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REASONS OF WEAKENING AND LOSSES OF HONEY BEE COLONIES*

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SUMMARY: In recent years, bee colonies are weakening and disappearing around the world, including Serbia. Very often they go unregistered because beekeepers considered they are a disgrace. The paper presents the main reasons of weakening and losses of bee colonies. The key problem remains the pathogens (parasites, bacteria, viruses...), global climate changes and often poor bee grazing, application of pesticides to protect crops and fruit, and others. With introduction of new laboratory diagnostics (PCR) in samples submitted from weakened and dead colonies in Serbia a new pathogens were established (*Nosema ceranae*) and almost all key virus: *Acute bee paralysis virus*, *Chronic bee paralysis virus*, *Sacbrood bee virus*, *Deformed wing virus*, *Black queen cell virus* and *Kashmir bee virus*. Reasons for bee colonies weakening and losses were analyzed and recommendations of biological methods for bees and brood combating against diseases with aim to protect and improve the health of honey bee colonies and gaining quality and safe bee products in Serbia are given.

Key words: Bee colonies, viruses, PCR.

Introduction

Decline in the number of colonies was observed in recent years in many countries around the world. Significant losses are recorded in the Republic of Serbia, where winter deaths during 2007/2008, 2008/2009 ranged from 30 to 70% of the bee colonies in some municipalities. The main cause of bee colonies decline was thought to be the parasites, such as mites, which is in the most cases associated with secondary infection caused by a bee viruses and the losses caused by *Nosema* spp. The results indicate the prevalence of parasitic mite, *Varroa destructor*, in almost every bee in each hive. An essential factor in the weakening of colonies was stress which they were exposed to. The most important stress factors are *Varroa destructor*, which is present in almost every hive; uncontrolled use of white sugar; pollen substitute and more intensive use of pesticides to protect crops and fruits. All this affect on reduce of the amount of proteins and protective substances in the body of the bees, this reducing the defensive ability of bees.

Numerous studies on the hive problems indicate that the losses are most often a combination of several causes, including disappearance of habitat, climate change, diseases, and the increasing use of pesticides to protect crops and fruit. The destruction of the natural habitat of bees is considered one of the major cause of reduction in the number of pollinators.

It is known that healthy bee colony requires a good quality bee pasture, which suppose to be a sources of natural nectar and pollen to prevent a lack of nutrients and improve the immune system of bees. Unfortunately, in past few years we are witness of problems resulting from poor pasture. In fact, due to climate change, the long dry periods and high temperatures, long period without bee pasture; resulted with poor quality summer bees, which can not properly prepare hives for winter time.

They create winter bees with a very low fat reserves and poorly expressed hypopharyngeal glands, and they can not survive the winter. Because of their strong instinct for maintaining species, parents in these societies starts to lay eggs very early, sometimes even in December and January. Cultivating honey bees brood requires lot of energy and exhausts the bees. If the bee community have enough food, pollen and honey, societe can slowly recover, but in hives where there was not enough food, mortality and other problems arise during the spring season.

In this period the presence of *Nosema exacerbatas*, involves the health of bee colonies, but we can notice the absence of diarrhea. The deteriorated state of health will be even worse to the spring, until the final decay.

Nosema is a parasitic disease of adult bees caused by microsporidial *N. Apis* [21] and *N. Ceranae* [5]. Taxonomy classifies the genus *Nosema* in the group of protozoa, but new molecular diagnostic methods found to show more similarities with fungi [16]. *Nosema sp.*, is a microscopic spore-forming parasite which attacks the lining of the middle intestine of worker bees, queens and drones [6]. *Nosema ceranae* compared to *Nosema apis* is a bigger health problem for entire bee community [9]. Infection with *Nosema apis* mostly occurs in early spring or late fall [3], and this cause of disease is considered as mildly virulent, without serious consequences for the colonies.

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Infection with *Nosema ceranae* can occur throughout the all year [9]. The disease is chronic and asymptomatic, and the cause is considered to be highly pathogenic for infected colonies [2]. The disease is spread all over the world [12], and make significant economic damages.

Losses are reflected in a reduced amounts in collected honey and other bee products, and decrement quality of fruits and crops [8].

