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THE MICROBIAL STATUS OF BEE FAMILIES *APIS MELIFERA* DURING THE WINTER PERIOD

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Introduction. *Bees are very important to balancing different ecosystems around the globe. It's worth noting that, like animals and humans, bees are affected by different pathogens, such as bacteria, viruses, ecto- and endoparasites, and fungi. Therefore, the health of bee families depends on the veterinary sanitary measures undertaken by the beekeepers in close contact with the veterinarians. The aim of the research is to analyze the impact of the microbial status of bee families during the winter period.*

Material and methods. *The research consisted of bee families from the experimental apiary of the Institute of Microbiology and Biotechnology of the Technical University of Moldova. Samples of dead bees, about 50 from each examined hive, were collected for bacteriological research. The morphological properties of the bacterial colonies were studied.*

Results. *The obtained results demonstrate that a diverse microbial microflora prevails in bee families during the winter period. The results of microscopic investigations confirmed the presence of bacteria E. coli, accounting for over 50% of bacterial forms, followed by Streptococcus and Staphylococcus with a level of 30%, fungal flora 15%, and up to 5% bacteria of the genus Salmonella spp.*

Conclusions*. The bacteriological research carried out in bee families after the winter period, reproduces the data of persistent bacteriocenosis in bee families during the cold period of the year and allows for predicting the risk of infectious diseases in bees.*

Cuvinte-cheie: *albine, bacterii, fungi, medii de cultură, colonii bacteriene.*

STATUSUL MICROBIAN AL FAMILIILOR DE ALBINE *APIS MELIFERA* ÎN PERIOADA IERNATULUI

Introducere. *Albinele sunt incredibil de importante pentru a echilibra diferite ecosisteme de pe glob. De menționat faptul că albinele asemeni animalelor și oamenilor sunt afectate de diferiți agenți patogeni – bacterii, viruși, ecto și endoparaziți, fungi. Din acest considerent, sănătatea familiilor de albine depinde de acțiunile sanitare veterinare care le întreprind apicultorii în strânsă conlucrare cu medicii veterinari. Scopul cercetării – analiza impactului statusului microbian al familiilor de albine în perioada iernatului.*

Material și metode. *Ca material de cercetare au servit familiile de albine de la stupina experimentală a Institutului de Microbiologie și Biotehnologii a Universității Tehnice din Moldova. Au fost prelevate probe de albine moarte, căte 50 de albine de la fiecare stup examinat, pentru cercetări bacteriologice. S-au studiat proprietățile morfologice ale coloniilor bacteriene.*

Rezultate. *Rezultatele obținute demonstrează că la familiile de albine în perioada iernatului predomină o microfloră microbiană diversă. Rezultatele investigațiilor microscopice au confirmat prezența bacteriilor E. coli cu peste 50% din numărul formelor bacteriene, urmate de streptococi și stafilococi cu o pondere de 30%, flora fungică – 15% și până la 5 % bacterii din genul Salmonella spp.*

Concluzii. *Cercetările bacteriologice efectuate la familiile de albine după perioada iernatuluiredau tabloul bacteriocenozei persistente la familiile de albine în perioada rece a anului și permit prognozarea riscului bolilor infecțioase la albine.*

INTRODUCTION

Bees are extremely important for balancing different ecosystems around the globe. Due to bees, many species of plants are pollinated in forests, grasslands, and a multitude of eco-sys-tems; resulting in the production of fruits that serve as food for many animal species and hu-mans. At the same time, bees produce a lot of important products like honey, propolis, which are beneficial for the human health and find applications in pharmaceuticals, cosmetics, bakeries, etc. (1, 2).

We have to think that if bees stop pollinating the fruits and vegetables which we eat, the world would lose a special nutritional supply for animals, and also all the elements in the chain that are ultimately consumed by humans. There are numerous studies that try to explain why bee populations and honey production are declining. However, the reason for this is not yet fully known. It is important to mentioned that there are a significant number of diseases, including infectious (bacterial, viral, fungal, etc.). These diseases are dangerous and affect both adult bees and their brood (Salmonellosis, Collibacilosis, Viral septicemia, American and European foulbrood, Aspergyllosis, etc.). For this reason, the health of the bee families and the efficiency of their activity depends on the veterinary sanitary measures undertaken by the beekeeper in close contact with veterinarians (3, 4, 5, 6).

At the moment there are programs for monitoring and sanitary veterinary supervision of beekeeping units at national level, which provide for control and surveillance measures, or eradication measures in case of the appearance of infectious diseases in bees (6, 7).

