

NOSEMA CERANAE FRIES ET AL., 1996 (MICROSPORA, NOSEMATIDAE) – A HONEY BEE PARASITE IN UKRAINE

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For the first time on apiaries of Ukraine in three regions (Kiev, Poltava and Zaporozhya) was found a Microsporidia *Nosema ceranae* (Microspora, Nosematidae) - the pathogen of so-called «Asian» Nosematosis, which is considered to be the one of the causes of mass death of bee families. Clinical signs and course of the Nosematosis in bee families where was found Microsporidia *N. ceranae*, were classical, namely weak development of families in the spring, in some families - the presence of traces of diarrhea. The attention is focused on the necessity to continue research directed on studying of the spread of Microsporidia *N. ceranae* on apiaries of Ukraine and its harmfulness for the beekeeping industry in mono and mixed parasitism along with Microsporidia *N. apis*.

Key words: bees, Nosematosis, Microsporidia, *Nosema ceranae*, spores, symptoms, diagnostics, Ukraine.

***Nosema ceranae* Fries et al., 1996 (Microspora, Nosematidae) – паразит медоносної бджоли в Україні. Єфіменко Т. М., Односум Г. В., Токарев Ю. С., Ігнат'єва А. Н.**

Вперше на території України на пасіках в трьох областях (Київській, Полтавській та Запорізькій) виявлено мікроспоридію *N. ceranae* (Microspora, Nosematidae) – збудника так званого «азіатського» нозематозу бджіл, що вважається однією з причин масової загибелі бджолиних сімей. Клінічні ознаки та перебіг нозематозу в сім'ях, в яких було виявлено мікроспоридію *N. ceranae*, були класичними, а саме: слабкий розвиток сімей весною, в окремих сім'ях – присутність слідів проносу. Акцентується увага на необхідності продовжити дослідження, направлені на вивчення поширення мікроспоридії *N. ceranae* на пасіках в Україні та її шкодочинності для галузі бджільництва при моно та змішаному паразитуванні разом з мікроспоридією *Nosema apis*.

Ключові слова: бджоли, нозематоз, мікроспоридія, *Nosema ceranae*, спори, симптоми, діагностика, Україна.

***Nosema ceranae* Fries et al., 1996 (Microspora, Nosematidae) – паразит медоносної пчели в Україні. Єфіменко Т. М., Односум А. В., Токарев Ю. С., Ігнат'єва А. Н.**

Впервые на территории Украины на пасеках в трех областях (Киевской, Полтавской и Запорожской) обнаружено микроспоридию *N. ceranae* – возбудителя так называемого «азиатского» нозематоза пчел, который считается одной из причин массовой гибели пчелиных семей. Клинические признаки и течение нозематоза в семьях, в которых было обнаружено микроспоридию *N. ceranae*, были классическими, а именно: слабое развитие семей весной, в отдельных семьях – присутствие следов поноса. Акцентируется внимание на необходимости продолжить исследования, направленные на изучение распространения микроспоридии *N. ceranae* на пасеках в Украине и ее вредоносности для отрасли пчеловодства при моно и смешанном паразитировании вместе с микроспоридией *Nosema apis*.

Ключевые слова: пчелы, нозематоз, микроспоридия, *Nosema ceranae*, споры, симптомы, диагностика, Украина.

Nosematosis is a widespread disease of the European honey bee *Apis mellifera* (Linnaeus, 1758), periodically causing mass death of bee families on apiaries (Гробов и др., 1987; Genetic..., 2009). Traditionally, the causative agent of nosematosis of the honeybee considered to be the one kind of Microsporidia - *Nosema apis* (Zander, 1909). Recent studies, however, showed a wide spread all over the world of another parasite, *Nosema ceranae* Fries et al., 1996 (*Nosema ceranae*..., 1996; Outcome..., 2007), the causative agent of so-called «Asian» nosema. *Nosema ceranae* is a microsporidia, originally described as a parasite of the Asian honeybee *Apis cerana* Fabricius, 1793 (*Nosema ceranae*..., 1996). Later the microsporidia *N. ceranae* was detected in the European honeybee on all continents, where the beekeeping is developed (Widespread..., 2007). Moreover, in a number of countries the causative agent of bees' nosematosis is registered exclusively or predominantly by *N. ceranae*. In particular, throughout the USA (Asymmetrical..., 2009) and the countries of the Balkan Peninsula (Dominance..., 2011) it is registered on one case of bees infection by *N.*

