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HEALTH CONDITION OF BEES INHIBITING WILD BEEHIVES AND LOGS LOCATED IN THE FOREST DISTRICTS OF NORTH-EASTERN POLAND

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ABSTRACT

The main objective of the research was to determine the overall health of bees that inhabit the wild beehives and logs located in Polish northeastern Forest Districts. The study was conducted in August 2015 in Augustow Forest District (1 wild beehive and 9 logs), Suprasl Forest District (3 wild beehives), Maskulinskie Forest District (1 log). In the samples with PCR methods (according to the OIE) and using specific primers species of *Nosema spp.* and the type of virus (*ABPV*, *CBPV* and *DWV*) were determined, the presence of american foulbrood AFB and *V. destructor* in brood and in bees were detected. In the collected material there were not found anatomical and developmental anomalies in worker bees indicating the pathognomonic symptoms of diseases (eg. paralysis, weight loss, etc.) or the presence of american foulbrood (AFB) in brood. However there were found *V. destructor* in five tested objects, and carried by this mite the virus of deformed wings *DWV* in four objects. It was also ascertained the presence of infection with a pathogenic fungus *N. ceranae* in two objects. Because of pioneering character of research it should be continued in the future.

Keywords: honey bee, beehive, pathogens.

INTRODUCTION

In many places in the World, also in Poland and Republic of Bashkortostan there are located National Parks in the area, where there are woods not subjected to human interference. In such natural conditions, honey bees inhabit so called wild beehives. Originally honeybee was a natural and permanent component of forest [Domanski, 2015]. The collapse of beekeeping in Poland, lack of nectar and parasitic disease (*varroa*sis) are the reason that now in Polish forests there are much less bees. They play in the woods numerous of useful functions, and their presence in forested areas, especially in protected areas, is crucial for the protection of endangered species of plants that require pollination [Domanski, 2015]. Therefore, should be made efforts to restore bees to forests and protect the natural colonies of bees that inhabit the wild beehives or logs.

Thanks to the initiative of Polish State Forests, in particular Augustow Forest District in the beautiful regions of Eastern Polish was launched project on “The traditional bee-keeping as a rescue for wild bees in the forests” aimed at the renewal of the population of wild bees. On the task are working together Forest Districts: Augustow (Augustow Primeval Forest), Browsk (Bialowieza Forest), Maskulinskie (Pisz Forest) and Suprasl (Knyszyn Primeval Forest), in the cooperation with the Warsaw University of Life Sciences - SGGW and the University of Bialystok. Knowledge of traditional beekeeping communicate beekeepers from the National Park Reserve Szulgan-Tash in Republic of Bashkortostan in the Urals in Russia, who are engaged in this for centuries (Figure 1, 2).

Figure 1. Employees of Polish State Forests and beekeepers from National Park of Bashkortostan, reserve Szulgan-Tash together in the Augustow Forest (photo Roman Rogodzinski).

Figure 2. Log in Augustow Forest District (photo Roman Rogodzinski).

During the process of restoring the bees to Polish forests it should be very carefully monitored the condition of bees in newly colonized beehives and logs. Survival rate on habitats offered by the nature and the control of the risks from commonly occurring pathogens are the basic tasks in this topic. The observations are difficult because the wild beehives and logs are often located at a fairly high altitudes and in areas with limited access. It's hard to find any information in the scientific literature about the pathogens in bees occurring in wild beehives. Therefore it is necessary to start monitoring of the basic and commonly occurring diseases of bees as a potential threats. Certainly it is needed to test the samples for the presence of *deformed wing virus* (*DWV*) because it indirectly indicates the presence the mite *Varroa destructor* in the studied colonies, which is a virus vector [Ball, 1985].

