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Stress Factors Affecting the Development of *Vairimorpha* (*Nosema*) spp. Disease in Bee Colonies

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Abstract: Microsporidia are intracellular parasites infecting a wide range of vertebrates and invertebrates. In bees, they cause a diarrheal disease called nosematosis, which represents a growing problem contributing to the decline of bee colonies. This parasitic disease infects the gastrointestinal tract of bees, disrupts their immune system, weakens them, and shortens their lifespan. There are two known species of this microsporidial parasite with a global distribution: *Vairimorpha apis* and *Vairimorpha ceranae*, with current research indicating that the species *V. ceranae* is gradually replacing *V. apis*. In this study, we focused on the detection and occurrence of *Vairimorpha* spp. in bee colonies kept in different environments, as well as on comparing the impact of hive location on its occurrence. The methods used included sampling from winter bee carcasses, microscopic examination of bee gut contents, and subsequent molecular duplex PCR analysis. Altogether, we examined 82 hives located at 4 different sites and focused on the impact of transportation as an abiotic factor disturbing bees leading to the development of *Vairimorpha* spp. disease. The results indicate an increased occurrence of this parasite in hives located in urban sites and hives situated in intravillage locations, which may be attributed to transportation and associated vibrations as an abiotic stress factor. Detection revealed that all samples were positive for *Vairimorpha ceranae.*

Keywords: *Vairimorpha* spp.; diagnostic; duplex PCR; Slovakia

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1. Introduction

Annual losses in bee colonies over the past few decades have been a major problem for all beekeepers. The syndrome called CCD - Colony Collapse Disorder appears to be the result of the accumulation of stressors that chronically weaken bee colonies. The immune system, metabolic pathways, and cognitive processes gradually weaken in the body under chronic stress until exhaustion and eventual failure (Chrousos 2009; Even, Devaud, Barron 2012).

Current epigenetic and nutrigenomic studies highlight the importance of interactions between microflora, climate, pathogens, toxins, nutrition (diversity and quality), and colony resilience to environmental changes (Dequenne, Philippart de Foy, Cani 2022).

Microorganisms responsible for the mortality of bee colonies include a wide range of bacteria, viruses, fungi, and protists, many of which are serious bee pathogens (Engel et al., 2016). *Paenibacillus larvae* and Melissococcus plutonius are highly prevalent bacterial pathogens causing American and European foulbrood (Genersch 2010; Forsgren 2010). If left untreated, they typically have a lethal outcome for the colony. In addition to bacterial pathogens, more than twenty different bee viruses have been described so far (McMenamin and Genersch, 2015), with most of them capable of causing various physiological changes (including severe physical deformities), behavioral changes, and reduced lifespan (Ryabov et al., 2023; Nguyen et al., 2024). However, individual hosts vary in susceptibility to specific infections, with many viruses persisting chronically and asymptomatically in bee colonies (De Miranda et al, 2013). The synergistic negative effects on bee health can result from combined exposure to pathogens and pesticides or concurrent infection by multiple pathogens. Many viral pathogens of bees also infect wasps or bumblebees, indicating a broader range of host species with overlapping geographic ranges (Streicher et al., 2024; Klocek et al., 2023).

Studies show that infection with microsporidia *Vairimorpha* spp., especially *Vairimorpha apis* and *Vairimorpha ceranae* is another important factor that contributes to the decline of the honey bee

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population. *V. ceranae* was originally discovered in Asian honey bees (*Apis ceranae*) and over the past few decades has spread to Western honey bee (*A. mellifera*) colonies worldwide (Parrella et al., 2024; Blot et al., 2023; Higes et al., 2006). *V. ceranae* has been found in hemolymph and various tissues, but it primarily infects the midgut of adult honey bees. By infecting individual bees in a colony, their lifespan is shortened, which results in a decrease in the colony's ability to provide and store food and thus maintain a healthy population (Alberoni et al., 2023). *V. ceranae* infection can not only disrupt the integrity of the midgut, but also affect energy metabolism and thus suppress the immune response of bees (Alberoni et al., 2023). Some researchers disagree that *V. ceranae* is more virulent than *V. apis* and may thus be responsible for bee colony losses (Liu et al., 2024; Li et al., 2024). They believe that the virulence of *Vairimorpha* spp. other factors also contribute (Zbrozek et al., 2023; Zhang et al, 2021). Thus, *V. ceranae* is generally considered to be the main biotic factor seriously threatening honey bees together with the ectoparasitic mite *Varroa destructor*. The latter parasitizes the fat body of honeybees and thus causes a shortening of the life span, a decrease in body weight and a reduced width of the acini of the hypopharyngeal glands (SD Ramsey et al., 2019; Bruckner et al., 2023). In addition, *V. destructor* can also transmit viruses such as deformed wing virus (DWV) (Oddie et al., 2023).

