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NOSEMOSIS IN HONEY BEES

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K e y w o r d s: Microsporidia, *Nosema apis, Nosema ceranae, Apis mellifera, Nosemosis*.

A b s t r a c t

Microsporidia (*phylum Microsporidia*) are single-celled eukaryotic organisms and obligate intercellular parasites that produce spores. They are classified under the *Fungi* kingdom. In honey bees (*Apis mellifera*), microsporidian infections are caused by *Nosema apis* and *Nosema ceranae*. Bees are infected per os by food contaminated with spores. Spores were observed in intestinal epithelial cells, the Malphigian tubule system, salivary glands and fat bodies. Nosemosis symptoms include digestive and absorption disorders because spores damage epithelial tissue of the alimentary canal that is responsible for food absorption. *Nosema* spp. spores are routinely determined under a light microscope, and they are identified to species level by PCR with the use of 16S rRNA primers designed for small subunits. Nosemosis treatments are regulated by European Union directives and recommendations of the World Health Organization.

NOSEMOZA PSZCZÓŁ

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A b s t r a k t

Microsporidia (gromada *Microsporidia*) należą do eukariotycznych organizmów jednokomórkowych, które są obligatoryjnymi wewnątrzkomórkowymi pasożytami wytwarzającymi spory. Zaliczane są do grzybów (*Fungi*). Przedstawicielem Mikrosporidii występujących u pszczoły miodnej (*Apis mellifera*) jest *Nosema apis i Nosema ceranae*. Do zarażenia dochodzi drogą *per os* po spożyciu zakażonego sporami pokarmu. Stwierdzono występowanie spor w komórkach nabłonka jelit, w cewkach

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Malpighiego, gruczołach ślinowych, ciele tłuszczowym. Objawy nosemozy związane są z zaburzonymi procesami trawienia i przyswajania pokarmu, ponieważ *Nosema* uszkadza nabłonek w przewodzie pokarmowym. Do rutynowego rozpoznania sporowców *Nosema* spp. używa się mikroskopu świetlnego, a przynależność gatunkową potwierdza się za pomocą metod PCR, wykorzystując startery zaprojektowane dla małej podjednostki 16S rRNA. Leczenie nosemozy jest objęte dyrektywami Unii Europejskiej oraz zaleceniami Międzynarodowej Organizacji Zdrowia.

Recent years witnessed an increase in bee mortality in many regions of the world. The drop in bee populations is associated with viral, fungal and parasitic diseases, pesticide poisoning, monoculture farming and pollen shortage. The direct cause of the colony collapse disorder (CCD), a phenomenon in which entire colonies abruptly disappear from a beehive without an apparent reason (HIGES et al 2008b, 2009), has not been identified to date. Similar changes in bee colonies had been reported earlier, but scientists were unable to find any links between those processes (HIGES et al. 2006, PORRINI et al. 2010). CCD is caused by a combination of many factors (COX-FOXTER 2007). One of the main causes of the syndrome are spores of the genus *Nosema* spp. (PAXTON 2010, CHAIMANEE et al. 2010), including *N. apis* and *N. ceranae* fungi (kingdom *Fungi*, phylum *Microsporidia*, family *Nosematidae*, genus *Nosema*). The above parasites cause nosemosis (*Nosemosis apium*), a microsporidian infection that affects adult bees (SNEATH and SOKAL 1973, FRIES 2010).

Microsporidia (class *Microsporea*) are single-celled eukaryotic organisms and obligate intercellular parasites that produce spores. They colonize both vertebrates and invertebrates, and their spores are characterized by the presence of polar fibers (GRACZYK et al. 2007). Microsporidia are widely distributed in nature, and they comprise more than 1200 species, of which at least 14 are dangerous for humans. Patients with impaired immune function, in particular HIV carriers, and organ transplant patients are particularly susceptible to microsporidian infections (DONG et al. 2010, GRACZYK et al. 2007).