V. destructor is a dangerous to bees parasite,

because the colonies infested with those mites die within 3-4 years [Fries et al., 1994; Shimanuki et al., 1994]. This is due to the fact that the *V. destructor* has a negative effect on the bees in many different ways. Feeding at the expense of bees and brood, mites pierce the mucous membrane of the host organism. Wounds are an open gate for a number of pathogens present in the body of the insect [Glinski, Jarosz, 1988], as well as for those carried by *V. destructor* [Ball Allen, 1988; Glinski Jarosz, 1990; Glinski Jarosz, 1991]. There are viruses, which multiplication favors the *varroa*sis presence. One of them already mentioned is *deformed wing virus* (*DWV*), but there are other such as virus of acute bee paralysis (ABPV - acute bee paralysis virus), which was discovered in 1963 during the work on the virus of chronic paralysis. This virus for many years, despite that occurred quite frequently, in adult bees in natural conditions did not caused any signs of infection nor dying infected insects. Only after the colonies of honeybees become host of mite *Varroa destructor*, virus was often the main cause of death of bees and brood in colonies heavily infested with this parasite [Topolska, 2007]. Another disease to which it is necessary to pay attention taking care of the health of swarms in natural beehives is widely known Nosemosis (*Nosemosis apium*) in honey bee *Apis mellifera*. It can be caused by two species microsporidia: *Nosema apis* and *Nosema ceranae*. *Nosema apis* was first described in 1909 [Zunino et al., 2009]. It causes the loss of worker bees in the wintertime and after overwintering, poor spring colony development and reduced honey production [Fries, 1997; Graaf et al., 1994). Within a beekeeping season the degree of infection the colony decreases [Bailey, 1959]. *N. apis* differs from a high virulence of *N. ceranae*, to *A. mellifera* and currently has commonly been found in honey bee colonies [Fries et al., 2006; Higes et al., 2006]. *N. cerance* causes a disease called type C Nosemosis [Higes et al., 2010]. According to Spanish researchers, this specie can cause massive death of entire bee colonies [Higes et al., 2008; Higes et al., 2009] due to the easy adaptation to the new host and high virulence. Another threat to the health of bees living in wild beehives and logs is American foulbrood caused by the *Paenibacillus larvae* spores. Colonies infection by the *Paenibacillus larvae* spores is not always a disease, but still a real threat. The primary way of infection of larvae is a food that contains spores of the bacteria. Pollution of the food originates directly from the cell walls of the honeycomb, or through the worker bees. To In some lines of honeybee it is reduced vulnerability to infection of *P. larvae* as an effect of an increased hygienic procedures of worker bees [Rothenbühler, Thompson, 1956]. Some role in the resistance plays an increase of the levels of microbial inhibitors, especially royalisin, in bees which are nursing brood [Rose, Briggs, 1969]. Systematic control of colonies during and after the end of the beekeeping season, careful overviews and quick reaction to the first signs of possible diseases is necessary for the survival of bees in newly colonized natural bee hives and logs. In view of the above mentioned potential threats for bees in wild bee hives resulting from the existence of pathogens the situation should be controlled all the time.

The aim of the study was to determine the overall health of bees that inhabit the wild beehives and logs located in the Forest Districts: Augustow, Suprasl and Maskulinskie. To estimate the chance of colony survival in new, specific conditions. The rationale for aims of the study beyond cognitive was also a practical aspect consists in the development of methods of collection from wild beehives and logs research material (bees, brood).

MATERIAL AND METHODS

The study was conducted in August 2015 in the area of state forests in Augustow Forest District, Suprasl Forest District and Maskulinskie Forest District. In each of the Forest District are freshly made wild beehives and logs on heights of 3 to 5 m.

- Augustow Forest District (NA) - 1 wild beehive and 9 logs
- Suprasl Forest District (NS) - 3 wild beehives
- Maskulinskie Forest District (NM) - 1 log

All objects mentioned above inhabited by bees in 2015 were included to studies. For this purpose, from each colony twice collected a sample of 60 living worker bees taken directly from beehive entrance outside or after opening wild beehive and also collected brood comb sized of 10cm x 10cm. Acquiring the brood combs smoke operations were limited to the absolute minimum. To obtain a brood samples pieces of external combs were first cut off to get inside the wild beehives in such a way to the least affect the construction of the colony. Next there were laboratory analysis of the presence of pathogens in samples of honey bees and bee brood to detect:

a. microsporidia of *Nosema* by microscopic method and species identification by multiplex PCR method

b. deformed wing virus (DWV) by RT-PCR method.

c. acute bee paralysis virus (ABPV) by RT-PCR method.

d. chronic bee paralysis virus (CBPV) by RT-PCR method.

e. *Paenibacillus larvae* in honey bee brood using diagnostic tests, Honeybee Foulbrood Test Kit - AFB Diagnostic Kit by VITA.

f. *Varroa destructor* in samples of bees and brood on the basis of characteristic symptoms (presence

of deformed insects) as well as development forms (females of parasite).

RESULTS

In the collected samples of worker bees in August in wild beehive No. 2 NS and in log No. 2 NA it was revealed the presence of *N. ceranae*. In the collected for testing samples of brood in August was detected and counted *V. destructor* in 5 objects. The most numerous of mites was characterized brood originated from log No. 8NA and wild beehive No. 1 NA. It is worth to notice that in the latter case, the results apply to drone brood. Throughout the study period there were no *V. destructor* in any of the samples of worker bees while deformed wing virus (DWV) was detected in two wild beehives (No. 2 and 3) located in the NS, and in two colonies originated from logs (No. 3, 6) located in NA. In the collected material there was not found of anatomical and developmental anomalies in worker bees indicating the pathognomonic symptoms of disease, eg. acute bee paralysis virus (ABPV) or chronic bee paralysis virus (CBPV). None of the tested samples of the honey bee brood showed the presence of *Paenibacillus larvae* which demonstrates the presence of American foulbrood (AFB) (Table. 1).

