

Nosema ceranae in Ukraine

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Nosematosis is a widespread disease of the European honey bee *Apis mellifera* L., periodically causing mass death of bee families on apiaries (Гробов и др., 1987; Bourgeois et al., 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, Feng, da Silva, Slemenda, Pieniasek 1996; Martin-Hernandez et al., 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* F. (Fries et al., 1996). Later the microsporidia *N. ceranae* was detected in the European honeybee on all continents, where the beekeeping is developed (Klee et al., 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 (Chen et al., 2009) and the countries of the Balkan Peninsula (Stefanovic et al., 2010) 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**).



Fig. 1. Distribution of two causative agents of nosematosis of a honeybee in Europe (Klee et al., 2007; Topolska, Kasprzak, 2007; Bacandritsos et al., 2010; Stefanovic et al., 2010).

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, Kyrgyzstan, etc. regions. 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, 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.

Researches became possible thanks to the contract about the creative collaboration between the Laboratory of Bee Pathology NSC "Institute of beekeeping them. P. I. Prokopovich" (Kyiv, Ukraine) and the Microbiomethod laboratory of All-Russia Institute of Plant Protection (St-Petersburg, Russia), supported by a grant of Russian Foundation for Basic Research (RFBR) №07-04-00269 and a grant of the President of the Russian Federation №MK-3419.2009.4, research of the Ukrainian group conducted exceptionally on the enthusiasm and scientific initiative.

The task of our research included:

- 1) Select samples of live and dead bees with symptoms and without nosematosis 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 nosematosis, 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).

Besides, the Laboratory of Bee Pathology (Ukraine) made an experiment on studying of the influence of doses of infection by spores of *N. ceranae* on longevity of bees.

Materials and Methods

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 nosematosis, 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 Zaporozhya (average sample of bees from the apiary of the suburb of Melitopol).

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 the 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; Zinatullina et al., 2011; Ignatieva et al., 2012).

The research results

In all analyzed samples of bees from the three regions of Ukraine (central - Kyiv and Poltava, and south - Zaporozhyia) 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).



Fig. 2. Infected regions of Ukraine by the spores of *N. ceranae*.

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 microsporidiologists 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; Zinatullina et al., 2011; Ignatieva et al., 2012).

It should be noted that the flow of the nosematosis on apiaries, where samples were taken, was classic, namely weak development of families in the spring, in some families - is a presence of tracks of diarrhoea.

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. Low doses (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**).

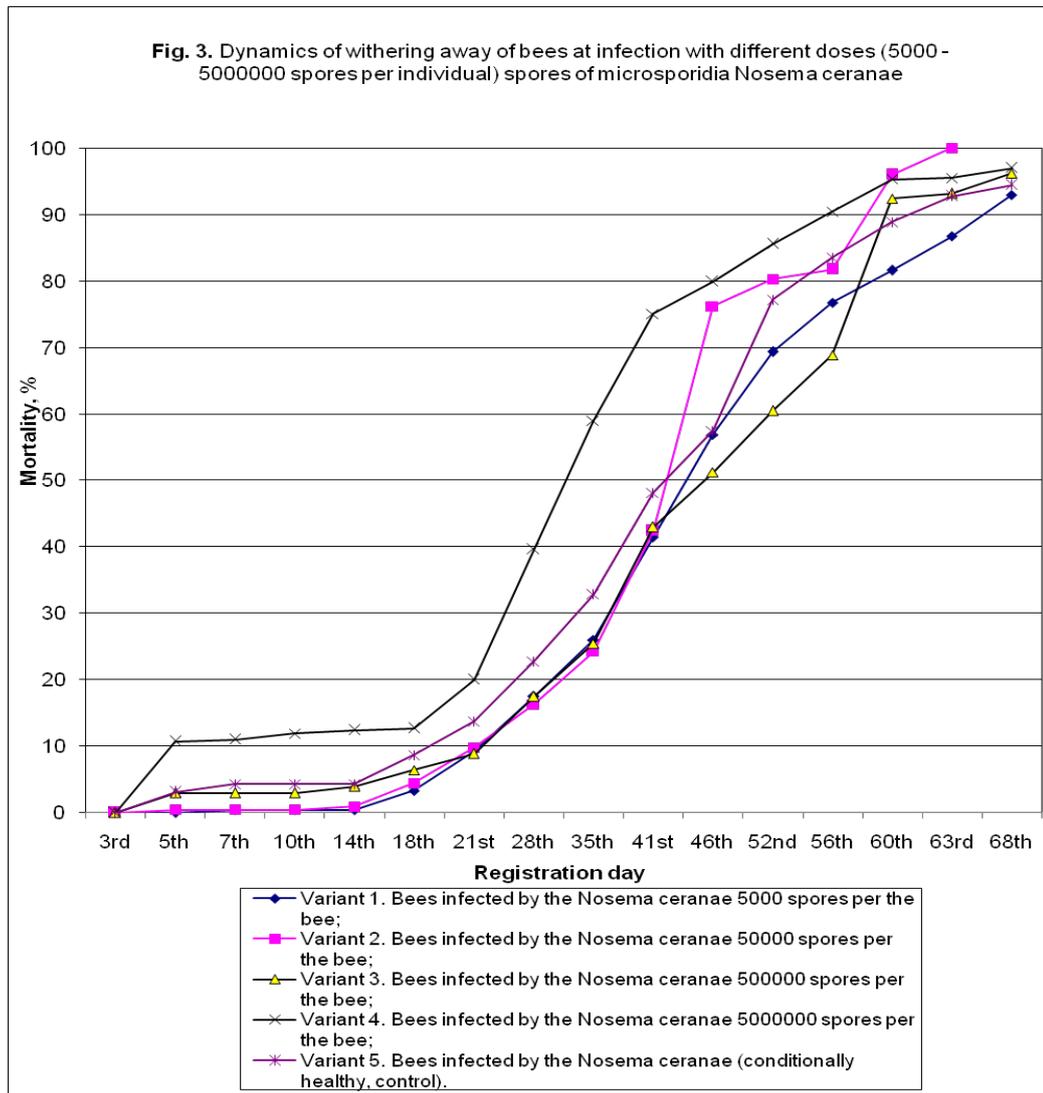


Fig. 3. Dynamics of withering away of bees at infection with different doses (5000 – 5000000 spores per individual) spores of microsporidia *Nosema ceranae*.

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 varroatosis caused by the mite *Varroa destructor*, the removal of which, as a rule is delayed until the end of honey extraction, and this is September.

I 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 to deactivate microsporidia spores, bacteria and fungi. In sick on nosematosis bee families it is desirable to replace the queen bee.

In summary, I 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.

Thank you for your attention!!!

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