

High Honey Bee Losses in the winter of 2016-2017 in Timiș County, Romania

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Abstract

Beginning with November 2016 beekeepers from Timiș county reported bee colonies losses. During the winter months the situation became more the situation has become more severe, increasing losses of bee families. From November 2016, until February 2017 were been received samples of dead bees from 23 different apiaries in Timiș county. The dead honey bees were analyzed in the way to discover what kind of causes produced all these losses. Was also proceeded to analyze the bacterial flora from bees intestine. Bacteriological examination of bees intestine and the cultivation on special medium of culture emphasized rich cultures of *Paenibacillus larvae subsp. larvae* (*Bacillus larvae*), etiologic agent for American foulbrood. These bacteria were been isolated from bee intestine in samples provided from 18 apiaries (78.26%). There are many causes of high degree of bee mortality in winter 2016-2017 but the isolation of *Paenibacillus larvae subsp. larvae* maybe was one of them.

Key words: American foulbrood, apiaries, bee colonies, beekeepers, *Paenibacillus larvae subsp. larvae* (*Bacillus larvae*).

1. Introduction

Romania is the largest producer of honey between Member States, indicating the latest European Commission report on the subject using the data in 2015. With a production of 35,000 tons, 75% more than the previous year, local farmers in 2015 have surpassed those in Spain and Hungary. On average, Romania has a constant around 20-25,000 tons. It does not see how to be produced 35,000 tons in 2015, which was a pretty good year. According with the INS data (National Institute of Statistics), in Romania there are about 1.4 million bee families and about 40,000 beekeepers. Year 2016 was a disastrous year for beekeeping, due to adverse weather conditions, critical situation that was overlapped with falling

prices in Europe, following a policy of supporting beekeepers promoted in Ukraine. Statistics show that Romanians consume on average 450-500 grams of honey per year per person, cannot be compared to two kilograms consumed in Germany and 1.5 kg in the Netherlands and Belgium. For this reason about half of the local production is exported. Overall, the EU produced in 2015 268,000 tons of honey to 240,000 tons a year ago and is the second largest world producer after China. But the EU is a net importer of honey, despite higher production [1].

Local production of honey in EU covers about 60% of consumption. The most important suppliers of honey for EU are China, Ukraine and Latin America, says the European Commission study. In terms of prices achieved, Romanian beekeepers receive the third lowest rate in the EU. In 2015, they received at the honey farm gate 4.35 euro per kilogram, given that the EU average reaches 5.76 euro per kilogram, according the

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European Commission's data. The most expensive honey is the UK (15.8 euro a kilo) and Montenegro (15 euro per kilo). The cheapest price of honey is in Sweden (4.30 euro a kilo) and Bulgaria (3.49 euro). Bees went into winter weakened, so that the population is much lower this spring and in these conditions the honey production will be lower too. Romanian market is flooded with imported honey from China, Mexico, Ukraine. Furthermore, while beekeepers honey from Romania for export should be subject of a severe analysis (for GMOs, antibiotics and pesticides) analysis that it costs a lot, only 5% of imported honey is analyzed. If 2015 was talked about a record production of honey in Romania, our country is number one in Europe, 2016 was a disaster. 2016 was the worst year of Romanian beekeeping from the last 35 years the agricultural sector is facing a high level of mortality among families of bees due to unfavorable climatic factors, but also due treating crops with pesticides or other harmful substances. In recent years, in our country important climate changes have occurred and farmers had to adopt technologies soil treatment with various substances in order to be productive. This, however, had a negative impact on the quality of life of bees and bee products [2]. Banat region is estimated by the point of view of beekeeping to be the most important area in the country. Timiș County ranked first by number of bees. About 40,800 honeybee families have the members of the Beekeepers Association Timiș branch. But many beekeepers are unaffiliated or other smaller organizations with other twenty thousand hives [3].

Every day, the number of bee families elite breed *Apis mellifera* *Carpatica* is declining. Currently, apiary elite Beekeeping Institute owns 1,099 bee colonies. Based on this genetic background, it provides biological material for apiaries in the country, which queens reproduce in turn. According to the National Agency for Livestock, until 2016, were authorized as apiary holding bee families breed *Apis mellifera* *Carpatica* 156 beehives multiplication holding 18,105 beehives and apiary elite with a staff of 1,099 bee families. Apiaries elite and multiplication only apiaries providing queens eligible to be settled by the measures stipulated in programs developed with funds from the national budget and the Community and the selling price of these include all expenditure on maintenance, selection and

improvement of the breed *Apis mellifera* *Carpatica*.

Romanian beekeeping has been severely affected last year on the one hand climate change and the other of attempts to introduce in Romania of hybrid species that cannot adapt to the conditions in our country, say researchers in the field. In the winter of 2016-2017 was recorded a high mortality among bee families, around 40% nationally. Only a few warmer days were enough for bees can come up in the so-called cleansing flight, but very low temperatures for a long time this winter affected many bee families. To these are added other causes that led to higher mortality in winter. Many honey families losses were been registered in 2016-2017 winter in Sălaj, Hunedoara, Argeș, Prahova, Olt, Bistrița Năsăud Counties, where beekeepers have expressed concern without knowing for sure which is the real cause of these losses [1,2].

