Praca oryginalna *Original paper*

Location of Nosema spp. spores within the body of the honey bee

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Summary

Nosemosis is a serious honeybee disease linked to Colony Collapse Disorder (CCD). It cause many changes at the individual bee level, which also affects the health of the entire bee colony. N. ceranae and N. apis are not tissue specific as was previously thought and besides the ventriculus epithelium their spore are also present in other tissue, such as Malpighian tubules, hypopharyngeal glands, salivary glands, and fat bodies. Emplacement of nosema infection in honeybee glands interferes with the production of the royal jelly, honey, bee bread. Moreover spores remaining in the honeybee glands are a potential reservoir of infection.

The aim of the research was to determine the correlation among the number of Nosema spores in whole bees, as well as in their ventriculus and hypopharyngeal glands. Nosema-infected honey bees were collected in the spring, when there should be a comparable degree of Nosema infection level in all tissues. Three independent experiments were conducted. In these studies the number of spores in the hypopharyngeal glands was the lowest and the highest results were observed for ventriculus samples. A large number of spores in the hypopharyngeal glands was also observed. This can be the cause of a reduction or loss of these glands' function; moreover, it may increase the horizontal transmission of the infection within a hive as well as to a queen bee.

Keywords: nosemosis, hypopharyngeal glands,ventriculus, Apis mellifera

Nosemosis (nosema) is the most common and widespread adult bee disease. It is caused by two species of the genus *Nosema*: *N. apis* and *N. ceranae* (Microsporidia: Nosematidae). The first description of European honeybee nosemosis comes just before 100 years (31). Initially it was thought that nosemosis is caused solely by one species – *Nosema apis*. But in 1996 a new species from Asia was described – *Nosema ceranae* (11), attacking *Apis cerana*. The first natural infection of *Apis mellifera* caused by *Nosema ceranae* was recorded among bees reared in Taiwan (18). Then infections were detected in Europe (13, 24), and also in Poland (28), in the United States (6, 7), in China and Vietnam (20). Moreover, *N. ceranae* can infect bumblebees (25). At present, serious infections of bees caused by *N. ceranae* are a worldwide problem (16, 19).

Nosemosis is linked to a devastating loss of honeybee colonies, which has been named honey bee Colony Collapse Disorder (CCD) in America and honeybee Colony Depopulation Syndrome (CDS) in Europe. This causes huge economic losses in the production of honey, bee products and shortages of food derived from plants pollinated by bees (oil products, fruits and vegetables) (2, 14, 15, 22).

Nosema-infected bees die very quickly – within 8-10 days, depending on the bee breed (23). To date, no effective drug was found for nosemosis, which also would be harmless to bees and did not penetrate into the honey.

Both *Nosema* species cause the infection of the ventriculus epithelium of adult bees, the symptoms of which are swollen abdomens. Multiplication of the parasite and cell damage leads to limited nutrient absorption, increased energy demands, may cause dysentery and a decrease in brood production (10, 21).

Nosema apis infections were thought to be restricted solely to the adult bees' ventriculus epithelium (9, 12) and *N. ceranae* to also other tissues, such as Malpighian tubules, hypopharyngeal glands, salivary glands,

and fat bodies (5). The latest studies have indicated both *Nosema* species in hypopharyngeal glands, mandibular glands, thoracic salivary glands and venom sacs (8). Therefore *N. ceranae* and *N. apis* are not tissue specific and spores remaining in the bee glands are a potential reservoir of infection (4, 8). Glandular secretion can be a route of horizontal transmission of the infection within a hive as well as to a queen bee. Such dispersal of bee viruses has been confirmed by numerous studies (1, 4, 27).

Changes in the structure of hypopharyngeal glands were observed in worker bees infected by *Nosema* (29); such as disappearance of the rough endoplasmic reticulum, Golgi complex, mitochondria, and pronounced myelinlike whorls of lysosome bodies. All these disintegrated the glands' cytoplasm and probably leads to the complete or partially inability to secrete royal jelly (29). Many microsporidian species infect and complete their life cycle in multiple tissues, e.g. *Nosema bombi*. Therefore all the changes in hypopharyngeal glands may be caused by nosemosis infection. Recent data, obtained by quantitative real-time PCR, are highly suggestive of the possibility that the thoracic salivary, hypopharyngeal, mandibular glands, venom sac and glands are infected in a similar level as intestines (8).