A growing body of evidence suggests that pesticides, specifically neonicotinoid insecticides, are among the main triggers of honey bee colony declines. After reviewing the risk of this group of insecticides to bee health, they were banned in Europe by the European Food Safety Authority (EFSA) (Bass and Field, 2018). However, several researchers have confirmed that bees show large differences in their sensitivity to different insecticides – including compounds belonging to the same class (Beadle et al., 2019; Iwasa et al., 2004; Manjon et al., 2018; Reid et al., 2020). For example, according to the official categories of the US Environmental Protection Agency (USEPA et al., 2014), in acute contact bioassays, honey bees (Apis mellifera) are >1000 times less sensitive to the neonicotinoid thiacloprid, while this group is classified as "virtually non-toxic". " and to the neonicotinoid imidacloprid are extremely sensitive, therefore this group is classified as "highly toxic". Also, while many pyrethroid insecticides are highly toxic to insect pollinators, tau-fluvalinate exhibits such low acute toxicity to bees that beekeepers use it as an in-hive treatment against parasitic Varroa mites (Bass et al., 2024). However, even lower doses of pesticides can have a significant impact on bee colonies. They can cause sublethal effects (e.g. reduced mobility, learning, memory, orientation, thermoregulation, foraging), which results in a decrease in the function of the entire colony and indirectly in their decline (Hester et al., 2023; Raine and Rundlöf 2024).

Currently, climate change is not only a threat to us humans, but also worries bees all over the world (Van Espen et al., 2023). Increased temperature brings many negative effects on food reserves (including honey production, increase in reserves for colonies and the composition of sugars in honey, it also affects interactions between plants and pollinators by reducing their visitation and the strength of interactions, it even causes colony mortality (bees die earlier, they are less resistant against winter, as their thermal tolerance decreases, they suffer from heat fluctuations) (Zapata-Hernández et al., 2024; Palmer-Young et al., 2023; Glass and Harrison, 2024; Manlik et al., 2023). A decrease in temperatures can cause increased mortality of colonies. Longer exposure to reduced temperatures affects their immunity and microbiome (Butolo et al., 2022). An increase in precipitation, which had a negative impact mainly on the hive's homeostasis (its internal temperature and relative humidity of the breeding ground and the forage area), food supply (the richness and diversity of pollen decreases, there is an increase in the weight of the bee colony and the average annual yield, there are also changes in the microbiome and a decrease in the interaction between plants and pollinators (Switanek et al., 2017; Abou-Shaara et al., 2017). A decrease in precipitation may result in reduced honey production and also reduce interactions between plants and pollinators (Jaworski et al., 2022). Changes in precipitation are also a starter for the reproduction and distribution of various pests, the emergence of diseases and changes in the geographical distribution of different lines of *A. mellifera* (Abou-Shaara and Darwish, 2021). However, the opposite view is held by Langowska et al., 2017, who argue that rising temperatures could be beneficial for bees, as they would extend spring foraging periods and thus be better prepared to take advantage of summer nectar and pollen flows.

Climate change and habitat fragmentation result in the inability of bees to gather enough food for their needs. Colonies need pollen to grow and reproduce, so a severe lack of food can cause a colony to fail drastically. This failure includes traits suggestive of social breakdown, including

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cannibalism and looting of resources from weaker colonies, as well as reduced brood care (Corby-Harris et al., 2022; Tong et al., 2019; Corby-Harris et al., 2022). The nutritional health of bees depends on the availability and collection of nectar, pollen, as well as their quantity and quality to meet the main nutritional requirements of carbohydrates, proteins, lipids, vitamins and minerals (Castaños et al., 2023). Mayack and Naug (2009) demonstrated a correlation between bees infected with the protozoan Vairimorpha ceranae and a higher level of hunger. Thus, these infected bees experience nutritional stress, which is a consequence of many pathogenic infections. Based on the feeling of great hunger, the bees are more inclined to go out of the hive for food. However, if bees infected in this way go in search of food in low-energy states, it is more likely that they will die during this act and will not return to the hive. The foraging behavior of honey bees is largely regulated by the nutritional status of individuals, independent of the amount of stored food in the colony. The nutritional and energetic stress of individuals due to infection thus provides a possible mechanism for the disappearance of bees from hives that still have intact honey reserves. If nutritional stress on individuals is a major contributor to colony loss, its effects are likely to be felt most severely at times and places where it is difficult for bees to find suitable food (Naug 2009).