The first microsporidian species of *Nosema bombycis* was discovered in the silkworm (*Bombyx mori*) and described by Nageli in 1857 (NAGELI 1857). In the same year, Donhoff performed a microscopic analysis of small formations isolated from the intestines of adult bees and identified them as fungi. Pasteur discovered many infectious diseases caused by pathogens, including *N. bombycis*, in silkworms. In 1909, Zander identified the spores present in bee intestines as parasites and named them *Nosema apis* (ZANDER 1909). It is generally believed that Danhoff and Zander identified the same parasite. *N. bombycis* is transmitted transovarially in 100%, whereas other *Nosema species* – in only 1.2% (HAN and WATANABE 1988) – Table 1.

Table 1

The presence of *Nosema* spp. species of insects

Host	Species *Nosema*
Mosquito (*Culex* spp.)	*N. algerae*
Mullbery silkworm (*Bombyx mori*)	*N. bombycis*
Honey bee (*Apis mellifra*)	*N. apis* *N. ceranae*
Locust (*Locusta migrateria*)	*N. locustae* *N. grylli*
Fly (*Drosophila melanogaster*)	*N. kingii*
Chrysolina beetle (*Chrysolina spp.*)	*N. coulloudi*
Wasp (*Vespula*)	*N. vepsula*
Moth (*Heterocera spp.*)	*N. lymantriae* *N. serbica*

In honey bees (*Apis mellifera*), microsporidian infections are caused by *Nosema apis* and *Nosema ceranae*. Honey bees initially colonized only Africa, the Near East and Europe. They were introduced to America, Australia and Asia by colonizers those regions. Today, honey bees inhabit geographically diverse areas, and the species of *A. mellifera* includes various African, Oriental and European breeds. Numerous breeding lines have been engineered by humans, in particular in European breeds (TOMASZEWSKA and CHORBIŃSKI 2000). Nosema infections were observed in the following bee species: *Apis mellifera, A. ceranae, A. florea* and *A. dorsata* (CHAIMANEE et al. 2010).

Development of Nosema spores in bees

Bees are infected orally by food contaminated with spores (CHEN et al. 2008, WEBSTER et al. 2004). The optimal temperature for spore growth in bee intestines is 30–34°C, and spores remain active for more than seven months. In bees, the spread of nosemosis is determined mainly by weather conditions during various seasons of the year. Nosemosis caused by *N. apis* develops on a seasonal basis, and the highest prevalence of the disease is noted in spring when bee populations increase. The pathogenic process is stabilized in summer when infection levels are low. A repeated increase in pathogen counts is noted in fall (GAJDA 2010, HIGES et al. 2006). Recent research indicates that *N. ceranae* infections are more prevalent in *A. mellifera* than infections caused by *N. apis*. Bees infected by *N. ceranae* quickly die, usually outside the hive, without displaying any clinical symptoms (CHEN et al. 2009, FORSGREN and FRIES 2010, HIGES et al. 2007, PAXTON et al. 2007). The prevalence

of *N. ceranae* infections remains similar throughout the honey season, which is the main distinguishing feature from infections caused by *N. apis* (KLEE et al. 2007, MARTIN-HERNANDEZ et al. 2007). The discussed parasites also differ in the length of their developmental cycle, which has been determined at five days in *N. apis* and three days in *N. ceranae*. Intestinal epithelial cells become infected already three days after the parasitic attack, and the insect usually dies within nine days, especially in the presence of other stressors such as bacteria or viruses. The analyzed pathogens were identified not only in intestinal epithelial cells, but also in the Malphigian tubule system, salivary glands and fat bodies (CHEN and HUANG 2010).