Considering the above, *the aim* of the investigations was to analyze of the impact of the micro-bial status of bee families during the winter period.

MATERIAL AND METHODS

The research material served the bee families from the experimental apiary of the Institute of Microbiology and Biotechnology of the Technical University of Moldova. After the winter period, three common samples were taken from the examined bee families. These samples comprised five hives selected through a random method. The general samples being composed of dead bees and remains from inside of the hives, for bacteriological research, in order to establish the presence and diversity of the bacterial flora from dead bees during the winter period.

For the isolation of bacterial and fungal forms from the collected samples, were used the common, selective and special nutrient mediums as: nutrient agar, RVS broth, XLD agar, Brilliance Salmonella Agar, Endo medium, Sabouraud medium. After incubation of the culture media at the thermostat (at 37±1˚C), were studied the morphological properties of the bacterial colonies that developed on the nutrient culture media. Smears were prepared from the obtained colonies and stained using the Gram method for microscopic investigations.

RESULTS

After examination of bee families to assess their condition after the winter period (fig. 1), priority was given to families with unsatisfactory physiological status (reduced mobility of the bee swarm, excessive bee mortality, diarrheal matter on the walls of the hives and on beehives). Bee samples for laboratory investigations were then randomly selected according to these criteria (fig. 2).

The results of bacteriological investigations confirmed significant growth of bacterial colonies on general culture media, as well as on special nutrient culture media. The most intensive growth of bacterial colonies was on the Endo medium, where specific microbial colonies for the *E. coli* bacteria genus were observed. The bacte-rial colonies were morphologically characterized with spherical and oval shapes, dark – red color, having a gloss with a metallic appearance. Colonies on this medium (on this medium) were predominant with the highest growth intensity across all culture media (fig. 3).

On the peptone agar medium there was a noticeable and intense growth of colonies of *Streptococcus* and *Staphylococcus* bacterial forms with a gray-white color and spherical shapes, uniformly placed on the entire surface of the Petri dish (fig. 4).

In the case of insemination on the Salmonella Shigiella Agar medium, initially were observed an uneven discoloration of the nutrient medium fol-

lowed by a moderate development of *Salmonella* spp. colonies on the entire surface of the Petri plate. These colonies exhibited a light pink color and a wet appearance, with oval shapes (fig. 5).

Figure 1. Examination of bee hives after winter period.

Figure 2. Preparation of samples taken from bee hives for bacteriological investigations.

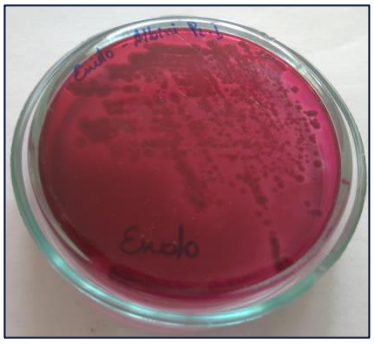

Figure 3. Colonies of E. coli on the nutrient medium Endo.

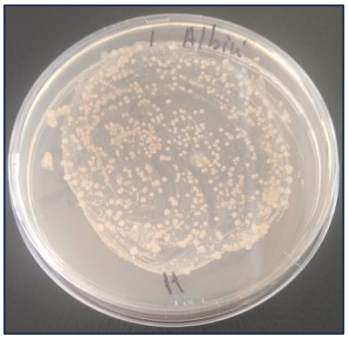

Figure 4. Colonies of Streptococcus and Staphylococcus on nutrient Agar.

Co-inoculations on Sabouraud medium showed an intensive growth of characteristic typical microscopic fungal colonies. These colonies developed over the entire surface of the Petri dish, had a characteristic filamentous-fluffy, with gray-white color and spherical shapes, placed on the surface of the substrate, concretized in the surface of the nutrient medium (fig. 6).

For the morphological-microscopic studies of microbial and fungal colonies with a typical, well-

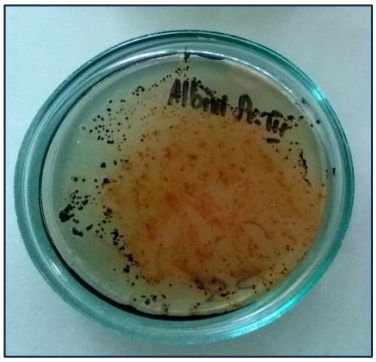

Figure 5. Colonies of Salmonella spp. on nutrient medium SSA.