On a global level, the extensive use of plastics has contributed to the production of a huge amount of hazardous waste and thus represents one of the most significant problems of today. Ubiquitous microplastics represent a potential threat to various species of plants and animals, therefore it also has an impact on bee colonies (Lin et al., 2024), specifically in the form of a decrease in the diversity of the intestinal microbiome, changes in the structure of the microbiome and changes in the expression of genes related to detoxification and immunity (Wang et al. al., 2021b; Balzani et al., 2022; Buteler et al., 2022). Also, atmospheric PM, primarily emitted by automobile traffic, combustion engines, but also coal mining and, last but not least, agricultural residues, affect worker bee navigation, prolong the duration of foraging and affect bee survival (Thimmegowda et al. 2020; Cho et al. 2021). Heavy metals such as copper, lead, or manganese also have a lethal or sublethal effect on bees (Al Naggar et al. 2020). The activity of pollinators was also largely influenced by noise, turbulence and dust on roadsides (Dargas et al. 2016; Phillips et al. 2021).

Many microorganisms are contained in various parts of larvae and adult bees, their food and plastic, and they play an important role in food digestion, pollination and antagonistic effect against various pathogens. In this context, bees are mainly associated with fungi, which can provide material for pollen degradation or help the maturation of royal jelly, while they can also be a source of food (Cui et al., 2022; Khan et al., 2020). Honey bees are therefore a potential model of fungus-host-symbiont interactions. Fungi are numerous in bee bread and nectar, but also from healthy honey bees Gilliam et al. (1979) isolated four fungal species, with *Candida* species detected predominantly from bees fed herbicide and antibiotics. Research in nosemosis field tests has revealed that the degree of *Vairimorpha ceranae* infection is related to yeast growth in the honey bee gut, suggesting that exposure to stresses such as infection can lead to structural changes in the gut microbial community (Yun et al., 2018). In this study, we aimed to compare the survivability of honey bees in different environments after infection with *Vairimorpha* species, taking into account different stressful factors.

A rodent in the hive can also be such a stressful factor. In picture no. 1 we see a shrew, which moved into the hive during the winter. During the spring inspection of bee colonies, we found a non-terrestrial infection in most of the bees, until finally this bee colony fell completely. So we think that this rodent caused stress in the bees, which culminated to a lethal end.

Image no. 1: The shrew at the bottom of the hive.

2. Material and Methods

2.1. Collection of Samples

In the month of March 2024, we took samples of winter carcasses of bees (Fig. 2) in the number of 30 pcs per bee colony from several locations where bee colonies were located. According to the type of biotope, we divided them into the following categories: A) urban biotope, B) rural biotope in the intravillage, C) rural biotope in the extravillage and D) forest biotope (Fig. 3 and Tab. 1):

2.1.1. Location no. 1: Municipal Bees (Košice - altitude 206 masl, Košice- Barca - altitude 210 masl, Michalovce - Altitude 113 masl):

All urban bee habitats are located between houses or blocks, within 5 meters of a busy road. Within 3 kilometers from Košice and 2 kilometers from Košice-Barca, as the crow flies, the largest eastern Slovakian plant U.S. is located. Steel Košice, producing mainly steel and sheets; at a distance of max. 1 km from both locations is the thermal power plant TEKO Košice. The company KOSIT, dedicated to the collection and processing of municipal waste, has its headquarters in the incinerator directly in the Košice-Barca area, as well as the industrial park Immopark, which produces electronic and mechanical components for the automotive industry. The company CASSPOS a.s. in Michalovce is focused on production various steel products and generally focuses on aluminum processing. Within this site there are still several smaller industrial plants, we have listed only the largest and closest ones.

Food for bees is provided in the form of various types of flowers found in city parks. Also, people living in houses or apartments grow garden crops, fruit trees, balcony flowers (but most of them are sprayed and fertilized).