For these reasons it is of interest to determine the correlation between the number of *Nosema* spores in a whole bee and the degree of functional changes in the glands. It is not clear if weakly infected bees show a change in the structure and function of glands or only a heavy infection causes such problems. Therefore the aim of the study was to conduct a the preliminary analysis to resolve the question. For this purpose the correlation among the number of spores in whole bees, and their ventriculus and hypopharyngeal glands was defined.

Material and methods

Nosema-infected Buckfast bees were collected from a stationary apiary in the northern part of the Lublin region in the spring, when there should be a comparable degree of *Nosema* infection level in all tissues (8). Three independent experiments were conducted. 20 whole bees (WB) were ground in 20 ml of sterile distilled water. The alimentary tracts of 20 bees were removed individually, ventriculus (VE) were gently washed in distilled water to prevent contamination by a haemolymph and crushed in distilled water.

Tissues of 20 hypopharyngeal glands (HG) were carefully separated under Olympus SZX 16 stereomicroscope, gently washed in distilled water to prevent contamination by the haemolymph and ground in sterile distilled water.

In order to obtain a comparable dilution of all samples of whole bees, isolated VE and HG were weighed (separately in every sample) and then the corresponding ratio of the appropriate amount of distilled water was used for grinding the VE and HG.

Estimation of *Nosema* spores per bee was accomplished using Olympus BX 61 light microscopy and a hemocytometer (3, 17). For each spore suspension, the averages of 2 estimates of intensity were used.

The results were statistically analysed with the SAS software (SAS Institute 2002-2003 SAS/STAT User's Guide Version 9.13, Cary, NC, Statistical Analysis System Institute) using the one-way ANOVA (a group effect was the experimental factor) and the HSD (honestly significant difference) test and correlation (26).

Results and discussion

Recently published findings showed seasonal patterns for *Nosema* infection levels in the intestines and glands. The number of spores in the intestines were higher in the spring and lower in the fall, while in the glands levels were high in the spring with a second peak in the fall (8). Therefore material gathered in the

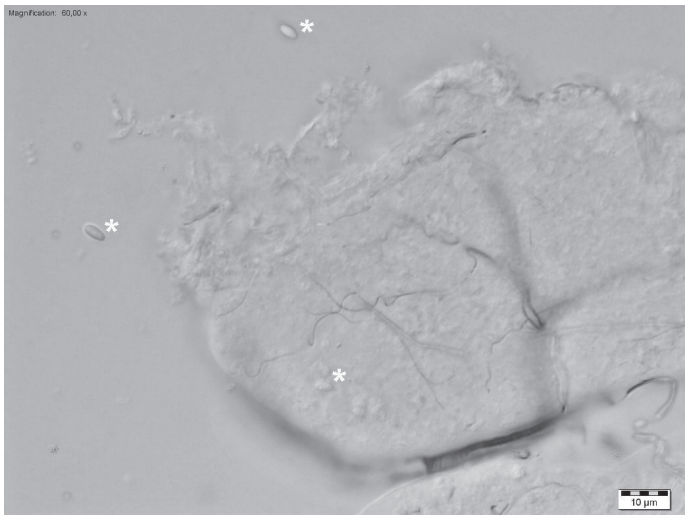

Fig. 1. *Nosema* spores (indicated by the asterisks*) in the hypopharyngeal glands (differential interference contrast – DIC microscopy)