A *Nosema apis* infection prevents the development of the glands that secrete royal jelly, leading to sick bees unable to feed the brood, and they become collectors very early [5]. Pathological changes in their mid-gut epithelial cells cause digestive and metabolic disorders, as well as malnutrition leading to the premature deaths of bees and decreases in population sizes of honey bee colonies [13]. The disease is often referred to as the "Silent Killer" [11], because the absence of obvious signs means the disease is often not noticed and because affected honey bees tend to die of exhaustion away from the hive.

It appears that *N. Ceranae* is an emerging pathogen that has increased its distribution and it may be displacing *N. Apis* in Europe. *N. Ceranae* is highly pathogenic, especially for new hosts like *A. Mellifera* [10].

In North America [20], as well as in Europe [14, 7] is observed that outbreaks of disease caused by the *Nosema ceranae* is more common in warmer, and disease caused by *N. Apis* frequently occurs in colder climates.

Nosema ceranae also has been confirmed in other organs and tissues of bees-malpighian tubules, adipose tissue and mammary gland [1]. Such altered tropism causes various pathological changes, which contributes to a worse clinical disease in which do not exist or are not visible signs of the disease typical to *Nosema*, as diarrhea [6, 10], it is even possible to register cases of constipation that occurs as a result of the accumulation of a large number of spores in the digestive tract. Degenerative pathological processes are inflation of the walls of small intestine, decreased absorption of nutrients or poor utilization of ingested food. Due to the deterioration of the infected epithelial cell function and reduces the secretion of digestive enzymes. This leads to a decreasing of reserves of proteins and fats, that affect the development of the glands that secrete royal jelly which is the main food for the brood and queen, this results with cannibalism.

The aim of this study was to analyze the key aspects of death of honey bee colonies in several apiaries where more than 90% of the colonies were weakened or completely died. In Vojvodina is proven 5 of 6 key bee virus [17]. On the other hand, the problem of *Nosema ceranae*, which is analyzed in the experimental apiary, 20 bee colonies were analyzed for the presence of *Nosema ceranae* by the method [19]. It has been found than 97 million spores per bee, but without the presence of viruses. Bee communities treated with therapeutic agents recovered very well.

Material and Method

As the material for this study used samples of bees, taken from 10 bee colonies from the locality that was extremely low and wet and 10 samples of bee colonies that is placed on more favorable area, and which one is treated with natural products KAS-81 (decoction obtained of pine shoots and wormwood) in the autumn.

For analyses was taken samples of 50 elderly bees from edges of frames. Microscopic counting of spores using hemocytometer was performed according to the instructions *Testing for Nosema Spores using Hemacytometer, Instructional Poster* [19]. From each sample it was taken 25 bees, and prepared a macerate from abdomens and distilled water at a rate of 0.5 ml / bee. On the prepared hemocytometer a drop of macerate was put with pipette. After filling the chambers, made a break of 63 seconds for distribution of the spores. Microscopic examination was performed at magnification of 400x. Counting of spores is conducted in 5 boxes and multiplied by 25,000, to calculate a total number of spores per bee.

Same samples of bees were analysed with the PCR method. It was made a composite sample by the apiary, abdomens were macerated with 10 ml of sterile distilled water. The suspension were filtered and centrifuged to 800 g for 6 minutes.

To extract DNA of *nosema*, it need to be stimulated germination of spores, with adding 200 microliters of freshly prepared buffer and incubated at 37 °C for 15 minutes. Phosphoric acid was used to adjust the pH of the buffer to 6.0. For DNA extraction was used a QUIAamp DNA Mini Kit [18], and to perform PCR reactions was used a HotSTARTag Master Mix Kit [18].

According to the OIE Manual [22] molecular weight of the obtained PCR products were identified by electrophoresis in 2% agarose gel with standard TAE buffer and the resulting bromide staining and visualization of UV illuminator.

Results and Discussion

With microscopic examination of macerated bee abdomens, it's identified the presence of *Nosema* spp. in every analysed sample made in both location. Results of counting of *Nosema* spores are presented in tables 1 and 2.