2.1.2. Location no. 2: Inner Village (Lipníky - Altitude 280 masl)

Site located in the immediate vicinity of road 1: class, where there is heavy traffic. The nearest industrial areas are 14 km away (ZEOCEM Bystré) - processing of clinoptilolite and 15 km to Prešov, where there are several plants, the largest of which is a clothing plant and an automobile plant. This area is characterized by agricultural activity, where rapeseed (which is sprayed) is grown for 3 km as the crow flies. Another option for bee food is gardens where people grow fruits, vegetables and ornamental plants. This is a locality with seasonal use of the laying conditions of the site, mainly in the first half of the beekeeping season (March - June of the calendar year) for obtaining mainly light (spring) types of honey, honey from fruit trees such as willow and also goldenrod honey.

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2.1.3. Location no. 3: Extravillain of the Village (Okrúhle- Altitude 280 masl)

The beehives are located in the garden behind the house, 500 m away from the 1st class road (with heavy traffic). The nearest plant is approx. 30 km from ZEOCEM Bystré and 50 km from Bukóza Vranov nad Topľou, approx. 30 km from Bardejov - textile industry. Agricultural crops are grown in the surroundings: organic peas, wheat (but bees do not pollinate them); in addition, only fruit trees and horticultural crops. It is a permanent habitat of bee colonies in the cadastre of Okrúhle village, district Svidník, in the area of the Ondavská vrchovina, in close proximity to the Forest Park called "Kerta v Okrúhlom". This location is mainly suitable for obtaining forest and mixed honeys. Picturesque forests alternate with mountain meadows and pastures. The principles of ecological agriculture are applied in this area, which strictly exclude the use of any artificial fertilizers or other agrochemicals. The phytodiversity of this park is very rich (40-50 species of trees): for example, *Acer platanoides*, *Fraxinus excelsior*, *Tilia cordata*, *Tilia plathyphyllos*, *Quercus petraea*, *Quercus robur*, *Picea abies*, *Pinus silvestris* and *Larix decidua.*

2.1.4. Location no. 4: Forest Bees (Komárnik- Altitude 420 masl)

It is a site located in the immediate vicinity of the forest, 2 km as the crow flies from a 1st class road (with heavy traffic). It is a locality of the Eastern Carpathians Protected Landscape Area, Dukla Forest Community of European Importance. 80% of the area consists of forest communities of the Eastern Carpathians, represented mainly by fir, beech and valuable deciduous trees. The nearest plant is over 100 km away. Agriculture in the area is not widespread, only hay is cut. Bees only use the surrounding leafy conifers and meadow flowers for laying eggs. In connection with the climate, the temperature is lower than in cities and more constant without higher fluctuations. The health status of bee colonies is checked annually by the relevant Regional Veterinary and Food Administration of Vranov and Prešov by taking samples of honeydew, which are evaluated by the State Veterinary and Food Institute in Dolní Kubín. It is a temporary habitat for bee colonies located in the region under Duklau, in the cadastre of the municipality of Nižný Komárnik, district Svidnik. This location is specific for its microclimatic conditions. It is part of the Eastern Carpathian Protected Landscape Area and at the same time the Dukla Area of European Importance. The dominant plant communities in the area are beech-fir flowering forests with a high proportion of white fir (*Abies alba*). The National Nature Reserve (hereinafter referred to as "NPR") Komárnická jedlina is located in close proximity to the site. These are intact fir-beech forests with admixtures of rare trees such as maples, elms, ash trees and linden trees. This NPR is classified as a European speciality. The territory was declared for the protection of biotopes of European importance, rare preserved relics of the natural fir-beech forests of the Eastern Carpathians with a very significant thickness and age differentiation. The 5th degree of nature protection with a continuous no-intervention regime applies here. This rare reserve of national importance is important for ensuring species diversity, ecological stability of the region and, last but not least, for the possibility of observing natural evolutionary processes in nature. This site is located in the outskirts of the village of Nižný Komárnik, 2 km from the international road. The radius of action of the bee colonies at this site is limited to 1.5 km due to the ruggedness of the relief. 80% of the area in the range consists of forest communities and the remaining 20% are meadow biotopes and riparian vegetation of the local stream "Šivárna." The territory is also sporadically visited by the brown bear (Ursus arctos) and therefore the bees must be protected by electric protection and fencing in accordance with § 45 par. 1 letter b) c) d) Decree of the Ministry of the Environment of the Slovak Republic No. 170/2021 of April 19, 2021, implementing Act No. 543/2002 Coll. on nature and landscape protection, as amended. No. 170/2021 Coll.