apis, in all the other samples, the number of which there are dozens, were identified as the infection by *N. ceranae*. Similarly in Japan, during the analysis of 336 bees samples from 18 prefectures *N. ceranae* is met in 6 samples from 3 prefectures, whereas *N. apis* is never met (Yoshiyama, Kimura, 2011). In Europe, bees' microsporidia infection is investigated in the majority of the EU countries. Pay attention to the distribution of *N. ceranae* in the European part of the Eurasian continent (fig. 1).

However, there remains a vast unexplored territory, stretching to the East and including the countries of Baltic states, Ukraine, Belorussia, the majority of Russia, Moldova, the countries of Southern Caucasus, Kazakhstan, Uzbekistan, etc. For today it remains under-researched, including a number of other issues, including: (1) how long microsporidia *N. ceranae* became widespread in the world as the parasite of *A. mellifera*; (2) what conditions are favorable for development of the nosematosis caused by *N. ceranae*; (3) the mutual relations between *N. ceranae* and *N. apis* at the joint infection of bees; (4) whether nosematosis caused by *N. ceranae* is a threat to the bee-keeping,



Fig. 1 Distribution of two causative agents of nosematosis of a honeybee in Europe (Widespread..., 2007; Kasprzak, Topolska, 2007; Sudden..., 2010; Dominance..., 2011).

Рис. 1 Распространение обоих возбудителей нозематоза медоносной пчелы в Европе (Widespread..., 2007; Kasprzak, Topolska, 2007; Sudden..., 2010; Dominance..., 2011).

etc. In connection with foregoing, an important theoretical and practical value is presented by researches on the study of prevalence of *N. ceranae* on apiaries in Ukraine. For this reason the aim of our researches was: to study – whether there is present microsporidia *N. ceranae* in Ukraine.

Materials and Methods

The **task** of our research included:

1) Select samples of live and dead bees with symptoms and without nose matosis symptoms from apiaries of geographically distant areas of Ukraine.

2) To receive detailed information about the peculiarities of bees' cultivation, apiary history, the condition of bee families, clinical symptoms of the nose matosis, held events for prevention and treatment of this disease (the Laboratory of Bee Pathology of Ukraine).

3) To perform microscopic and molecular diagnostics of the selected samples of bees about their infection with microsporidia *N. ceranae* (the Microbiomethod laboratory of Russia).

4) To study the influence of doses of infection by spores of *N. ceranae* on a bees' lifespan (Laboratory of Bee Pathology, Ukraine).

Wishing to find out whether *N. ceranae* is present in samples of bees in apiaries of

Ukraine, last spring we sampled podmore bees in a large extent affected by nose matosis, from three regions of Ukraine – Kiev (average sample of bees from three apiaries of the suburb of Kiev), Poltava (average sample of two apiaries of Gadyach) and Zaporozhyha (average sample of bees from the apiary of the suburb of Meliopol).

After the microscopic analysis the part of the material was transferred to St. Petersburg, and other spores were isolated and purified by standard techniques for laboratory researches. In St. Petersburg morphometric analysis of spores of the parasite was carried out by microscoping of the drop of suspension of spores in the bright field of the light microscope Carl Zeiss Axio 10 Imager M1 in appendix of Carl Zeiss AxioVision Rel. 4.6. For these purpose bees abdomens from the podmore samples were triturated in a porcelain mortar along with distilled water at the rate of 1 ml per abdomen.

As a standard it used samples of spores of *N. apis* and *N. ceranae*, specifically which is set by the methods of the light microscopy and molecular phylogeny (Tokarev et al., 2010; «Asian»..., 2011; Ignatieva et al., 2012).

