Short Communication

AN ANALYSIS OF THE PREVALENCE OF *LOTMARIA PASSIM*, *CRITHIDIA MELLIFICAE* AND *NOSEMA* SPP. PATHOGENS OF FREE-LIVING BEE COLONIES

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Abstract

Honey bees (*Apis mellifera* L.) may inhabit abandoned beehives in trees in the city and forests. Bees that naturally occupy abandoned nests, cavities, or hives are not managed by humans and are not treated for pathogens; consequently, they may be particularly susceptible to diseases. The aim of the study was to evaluate the prevalence of *Lotmaria passim*, *Crithidia mellificae* and *Nosema* spp. in free-living bee colonies through polymerase chain reaction (PCR). The study was conducted on samples of adult bees, pupae, larvae, bee bread and honey. The pathogens were detected in two samples. The predominant pathogen was *L. passim*, which was found in adult bees and four-day-old larvae. In addition, *Nosema apis* was found only in bees. The results of this study indicate that pathogens may be present in apparently healthy bee colonies and their larvae. Early detection of pathogens in adult bees and larvae can enhance the effectiveness of veterinary treatment in apiaries.

Keywords: *Crithidia mellificae*, *Lotmaria passim*, free-living bee colonies, *Nosema* spp., PCR

INTRODUCTION

Pollination is a crucial ecosystem service for both natural and agricultural environments, contributing to biodiversity (Porto et al., 2020). Bees are among the most effective pollinators of entomophilous plants worldwide, and the high quality of crops pollinated by bees brings both environmental and economic benefits. The main transmission routes among bees include direct contact, ingestion of fecal matter, pollen, nectar contaminated with pathogens, and bee bread and honey (Babin et al., 2024). Many honey bees exploit the same floral resources, allowing horizontal transmission of pathogens, especially honeybee pathogens of, between wild and managed honey bees (Dalmon et al., 2021). Bees not managed or treated for pathogens are likely to be more susceptible to viral, bacterial, and parasitic diseases, which lead to their demise (Martin et al., 2012). Flowers increase the transmission risk by exposing pollinators to infections during foraging (Vejnovic et al., 2018; Gómez-Moracho et al., 2020).

Infections caused by *Lotmaria passim*, *Crithidia mellificae*, *Nosema apis*, and *N. ceranae* are common in bee colonies. These pathogens negatively impact the health of worker bees, compromising the activity and performance of bee colonies, and alsonegatively impact the health of worker bees. They contribute significantly to the decline and loss of bee colonies and also have profound negative effects on bee behavior, physiology, and immune function, ultimately reducing colony vitality and resilience (Runckel et al., 2011; Schwarz & Evans, 2013). These protozoa have been extensively researched due to their detrimental effects on the behavior, physiology, and immune function of bees (Phiri et al., 2022). *Nosema apis*

and *N. ceranae* are highly specialized unicellular eukaryotic parasites (Adl et al., 2005).

Bees become infected by ingesting spores with water or pollen (Chen et al., 2008). The literature provides minimal data on the sources of bee infections with *L. passim*, *C. mellificae*, and *Nosema* spp. spores or their transmission routes. Honey and bee bread have been identified as potential sources of infection (Sokół & Michalczyk, 2016). The apiary and bee colony disease is usually transmitted via combs infected with spores, water, and between insects through trophallaxis. (Higes et al., 2013). The objective of this study was to determine the age at which honey bee workers, larvae, bee bread, and honey are most susceptible to the pathogens *L. passim*, *C. mellificae*, and *Nosema* spp. in free living bee colonies.

MATERIAL AND METHODS

The study was conducted on samples of bees, pupae, larvae, bee bread, and honey from combs, with pooled samples consisting of twenty each collected from free living bee colonies. The samples for research came from free living bees that had colonized an empty, abandoned hive in Spręcowo in the Warmian-Masurian Province. The brood samples were collected through the removal of the brood combs directly from the hive. Bees were sampled only from naturally colonized sites, and no clinical symptoms of honey bee diseases, such as diarrhea or inability to fly, were observed in these colonies. Brood samples (20 individuals each) aged larvae (4, 8, 10 days), pupae (12, 14, 18 days) and adults (21 days) were collected. The age of individual developmental stages of bees was determined based on the course of individual development and the duration of development, expressed in days. Brood samples were collected with the use of sterile forceps and placed into individually labeled containers. Adult bees were sampled randomly and transferred into labeled cages. All collected samples were immediately transported to the laboratory for further analysis.