In all beehives, we collected bee carcasses from the bottom of the hive, at the same time we observed and evaluated the strength of infection of the beehives, according to the number of occupied frames, by checking the strips of grind on the underlays.

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Image no. 2: Dead bees at the bottom of the hive.

Image no. 3: Observed habitats: A) Urban habitat; B) Rural site; intravilan; C) Rural location of extravillans; D) Forest habitat.

Table 1. Number of bee colonies per individual site.

Habitat according to the location of bee colonies	Numbers of bee colonies
A) City bees	22
B) Rural bees in the inner city	27
C) Rural bees in extravilla	22
D) Forest habitat	11

2.2. Sample Processing and Detection of Vairimorpha spp.

2.2.1. Microscopic Diagnostics

We cut the abdomens from the bee carcass samples and mixed them in a mortar with distilled water, then crushed the abdomens. We followed the Pohl 2005 methodology.

We observed the samples under a microscope at 40x magnification and counted the number of spores in the specimen. We counted spores in 5 fields of view and after averaging, we evaluated the strength of the infection according to Table no. 2

Table 2. The strength of the Nozemosis infection in relation to the number of spores.

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Strength of infection	Number of spores
Negative sample	0 in the field of view
Weak nozem infection +	1-19 spores per field of view
Moderately strong nozem infection ++	20-100 spores per field of view
Strong nozem infection +++	Over 100 spores per field of view

2.2.2. Molecular Diagnostics

We isolated DNA from crushed bee abdomen samples using the commercially available DNA - sorb- AM isolation kit from AmpliSense and following the manufacturer's instructions. We used a PRECELLYS tissue homogenizer with a program of 6500 rpm for 2x 45 seconds to break the hard shell of the spores.

In the next step, we created a PCR mix for each sample, which consisted of PCR water, master mix and 10 µM mediums for both species of *Vairimorpha* spp. (table no. 3). We added the template to the mix and vortexed thoroughly.

Table 3. Components of the PCR mix and their amounts.

Components of the PCR mix	Quantity
PCR water	11,5 µl
Firepol Master Mix (Solis Biodine)	4 µl
APIS FOR(5′-GGGGCCATGTGTTTGACGTACTATGTA-3′)	0,5 µl
APIS REV (5′-GGGGGGCGTTTAAAAATGTGAAACAACTATG-3′)	0,5 µl
MITOC FOR (5′CGGCGACGATGATGATGATGAAAATATTAA-3′)	0,5 µl
MITOC REV (5′-CCCGGTCATTCTCAAAAAAAACCG-3′)	0,5 µl
Template	2,5 µl

Duplex PCR was performed using a VWR RISTRETTO thermal cycler. The initial denaturation took place for 4 min. at 95 °C, followed by 28 cycles of denaturation at 95 °C (25 s), annealing at 58 °C (45 s) and polymerization at 72 °C (2 min). The final extension step lasted 7 min. at 72 ° C. To prove the presence of DNA in the investigated samples, we used gel electrophoresis, using a 1.5% agarose gel. After evaluating the results with a UV transilluminator, the DNA concentration was measured with a NanoDrop and the PCR products were sent for sequencing. The obtained sequences were compared with the sequences stored in the gene bank using the BLAST program.

3. Results

3.1. Microscopic Diagnostics

Using a light microscope with 400x magnification, we detected the presence or absence of spores of *Vairimorpha* spp. in bee colonies. In 41 (50%) samples we detected spores of *Vairimorpha* spp. and in 41 (50%) samples there were no spores found (Fig. no. 5 and Tab. no. 4)

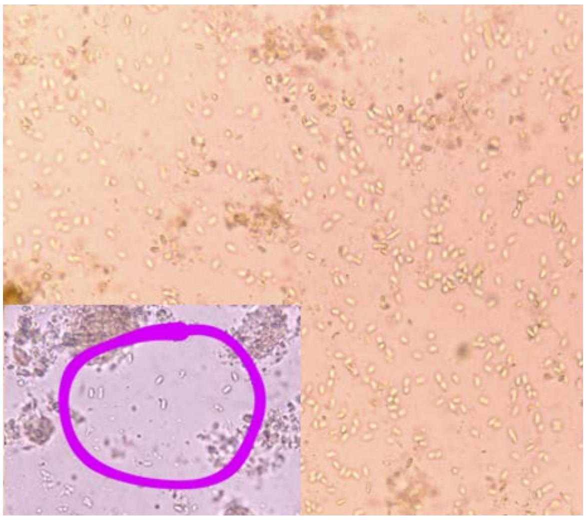

Image no. 5: Spores under the microscope at 400x magnification.