The research results

In all analyzed samples of bees from the three regions of Ukraine (central - Kyiv and



Fig. 2. The regions of Ukraine which snfected by the spores of *N. ceranae*.

Рис. 2. Регионы Украины зараженные спорами *N. ceranae*.

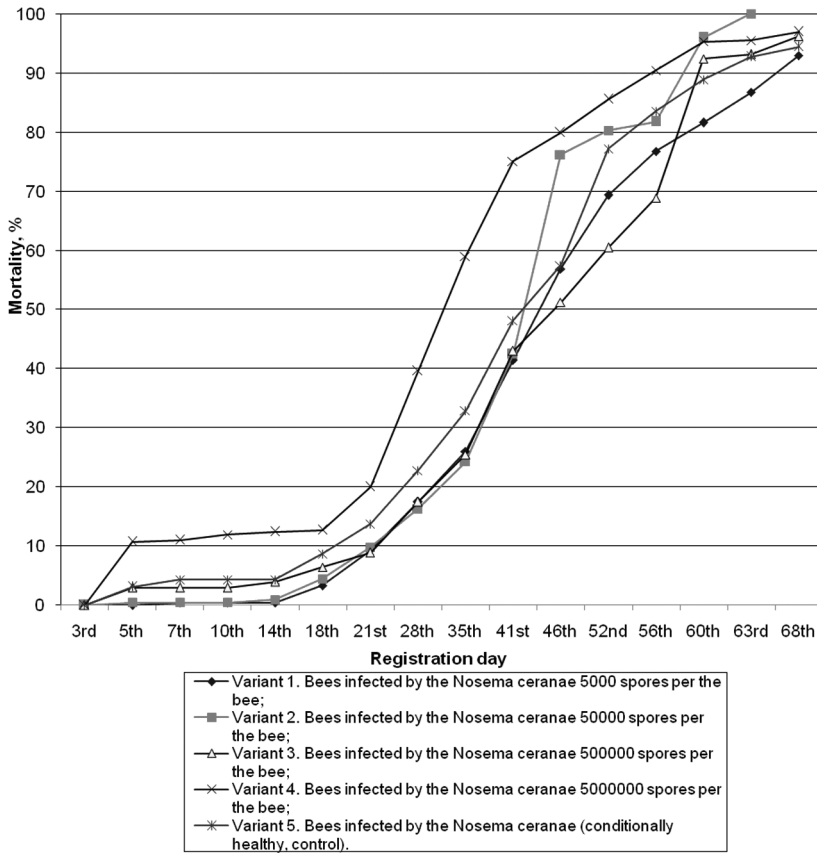


Fig. 3 Dynamics of withering away of bees at infection with different doses (5000 – 5000000 spores per individual) spores of microsporidia *N. ceranae*.

Рис. 3 Динамика отмирання пчел при зараженні різними дозами (5000-5000000 спор на особь) спор мікроспориї *N. ceranae*.

Poltava, and south - Zaporozhyha) were found spores of microsporidia from 10 to 500 spores per field of the view (at a magnification of 400 times). The morphometric analysis showed the accordance of size characteristics of identified spores (length, width, length-to-width and shape) to the indicators specific to *N. ceranae*. As statistically reliable distinction of dimensional characteristics of morphotypes, spores which belong to *N. apis* and *N. ceranae*, were established by a method of sequencing of ribosomal RNA gene region, so the revealed spores where identified as *N. ceranae*. There are marked fields on the map of Ukraine where is established the infection of bees by the spores of *N. ceranae* is established (fig. 2).

Furthermore, laboratory experiments showed that the feeding bees along with sugar syrup by low doses of *N. ceranae* spores (up to

10^5 spores on the bee) prolong the life of the bee and high doses (10^6 - 10^7 spores on the bee), vice versa, reduce it (fig. 3).

Discussion

Considering, that there were spores of *N. ceranae* in the analyzed samples of bees from the three regions of Ukraine it is possible to assume that this parasite is widespread on apiaries in Ukraine, irrespective of a climatic zone. We plan further joint researches on studying of this question with group of microsporidologists from the All-Russia Institute of Plant Protection, which have earlier shown the prevalence of *N. ceranae* in the south regions of Russia (Tokarev et al., 2010; «Asian»..., 2011; Ignatieva et al., 2012).