2. Materials and methods

From mid-November 2016 until February 2017 in Microbiology laboratory was been received a lot of dead bees provided from different apiaries in Timiș County. Beekeepers were desperate because they noticed a large number of dead bees, in some cases whole families, something unprecedented in winter. After an examination from each lot of received dead bees were sampled the intestinal tracts content, from esophagus to rectum. Then intestinal content was been inoculated in liquid medium of culture and incubated 24 hours at 37 °C. After 24 hours the liquid culture mediums had moderate turbidity, deposit at the tube basis and surface rings adherent on tube walls, more and less developed [4]. From these mediums of cultures were been made microscopic preparations Gram method stained. Gram staining: smears were made from inoculated liquid medium of culture after 24 hours of incubation on a slide. For Gram staining, the slide was flooded with crystal violet for 1-2 minutes, washed then flooded again with Lugol's iodine solution for 1-2 minutes, decolorizing agent was used as acetone alcohol for 5 seconds, the final steps involved applying diluted Ziehl fuxin, after each steps the slide was rinsed with water for 5 seconds [5]. The prepared slide was then examined under microscope (Novex, Holland) and photographed . The cultures

provided from inoculated liquid medium was then cultivated on Mueller-Hinton-yeast-phosphate-glucose-pyruvate (MYPGP) liquid broth agar at 37°C for 48 hours (Dingman and Stahly, 1983). This medium of culture contains per liter: 10 g Mueller-Hinton broth (Oxoid CM0405), 15 g yeast extract, 3 g K₂HPO₄, 1 g Na-pyruvate, 20 g agar, autoclaved at 121°C/15 minutes, then added 20 ml 10 % glucose (autoclaved separately) for *Paenibacillus* genus [4].

3. Results and discussion

The bacteriological examination results were emphasized by observation of developed colony morphology and microscopic examination of bacterial smears. After 48 hours of incubation at 37°C in MYPGP medium were observed small bacteria colonies, regular, raised or flat, mostly rough, color ranging from white to beige. All these morphological characteristic are attributed to *Paenibacillus larvae subsp. larvae* (*Bacillus larvae*), the etiologic agent of American foulbrood. In Gram stained smears the bacterium was identified as *P. larvae* with Gram positive rods 0.4-0.6 µm wide and 1.4-6 µm long (Photo 1). Table 1 shows the results of bacteriological examination due in the way of *Paenibacillus larvae subsp. larvae* isolation.

Samples of dead bees arrived from 23 apiaries from November 2016 until February 2017. From 23 apiaries, in 18 was identified after

bacteriological examination, *Paenibacillus larvae subsp. larvae* (78.26%). However bees died in a great number from all studied apiaries whether or not this bacteria was identified (Photo 2).

Table 1. The results of bacteriological examination

Apiary	<i>Paenibacillus larvae</i> isolation results
1	+
2	+
3	+
4	+
5	-
6	+
7	+
8	+
9	+
10	+
11	-
12	-
13	+
14	+
15	+
16	+
17	+
18	+
19	-
20	+
21	+
22	+
23	-
Percent of positive results	78.26%

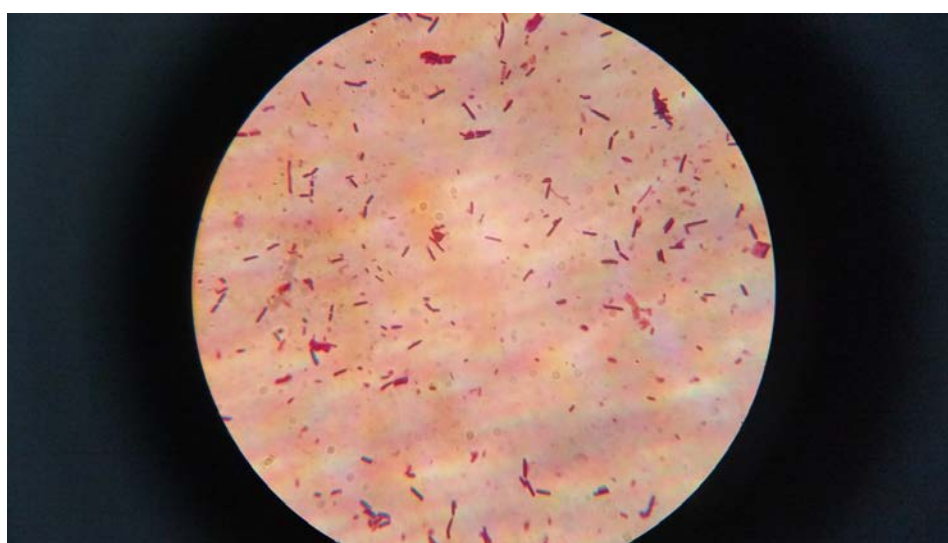


Photo 1. Microscope examination of *Paenibacillus larvae* on Gram staining smears