Table 4. Evaluation of negative and positive samples with differentiation of infection strength.

	negative	+	++	+++	Positive
City	5	5	3	9	17/22
Inner city	7	6	6	8	20/27
Extravilla	18	0	0	4	4/22
Forest	11	0	0	0	0/11
Total count	**41**	**11**	**9**	**21**	**41/82**

3.2. Molecular Diagnostics

The results of the microscopic method correlated with the results of molecular diagnostics, except for one case of a sample taken from a city district, where we evaluated one sample microscopically as negative, and after examination with molecular methods, we found it positive. In all positive samples for the detection of *Vairimorpha* spp., which underwent DNA isolation and subsequent amplification with the help of primers for both species *Vairimorpha apis* (amplicon 321 bp) and *Vairimorpha ceranae* (amplicon 218 bp) after loading on a 1.5% agarose gel and subsequent visualization the species *Vairimorpha ceranae* was detected using a UV transilluminator.

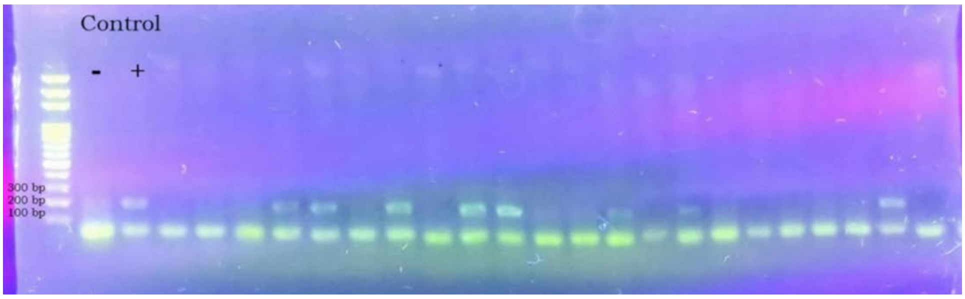

Image no. 6: Samples positive for the disease *Vairimorpha ceranae* (amplicon 218 bp).

3.3. Proportion of Positive Cases with Respect to Habitat

After examination of urban bees, 18 (81.81%) samples were positive and 4 (18.19%) were negative. Of the rural bees located at the site in the inner city, 20 (74%) samples were positive and 7 (16%) were negative. Of the samples of rural bees located at the site in the extravillan, 4 (18.18%) were positive and 18 (81.82%) were negative. Of the 11 samples of bees located in the forest habitat, all bee colonies (100%) were free of *Vairimorpha* spp. (see table no. 5 and picture no. 7)

Table 5. Proportion of positivity and negativity of bee colonies on *Vairimorpha* spp. to a specific location.

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Location	Number of positive colonies	Number of negative colonies
City bees	18	4
Rural bees - intravillan	20	7
Rural bees - extravillan	4	18
Forest bees	0	11

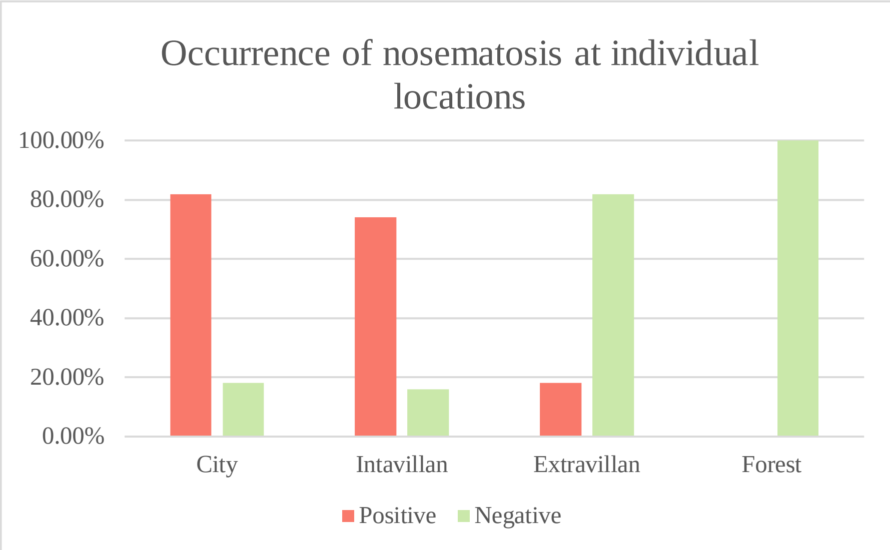

Image no. 7: Graph of the incidence of nosematosis at individual sites.