Table 1.

Pathogens found in samples of brood and worker bees originated from logs and wild beehives from north-eastern Polish Forest Districts

The name of the studied object	Type of pathogen							
	V c	V p	AFB	N a	N c	A	C	D
beehive nr 1NS	-	-	-	-	-	-	-	-
beehive nr 2 NS	-	-	-	-	+	-	-	+
beehive nr 3 NS	5	-	-	-	-	-	-	+
log nr 1NM	-	-	-	-	-	-	-	-
beehive nr 1 NA	16	-	-	-	-	-	-	-
log nr 1NA	-	-	-	-	-	-	-	-
log nr 2NA	-	-	-	-	+	-	-	-
log nr 3NA	0	-	0	-	-	-	-	+
log nr 4NA	2	-	-	-	-	-	-	-
log nr 5NA	-	-	-	-	-	-	-	-
log nr 6 NA	2	-	-	-	-	-	-	+
Log nr 7 NA	-	-	-	-	-	-	-	-
log nr 8 NA	17	-	-	-	-	-	-	-
log nr 9 NA	0	-	0	-	-	-	-	-

Explanation: NA - Augustow Forest District; NS - Suprasl Forest District; NM - Maskulinskie Forest District; N (*Nosema*): a - *apis*, c - *ceranae*, (-) - no infection; V (*Varroa destructor*): c- on brood, p - on bees (nr of pcs.); A - ABPV, C - CBPV, D - DWV, (-) - no virus; AFB (American foulbrood): + or -.

DISCUSSION

In the available literature it is lack of information on pathogens of bees colonizing logs and wild beehives. The studies are pioneering, there were obtained preliminary results that require follow-up and further observation. However it is clear, that control the health of the bees is necessary because pathological condition can cause a huge loss, and in extreme cases leads to complete destruction. The extinction of bees pollinating most crops can cause catastrophic consequences [Glinski, Kostro, 2007]. According to Sokol et al. [2016] the possibility of monitoring the bee condition in wild beehives and logs is important not only cognitive, but it can help to detect diseases directly being life-threatening for bee colonies and, indirectly can be a contribution to the development of procedures preventing uncontrolled development and spreading diseases. Development of the management the infected colonies of bees in such objects, and the way the sampling for analysis, requires further analysis.

CONCLUSIONS

1. In the tested material there were detected mites of *V. destructor* and also transmitted by them viruses of deformed wings DWV. In the material it was also found infection with a pathogenic fungus *N. ceranae*.
2. There was not ascertained the presence of the virus of the acute bee paralysis (ABPV), chronic bee paralysis virus (CBPV) and the presence of American foulbrood (AFB) of brood.
3. It was found that the condition of colonies in newly colonized wild beehives and logs was good and generates chances for survival.
4. The procedure (algorithm) for sampling aimed to harmonize the activities in each wild beehive, shorten the interference in the colony and to ensure the greatest possible safety of person retrieving

sample.

5. Because the studies are pioneering it should be continued in the following months and years.

6.

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СОСТОЯНИЕ ЗДОРОВЬЯ ПЧЕЛ, НАСЕЛЯЮЩИХ БОРТИ И КОЛОДЫ В ЛЕСНОМ РАЙОНЕ СЕВЕРО-ВОСТОКА ПОЛЬШИ

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АННОТАЦИЯ

Основная цель исследования заключалась в определении общего состояния здоровья пчел, обитающих в бортях и колодах, расположенных в северо-восточных лесных районах Польши. Исследование было проведено в августе 2015 года в лесничестве Августова (1 борть и 9 колод), в лесничестве Супрасле (3 борти), в лесничестве Маскулинские (1 колода). В образцах, с помощью ПЦР (в соответствии с требованиями Всемирной организации здоровья животных), были обнаружены *Nosema spp.*, вирусы (*ABPV, CBPV* и *DWV*), американский гнилец AFB и *V. destructor*. Среди образцов не были обнаружены анатомические и физиологичесике аномалии - симптомы для вышеперечисленных аболеваний (такие как паралич, потеря массы). В потомстве не было обнаружено присутствие американского гнильца (AFB). Однако был обнаружен *V. destructor* в пяти образцах, и переносимый клещом вирус деформации крыла *DWV* в четырех образцах. Была также обнаружена инфекция *N. ceranae* в двух образцах. Исследования должны быть продолжены в дальнейшем.

Ключевые слова: медоносная пчела, улей, патогены