Photo 2. Dead bees

The isolation of *Paenibacillus larvae subsp. larvae* from dead bees intestinal tract content demonstrate that these colonies are infected, being known that adult bees recognized diseased larvae, remove or cannibalize them, when they cleaning brood cells. In this way adults honey bees are exposed to bacteria, in spore or vegetative stages. In winter season honey bees must bear the American foulbrood infestation pressure for five months because low temperature limited frequent possibilities to defecate. Riessberger-Gallè and collaborators in 2001 [6] identified in workers bees gut numerous spores of *Paenibacillus larvae subsp. larvae*, but vegetative rods were never found in the content of alimentary canal. They demonstrated the existence of a substance in the midgut of honeybees that is responsible for the resistance of adult honeybees to infection with *Paenibacillus larvae subsp. larvae* and can be produced by bees even those are malnourished. They considered that substance to be a small peptide or polar lipid capable to exhibit bactericidal effects. Another explanation, for nurse bees may be free fatty acids derived from pollen with antibacterial effect of gut content [7]. This resistance mechanism was not induced by an advanced infection with etiologic agent of American foulbrood. Another explanation for such a big number of dead honeybees, especially from apiaries free for *Paenibacillus larvae subsp. larvae* infection is the low temperature a long period, as it was this winter. It is possible to die as

a result of starvation, even with honey stored outside of the cluster because when it get cold bees are not capable to move enough to reach the food. It is possible that so many losses in apiaries to be the result of inadequate supply of stored food over the winter period. Honeybees in winter stay in cluster within hives and vibrate to keep warm. To maintain an adequate temperature in hives bees require constant and large supply of food to be stored in their hives in all winter period [8]. Normally beekeepers once expected to lose perhaps 10% of their bees in an average year. Honeybees have one purpose, a primary objective during the winter months: to keep the queen alive and thousands of worker bees may die in the process. Honeybees have a great adaptation to harsh winter conditions, keeping the queen in the center of so called winter cluster, the worker bees rotate from outside to inside of the cluster such that no individual bee stays in outside and get too cold. The more cold is outside, the more compact cluster becomes. However, all these conditions cannot be held if the beekeeper hasn't prepared the hives correctly maybe because ignorance or bad management. Hives must be protected externally with insulating materials for preventing the penetration of cold and wind. Another reason of winter losses is related by the queen. A queen in her second full season has 50% chances of failing over the winter, while a young and strong queen has a far better

chance of heading a strong hive in the early spring, leading to bigger crops. Wet hives can also be a cause of winter losses, with many problems of ventilation [9]. Warm air condenses on cold surfaces and forms ice, this then melts in the spring and often drips back into the cluster, a simple top entrance would help. That same top entrance will also prevent suffocation in the event of ice build up on the entrance reducer. Another cause might be the stock bred for use in an area climatically similar to where they are to be hived, well adapted to weather conditions. Periodically the families are listening by beekeeper bringing ear nearby bee entrance of hive. Very low hum like the rustling of dry leaves indicate that families of bees are deprived of food and it imposing replenishing food with honey combs or sugar.

4. Conclusions

Last year was one of the worst years for Romanian beekeeping. Beginning in spring with frost of Acacia and continuing in summer with rain, wind and drought for sunflower cultures, was leading to a high mortality of bee families. But it was not enough because winter came with very low temperature for a long time and did not allow the bees to leave the hive to fly cleaning, which is done only when the temperature is above 10°C. A percent of 78.26% from dead honeybees received in microbiology laboratory, from mid-November 2016 until February 2017 were founded after bacteriological examinations of intestinal tracts content, from esophagus to rectum contaminated with *Paenibacillus larvae subsp. larvae*, etiologic agent of American foulbrood. But isolated bacteria mostly affect bee larvae and none apiaries from which bees originated has not been faced with this disease recently. The explanation can be that these adult bee colonies are infected, but disease was not clinically expressed, adult bees recognized diseased larvae, remove or cannibalize them, when they cleaned brood cells. In this way adults honey bees are exposed to bacteria, in spore or vegetative stages but researchers found a substance, a small peptide, or polar lipid, or free fatty acids derived from pollen with antibacterial effect of gut content, capable to exhibit bactericidal effects and to prevent disease outbreak with the coming warm season. It is understood that the presence of *Paenibacillus larvae subsp. larvae* did not cause the death of

bees, the bacteriological isolation in intestinal content of adult bees showed only that there is a contamination which will be carefully monitored in time. If studies on mechanisms of resistance of adults to *Paenibacillus larvae subsp. larvae* will apply in this case it is certain that the disease will not occur in future. Studied honeybees it is possible to be killed by starvation, even with honey stored outside of the cluster because when it get very cold bees are not capable to move enough to reach the food. It is also possible that so many losses in apiaries to be the result of inadequate supply of stored food over the winter period. The low temperatures of the 2016-2017 winter found unprepared the beekeepers which hasn't prepared the hives correctly, externally protecting with insulating materials for preventing the penetration of cold and wind, or hives were wet, maybe because ignorance, lack experience or bad management. Also, all winter period beekeepers must noticing any change, any sound, watching the hive and listening nearby bee entrance of hive.

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