge still exist. Further research is needed to fully comprehend the intricacies of Nosema infection, its transmission dynamics, and its effects on individual bees and entire colonies. The future prospects for addressing Nosema disease in honeybees are promising. Innovative approaches, such as the development of Nosema-resistant honeybee strains and the use of probiotics to manage infection, hold significant potential. Additionally, molecular biology and genomics advancements offer new tools for investigating the genetic basis of Nosema resistance and susceptibility. Furthermore, incorporating integrated pest management strategies and sustainable beekeeping practices can contribute to reducing the prevalence of Nosema disease.

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Statement of conflict of interest

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