4. Discussion

On the infection of bees with *Vairimorpha* spp. several factors are involved, which must be followed in order to preserve a large bee colony. The most important prevention against this bee disease includes suitable environmental temperature, adequate number of bees in the hives, selection of habitat for wintering honey bees enabling earlier and more frequent flying, which would prevent overcrowding of the hive environment and the spread of infection at the same time. Replenishment of winter supplies, hygienic feeding and watering, regular disinfection of bee colonies, filtration, burning of dead individuals and sanitation are also important (Galajda et al., 2021). Failure to comply with the conditions for proper beekeeping can lead to bacterial or viral infection, which are other important factors in the reduction of the bee population (Genersch 2010; Forsgren 2010; Ryabov et al., 2023; Nguyen et al., 2024). Even today, very popular pesticides are an important factor affecting the health of bees (Hester et al., 2023; Raine and Rundlöf 2024). In our study, we therefore focused on comparing the occurrence of nosematosis in bees occurring in different habitats. As the results show, the infection most often occurred in bees kept in cities (81.81%), followed by the intravillage area of the village (74%), extravillage area (18.18%), and we detected no bees positive for the Vairimorpha species in the forest habitat. It can therefore be concluded that the urban environment was the least suitable for bee colonies due to the highest level of stress factors influencing the occurrence of nosematosis. Several researchers also dealt with the issue of comparing urban and rural environments. It has been concluded that urban environments are suitable in terms of flower diversity and higher temperature (Prendergast et al., 2022), while the situation of bees in the countryside is correlated with the city (Amado De Santis and Chacoff, 2020). That urban areas are probably more suitable for bees than agricultural areas was proven in their work by Geslin et al. (2013), who noted a large difference between food supply diversity and bee richness. Mahé et al. (2021) in their study found a higher concentration of insecticides in urban foragers and a higher content of metals in their larvae compared to rural bees. Also, bees living near the main Italian highway Autostrada A1 showed contamination with nanoscale Fe oxides/hydroxides and barite, as well as collected pollen and honey produced by the bee colony (Papa et al., 2021). Another study (Taylor et al., 2023) investigating trace element contamination in honey bees inhabiting urban areas around the largest and longest operating nickel smelter in the South Pacific at Nouméa, New

Caledonia reported elevated concentrations of potentially toxic trace elements including cobalt, chromium and nickel in bees. Concentrations of these metals decreased with distance from smelting operations, suggesting a relationship between environmental contamination and uptake of trace elements by bees. The bee colonies studied by us were influenced not only by industrial centers, but also by climate, pesticides, food options, and last but not least, vibrations and shocks caused by road traffic. With this, we would like to appeal to beekeepers and the scientific community to pay more attention to this issue and to investigate road traffic as a stress factor affecting the health of bee colonies.

5. Conclusions

Based on the global decline of pollinators and insects in general (Potts et al. 2016; McDermott 2021), the health of bees has long been the subject of debate in both the beekeeping and scientific communities (Hassler et al. 2021). Productive beekeeping requires that bee colonies have enough pasture from spring to fall. Regular migration significantly supports the development of the bee colony, improves its health and the supply of bee colonies. Many studies deal with various biotic and abiotic stressors affecting bee colonies. However, based on our results, we believe that further research would be needed to investigate other potential factors that may plausibly influence bee physiology and thus lead to the spread of *Vairimorpha* spp. We think that the impact of road traffic, especially in terms of vibrations and shocks, is generally under-researched so far. However, in general, the combined effect of multiple stressors is certainly more harmful than a single stressor (Goulson et al. 2015; Goulson and Nicholls 2022). It can be argued that the interaction between multiple stressors that vary spatially and temporally is a key factor underlying the issue of global honey bee health.

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