The developmental cycle of *Nosema* spp. takes place in several stages. The spore ejects a polar filament upon entering the insect's middle intestine. A planont, a motile amoeboid form of the parasite measuring approximately 1 μ, emerges from the capsule. Initially, the planont has two nuclei that are later merged. The planont penetrates mid-intestinal epithelial tissue where it feeds and loses motility. The fungus grows and begins to divide. Multiplying cells are known as meronts. Meronts have the size of 3.3–7.5 μ. Multiplying meronts fill the entire epithelial cell, destroy its protoplasm and, in some cases, damage the nucleus. In an unsupportive environment, meronts cease to multiply and turn into spores. This transformation takes place in three ways. In the first case, the meront nucleus becomes elongated and narrow, and it divides to produce two daughter cells. Each daughter cell gives rise to a sporoblast that is transformed into a spore. In the second case, the meront nucleus becomes elongated and undergoes multiple division. This process gives rise to multinucleate meronts, and the number of produced spores is equal to the number of nuclei. In the third case, the meront preserves its round shape, the nucleus undergoes multiple division to create multinucleate plasmodium. The number of spores formed inside the plasmodium is equal to the number of nuclei. Unlike meront nuclei, sporont and sporoblast (intermediate forms between a meront and a spore) nuclei do not have a protective capsule, and they comprise numerous separate granules grouped in clusters. Developing spores fill the entire mid-intestinal epithelial cell that eventually dies and exfoliates into the intestinal lumen where it disintegrates or is evacuated with other spores (DIDIER et al. 2000) – Figure 1.

The symptoms of *N. apis* infections include digestive and absorption disorders because spores damage epithelial cells of the alimentary canal that is responsible for food absorption. Infected bees excrete large amounts of sweet watery stool (undigested food). The feces of diseased individuals contain large numbers of spores, and they are the main source of infection. Infected feces are excreted by bees during flights to the apiary and water sources, and in unfavorable weather conditions, also inside the hive – on honeycombs, honey

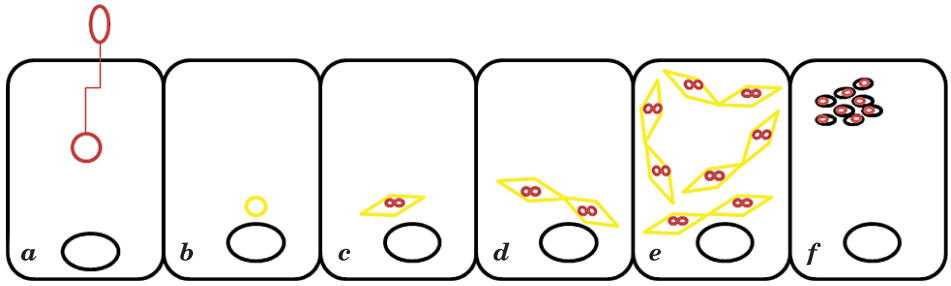

Fig. 1. Development scheme *N. apis* i *N. ceranae* in the midgut epithelial cells of bees: *a* – penetration of polar spores into the host cell; *b* – sporoplazma (round-shaped) in the epithelial cell; *c* – development meronts; *d* – division and the formation meronts; *e* – division meronts; *f* – formation oval sporonts

supers, frame bars, walls and the bottom board. In most cases, the disease spreads when healthy colonies are merged with infected colonies, and when contaminated hive equipment and food reserves are used. Infections are potentiated by stressors, such as the loss of the bee queen, changes in the hive microclimate and the presence of weak colonies that are unable to accumulate the required food reserves, mainly pollen. Symptoms of disease include swollen abdomen and grayish-white discoloration of the middle intestine.

Four stages of *N. ceranae* infection have been identified in *A. mellifera* bees (HIGES et al. 2008). The first stage is asymptomatic, and it lasts from spring to early fall. There are no discernible changes in the size of hive colonies or broods. The second stage is colony replacement, and it is observed between late fall and winter. Bees begin to die when the temperature drops (energy stress), and the queen attempts to make up for that loss by laying more eggs. The size of the bee population remains unchanged, but the brood increases. The queen begin to lays eggs in winter, which is often mistakenly interpreted as a sign of colony health. The third stage is false recovery when hive populations are high and all frames are filled with brood. Despite the large size of colonies, bees do not swarm. The last stage is depopulation, namely the sudden collapse of entire colonies. A small number of bees, the queen and infrequent brood survive the infection. Substantial food reserves are accumulated in the hive. The depopulation stage is observed mainly in fall or early winter. Less virulent infections may lead to colony collapse in spring.