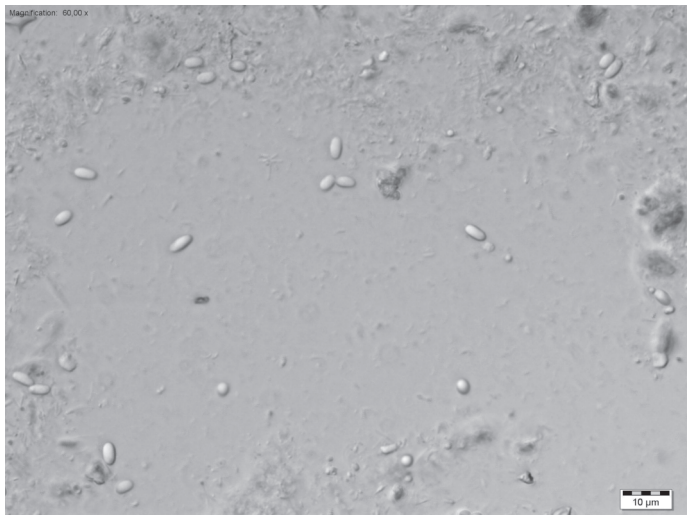

Fig. 2. *Nosema* spores in the ventriculus (differential interference contrast – DIC microscopy)

Tab. 1. Number of *Nosema* spp. spores in different parts of bees' body (mln)

Part of bees body	Mean	CV	SE	Min	Max.
WB	35.63[a]	21	1.67	30.50	56.80
VE	47.68[b]	11	1.26	40.20	62.30
HG	22.46[c]	16	0.83	18.70	32.40

Explanations: WB – whole bees; VE – ventriculus; HG – hypopharyngeal glands; CV – coefficient of variation; SE – standard error; Min. – minimum value of the features; Max. – maximum value of the features; a, b, c – significant differences in the columns at $p \leq 0.05$

Tab. 2. Correlation between number of *Nosema* spp. spores and the part of bees body

Part of bees	WB	VE
VE	0.95*	
HG	0.98*	0.97*

Explanations:* – significance at $p \leq 0.05$

spring should have a comparable degree of *Nosema* infection level. In our study, similarly as in Copley and Jabaji's (8) research, the number of spores in HG (Fig. 1) was the lowest and the highest results were observed for VE samples (Tab. 1, Fig. 2).

The coefficient of variation (CV) has the highest value for WB and the lowest for VE. For this reason, counting spores in the VE can be recommended for an estimation of the degree of *Nosema* infection. However, due to the problems with preparation of VE this method is not widely used. In WB, counted number of *Nosema* spores are the average of the infected and uninfected tissues (such as muscles or nervous system).

Data indicate that spores number in glands is correlated with the overall level of bee infections (Tab. 2). According to data obtained in the conducted experiments, a large number of spores in the hypopharyngeal glands can lead to reduction or loss of their function, such as royal jelly secretion, honey and bee bread production. This corresponds to recent data gained by quantitative real-time PCR (8).

Recently published research confirms that spores and/or the vegetative stage of *Nosema* species are present within the glands; however, evidence that spores become replicated in these tissues is still lacking (8). This may explain the lower number of spores in the HG than in the VE, where *Nosema* undergoes the full cycle of development and where new spores are released. Nevertheless, the observation about the lower number of spores in HG than in VE should be confirmed by other research. Especially if the study on changes in HG structure after *Nosema* infection (which suggests the complete infection of these structures) would be taken into account (29). Moreover, the changes in the hypopharyngeal glands might not be caused only by the nosemosis but are the result of malnutrition and exhaustion of infected bee organisms. Glands would then act only as a reservoir of the *Nosema* spores.

Conclusion

Nosemosis is a serious disease of honey bees. It causes a number of changes in the colony as well as at the individual bee level (increased winter mortality, reduced honey yield, poor spring colony build-up and reduced lifespan of bees; it is the reason for faster physiological ageing, supersedure of infected queens, atrophy of hypopharayngeal glands). Combating this disease is difficult because spores are resistant to environmental conditions and under favorable circumstances retain the ability to cause the infection even after 6 years (30). *Nosema*-infected bees die in a very short time, within 8-10 days. To date, no effective drug has been found for nosemosis which would be harmless to bees and also did not penetrate into the honey. Therefore each aspect of this disease should be studied: especially infected glands, which can act as reservoir for both *Nosema* species and may increase the likelihood of this disease's dispersal via horizontal transmission within the hive as well as to the queen.

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