In apiary at first location in the hive number 1, were examined the highest number of spores, 95.85 million, that indicates a very high level of infestation of bee society with *Nosema ceranae*, and in almost all examined hives the number of spores was significantly high. The apiary 2, which is located in a dry, sunny place, but also in Vojvodina, number of spores of *Nosema sp.* is significantly smaller, that supports the opinion that by humidity and moisture in

the hive envelope a development of Nosemosis. *N. Apis* were not found in examined samples. The tested samples were negative for the presence of bee viruses.

Table 1. Results of epizootic and laboratory analyses the number of *Nosema* spp. Location 1

No. of hive	No. bee street	No. of bee brood	Food supply	Fresh pollen	No. of spores of <i>Nosema</i> spp.
Hive 1	9	4	>15 kg	good	95.850.000
Hive 2	10	4	>15 kg	good	5.475.000
Hive 3	1-2	2	>10 kg	weak	15.900.000
Hive 4	12	8	>15 kg	good	1.150.000
Hive 5	6	3	>15 kg	good	2.525.000
Hive 6	7	4	>8-9 kg	good	13.450.000
Hive 7	died	-	-	-	44.350.000
Hive 8	6	3	>10 kg	good	7.925.000
Hive 9	8	4	>8-9 kg	good	725.000
Hive 10	7	5	>10 kg	good	3.900.000

Table 2. Results of epizootic and laboratory analyses the number of *Nosema* spp. Location 2

No. of hive	No. bee street	No. of bee brood	Food supply	Fresh pollen	No. of spores of <i>Nosema</i> spp.
Hive 1	5	4	>6 kg	obilan	2.775.000
Hive 2	10	5	>10 kg	obilan	0
Hive 3	8	4	>10 kg	obilan	1.425.000
Hive 4	9	4	>15 kg	obilan	775.000
Hive 5	9	5	>12-15kg	obilan	950.000
Hive 6	7	3	>10-12 kg	obilan	1.475.000
Hive 7	10	4	15 kg	obilan	0
Hive 8	10	6	>10 kg	obilan	1.425.000
Hive 9	10	5	>8-9 kg	obilan	50.000
Hive 10	10	5	>10 kg	obilan	5.950.000

The best time to prove *N. Apis* and *N. Ceranae* in honey bee colonies is the beginning of the active season after winter, when we still can find the old winter bees. According to the literature parasitized bees only with *N. Apis* can be found in apiaries in Canada, Sweden, Ireland, and UK, but there are a great possibility that the disease will further deteriorate under the influence of a growing number of mixed infections, which are more complicated by the presence of the virus, especially the black queen cell virus and Kashmir bee virus. The rapid progress of the process of replacing of the *N. Apis* to the *N. Ceranae* in the bee population, it is considered that *N. Ceranae* is more virulent and dangerous to honey bees [16]. Altered tropism of *N. Ceranae* causes various pathological changes in which there are no characteristic symptoms of classical Nosemosis.

Treatment of bee colonies is very difficult and unpredictable, because the only registered drug, fumagillin is banned because of its carcinogenic and other adverse effects. Application of herbal preparation, KAS-81, in the prevention of feed supply in spring and autumn, especially in the period when the winter bees are born, gives good results, but it's hard to acquire for the most of beekeepers.

Determining the true state of disease in the hive is of great importance, therefore it is necessary to accept the method of spores counting as a valid diagnosis instead of the current flat-rate estimates and of course confirmation by PCR.

This is of big importance because it has been found that bee colony survives and lives with the presence of spores in the body, but it is only necessary to minimize the number of spores.

According to the Canadian Association of Professional Beekeepers [23] is considered to be necessary to treat the colony when it finds one million or more spores per bee, and if the number of spores is under the 10,000 per bee, spores is not even found under the microscope and then issued a finding "notdetected-ND" which does not mean that there is no infection [15].

The future of beekeeping and guidance activities

Fight for survival in the beekeeping production, with smaller or bigger losses, is old as a beekeeping, but parasite *N. Ceranae*, is potentially very dangerous for the bee as an individual, as well as the entire bee colonies and

beekeeping in general. *N. Ceranae* synergistically with other pathogens, especially viruses and mites, with the loss of natural habitat and lack of floral resources, is a dark future for the survival of the most important pollinators on Earth. Predicted climate changes leads to increased influence on the development of *N. Ceranae*, which is very well adapted to the warm climate, unlike *N. Apis*, which were a problem for bee community only during the winter in cold and humid climates.

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