Nosema spp. spores are routinely determined under a light microscope. Analyses are generally performed in early spring on samples collected from winter hive debris (mostly worker bees) (TOPOLSKA and KASPRZAK 2007, MICHALCZYK et al. 2011). *Nosema* spp. parasites are difficult to identify under a light microscope due to minor differences in the anatomy of *N. apis* and

N. ceranae spores. *N. ceranae* spores have the length of 3.3–5.5 μm and the width of 2.3–3.0 μm. *N. apis* spores are larger with the length of 4.6–6.4 μm and the width of 2.5–3.4 μm, they have a regular, cylindrical shape with one slightly tapering end, and they strongly refract light under a light microscope (FRIES et al. 2006). *N. apis* and *N. ceranae* spores have a similar morphological structure, and the main difference that can be observed under an electron microscope is the length of the polar filament (FORSGREN and FRIES 2010). For this reason, spores are identified to species level by PCR with the use of 16S rRNA primers designed for small subunits (HIGES et al. 2006, KASPRZAK and TOPOLSKA 2007). The presence of spores in a bee colony can also be determined in a field test that involves the preparation of mid-intestinal specimens and observations of their color. Healthy bee intestines are yellow to brown in color, whereas infected intestines turn white.

In nurse bees, microsporidian infections inhibit the development of pharyngeal glands that secrete royal jelly, which could disrupt the feeding patterns of queen bees and the brood (GLIŃSKI and RZEDZICKI 1993). *N. apis* infections shorten the average life of worker bees by 20–50% and of queen bees by 30–75%, they lower honey production by 60% and wax production by 25%. In diseased colonies, the brood can be reduced by even 50%, and highly virulent infections may lead to ovarian damage and infertility in queen bees (WEBSTER et al 2004, SAGASTUME et al. 2011).

N. ceranae infections develop rapidly and are highly lethal. Bees die within 8 days after exposure to the pathogen (HIGES et al. 2007). Recent research demonstrated that *N. ceranae* had developed more effective mechanisms of adaptation to changing temperatures than *N. apis*. At temperatures that limit fungal development (25 and 37°C), *N. ceranae* is able to complete its lifecycle, whereas the lifecycle of *N. apis* is inhibited. At the optimal temperature of 33°C, *N. ceranae* is able to destroy 2–3 times more intestinal epithelial cells than *N. apis* (FENOY et al. 2009). Infections caused by *N. ceranae* in bee colonies last one year and can remain asymptotic, whereas the disease spread by *N. apis* disappears in warm months of the year, often at the beginning of the honey season. *N. ceranae* infections do not produce diarrhea, which always accompanies *N. apis* infections, and they are often referred to as „dry" nosemosis (FAUCON 2005, MAYACK and NAUG 2009). In Poland, worker bees are more frequently infected with *N. ceranae* than *N. apis* spores. In a multiplex PCR analysis of 1000 winter hive debris samples, the presence of *Nosema* spp. DNA was found in 806 samples (80.6%), including *N. ceranae* in 206 samples (20.6%), mixed infections (*N. apis* / *N. ceranae*) were noted in 600 samples (60%), 194 samples (19.4%) were free of the analyzed pathogens, and none of the examined samples were infected by *N. apis* only (MICHALCZYK et al. 2011).

Nosemosis treatments are regulated by European Union directives and recommendations of the World Health Organization. The use of pharmacological products such as Fumagilin DCH and Fumidil B is discouraged because their residues may contaminate bee products. Sanitary and preventive methods are recommended to minimize the risk of disease, including stimulating flight activity in early spring, providing hives with adequate sunlight exposure and positioning hives at a distance to prevent bees from entering the wrong hive. In spring, hive debris should be removed to prevent bees from coming into contact with infected individuals in the bottom board. Hive walls and frames contaminated with feces also contribute to the spread of disease, and they should be chemically disinfected. In early spring, healthy bees can be fed water syrup with pollen to stimulate their alimentary canal, and diseased bees should be administered dietary supplements, such as Api-Herb, Nozevit, Vita Feed Gold, Protofil and Noestat. Treatment of nosemosis is difficult and often ineffective. Continuous efforts are being made to identify new substances that effectively treat Nosema infections and are safe for bees and consumers of bee products.

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