Additionally, bee bread and honey from combs were collected, and about 5 g of bee bread stored in newly built marked combs were collected. Fifteen samples of bee bread and 15 mg of honey were used for analysis. The collected samples were analyzed using molecular methods to identify *Nosema* spp., *C. mellificae*, and *L. passim*. Whole bees, brood, bee bread, and honey were homogenized with the use of a homogenizer. For DNA extraction, all homogenates from each sample were used. According to the manufacturer's instructions, genomic DNA was isolated using the Sherlock AX kit (A&A Biotechnology, Gdynia, Poland). The presence of *L. passim*, *C. mellificae*, was determined by real-time PCR.

These pathogens were detected with the use of the FastStart Essential DNA Green Master kit (Roche). The reaction mixture had a final volume of 20 µL and contained approximately 150 ng of extracted genomic DNA (1 µL), 10 µL of FastStart Essential DNA Green Master, 0.5 µL of each primer, and PCR-grade water (FastStart Essential DNA Green Master H_2O) added to reach the final reaction volume. Real-time PCR assays were carried out with the use of a LightCycler Nano System (Roche) (Vejnovic et al., 2018). The presence of two microsporidian parasites, *N. apis*, and *N. ceranae*, was determined by duplex PCR. Duplex PCR was performed with the use of HotStarTaq Plus DNA Polymerase (Qiagen) and the HotStarTaq Plus Master Mix Kit (Qiagen). The reaction mixture had a final volume of 20 µL and contained approximately 120 ng of extracted genomic DNA (1–3 µL), 10 µL of 2× HotStarTaq Plus Master Mix, 2 µL of 10× CoralLoad Concentrate, 0.1 µL of each primer (final concentration 0.5 µM), and RNase-free water added to reach the final reaction volume (Martin-Hernandez, 2007).

RESULTS AND DISCUSSION

After examining nine different samples (bees, brood, bee bread, honey), we found pathogens only in two samples. *Nosema apis* was detected in bees samples, and *L. passim* in two samples (bees and larvae - 4 days). Coinfections with only *N. apis/L. passim* were noted in bee sample (Tab. 1).

Table 1.

Occurrence of pathogens in the tested samples

Samples	Pathogens			
	N. apis	*N. ceranae*	*L. passim*	*C. mellificae*
bees	+	-	+	-
pupae	-	-	-	-
larvae	-	-	+	-
bee bread	-	-	-	-
honey	-	-	-	-

The pathogens (*L. passim, C. mellificae, Nosema* sp.) carried by infected bees may persist on flowers, where they pose an infection risk to susceptible pollinators of other species, depending on the number of floral visits. According to multiple researchers, *L. passim* can compromise the health of honey bees, and coinfections with other pathogens may lead to colony collapse. Tritschler et al. (2017) reported a relationship between *L. passim* and *Nosema* in bees, while Vejnovic et al. (2018) attributed observed variations in the prevalence of *Nosema sp*. and *L. passim* to different methodological approaches. This study identified only one sample with a coinfection of *N. apis* and *L. passim*. An intriguing observation is the presence of bee pathogens in pollen, bee bread, and honey. Higes et al. (2013) explained the presence of *Nosema sp*. spores in pollen and bee bread as resulting from self-infection of foragers during pollen collection. Pollen stored in comb cells as bee bread, infected during foraging, may serve as a potential infection source for new generations of bees (Martin-Hernandez et al., 2007). In this study, spores of *N. apis* was found. The presence of *Nosema* spores in worker bees may also be associated with subclinical disease symptoms or recovery after infection in at least some bees, as the bees studied showed no signs of disease. Bees can be easily infected by ingesting food containing spores, but the infection's progression depends on the age of the insects.

Beekeepers living in Chile reported increased colony losses accompanied by reduced activity of honey bees. These mortality events were likely driven primarily by the presence of *N. ceranae*, although *L. passim* was also highly prevalent, detected in up to 90% of the analyzed samples (Rodríguez et al., 2014). In Belgium, Ravoet et al. (2013) identified *C. mellificae* as an emerging pathogen contributing to honey bee colony declines. Gómez-Moracho et al. (2020) studied the impact of *L. passim* and *C. mellificae* on honey bee health and found that both protozoans significantly reduced bee lifespan, with *L. passim* showing higher virulence than *C. mellificae*. Cultured on different media, the pathogens were used to infect bees, and infected individuals died earlier than uninfected controls, confirming the pathogenic potential of both species. Some authors Armitage et al. (2022) have shown that multiple infections often reduce pathogen virulence due to antagonism, while in other cases, synergistic interactions may increase virulence and reduce host lifespan, highlighting the potential long-term effects of these infections. The potential spillover of honey bee pathogens may have undetected yet significant impacts on the health and functioning of ecosystems, underscoring the importance of ongoing research in this field. Free-living colonies can infect colonies of bees kept by beekeepers. Colonies comprise larvae, but larvae themselves are found rarely outside the colony.

DISCLOSURE STATEMENT

No potencial conflict of interest was reported by the authors.

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