It should be noted that the flow of the nose-matosis on apiaries, where samples were taken, was classic, namely weak development of fam-

ilies in the spring, in some families - is a presence of tracks of diarrhea.

We pay attention to the fact that the presence of traces of diarrhea is not always a characteristic feature of nosematosis as at parasitizing by *N. apis* (observations of 20 years ago), so at parasitizing by *N. ceranae* (observing the last 10 years). The clinical signs of this disease first of all depend on the dose of infection of bees by the spores of parasite, whether it is a microsporidia *N. apis* or *N. ceranae*.

It is desirable also to underline that both these parasites do not have their own mitochondria and live exceptionally due to the energy of the host, which is why the disease occurs usually in the spring – in the period of the high energy loss, which is connected with the beginning of growing of the brood and the need to maintain an optimal climate in the nest.

The manifestation of the disease in the other time of the year, mainly in the autumn, according to our observations, it is extremely rarely at infection with two species of microsporidia - and *N. ceranae*, and *N. apis* - and it is conditioned by a high percentage of Varroosis caused by the mite *Varroa destructor* Anderson & Trueman, 2000, the removal of which, as a rule is delayed until the end of honey extraction, and this is September.

We wish to stop also on medical and prophylactic measures at bees nosematosis.

Antibiotics are forbidden for the use in the countries of Europe and in Ukraine. And it is quite justified. Data of our experiments testify that neither fumagillin, nor tetracycline or other antibiotics don't suppress completely the development of any of microsporidia, and their medical effect is caused generally by suppression of intestinal microflora, which is involved in pathological process during the nosematosis. That is why the Laboratory of Bee Pathology NSC «Institute of beekeeping them. P. I. Prokopovich» for over 10 years worked under the selection of plants, extracts from which have antiseptic and antiparasitic effect and in no way inferior to the effectiveness of fumagillin or other antibiotics. We recommend a number of plant extracts for the additional fertilizing of bees in autumn – during the replenishment of food stocks for the winter, and also in the spring – in a period of increase of bees to the spring

honeyflow. In particular, high efficiency was obtained when feeding 0,1% of an extract (by the dry substance) of the wormwood *Artemisia absinthium* L. (no less than 30% of a medical forage from the general stock of forages for the winter).

Disinfection of those beehives which occupied by the bee families with clinical signs of nosematosis has to be obligatory reception on an apiary. For this purpose it is enough to use 4 - 5% alkaline solution which completely allows deactivating microsporidia spores, bacteria and fungi. In sick on nosematosis bee families it is desirable to replace the queen bee.

In summary, we wish to underline that no matter how long ago did not work out the host-parasite relationship between the honey bee and microsporidia *N. ceranae* or *N. apis*, this relationship is only at the first sight might seem to be “peaceful”, because any stress factor like infection of bee pathogens of the other nature, a high degree of bees invasion by the mite *Varroa destructor*, feed with the admixture of honeydew, the toxic load on bees from pesticides, weak families, unable to support the microclimate of the beehive, late feeding to bees of sugar syrup can easily shift this balance and cause mass death of bees.

For this reason it is so important to take this disease seriously. We consider a necessity to continue studies of the distribution of microsporidia of *N. ceranae* on the territory of Ukraine and its harmfulness for beekeeping.

Conclusion

For the first time on apiaries of Ukraine geographically remote regions was found a Microsporidia *N. ceranae* - bees' Nosematosis pathogen, which is known as «Asian».

Clinical signs and course of the Nosematosis in bee families from which were selected samples were classical, namely weak development of families in the spring, in some families - the presence of traces of diarrhea.

We consider a necessity to continue research directed on studying of the spread of Microsporidia *N. ceranae* on apiaries of Ukraine and its harmfulness for the beekeeping industry in mono and mixed parasitism along with Microsporidia *N. apis*.

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