Figure 6. Colonies of fungals on nutrient medium Sabouraud.

devel oped morphological structure, smears were prepared, stained according to the Gram method and examined under a biological microscope with a magnification of 10x100.

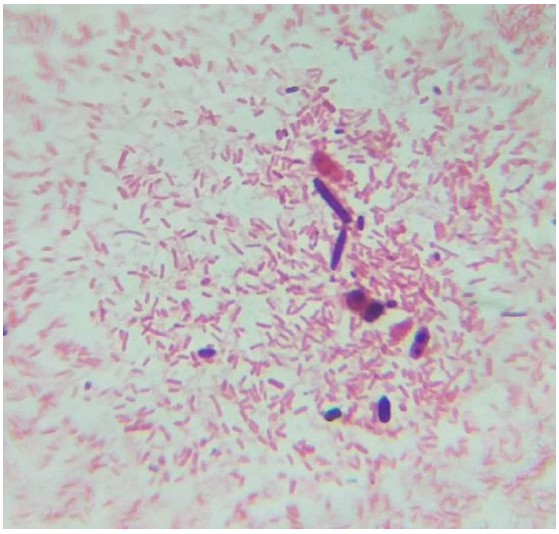

Figure 7. Association of microbial forms (*E. coli, Salmonella* spp., yeasts).

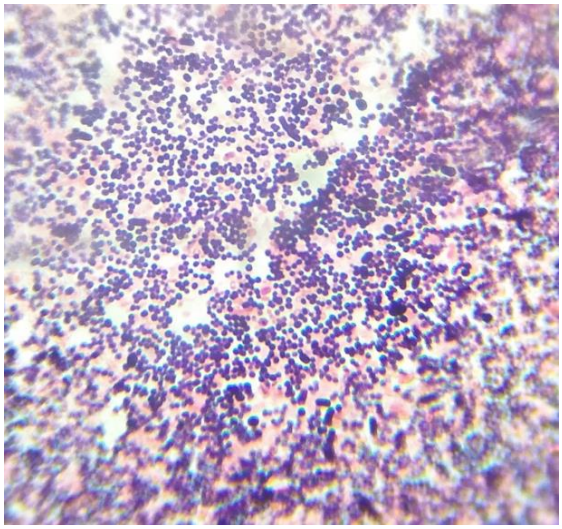

Figure 8. Association of bacterial forms (*Streptococcus, Staphylococcus, and Bacillus).*

The microbiological study results confirmed that bee families after the winter period have a predominant associative bacterial flora. This combination of bacterial forms includes coliform bacteria from genera such as *Escherichia* and *Salmonella* (fig. 7) along with an association of bacteria from genera *Streptococcus, Staphylococcus*, bacillary flora and some forms of fungi (fig. 8).

The results of microscopic investigations confirmed the presence of bacteria of the genera *E. coli* which represented over 50% of the number of all bacterial forms, followed by *Streptococcus* and *Staphylococcus* in a range of 30%, fungal flora 15%, and up to 5% forms of bacteria from the genera *Salmonella* spp.

DISSCUTIONS

One of the riskiest periods for the survival of bee families is the cold period of the year, known as the winter season. Beekeepers should fundamentally prepare for the winter of bee families. The bacterial flora through which the bee family passes during the winter period is decisive for their health.

Another equally important factor is the volume and quality of food (quality honey) for winter period. As a rule, because the winter period is often unpredictable in duration, the food reserve should include a surplus of 15-20% beyond the ordinary needs of a bee family. In addition, the composition of the microbiocenosis of bee families is the basic health indicator of the bee families during the winter period. For this reason, it would be highly recommended to take samples from the bee families before the wintering period. This would allow the analysis of the microbiological risks that may persist or become important factors affecting the viability of the bee nest during the winter period.

In the microbiological study carried out, it was demonstrated that the bacterial flora in bee families after the winter period is polymorphic, consisting of bacterial forms from genera, *Escherichia, Salmonella, Streptococcus and Staphylococcus* in combination with microscopic fungal forms. The results obtained demonstrate that a considerable percentage of the dead bees are the result of the presence of conditionally pathogenic bacterial forms that, under the action of some favorable factors, become pathogenic for some bee families. This is exemplified by a 25-30% increase in mortality compared to other bee families, where the intensity of the bacterial flora is much reduced. The data obtained suggest that periodic monitoring of bee families and the implementation of veterinary sanitary measures would minimize the incidence of risk factors favoring the increase in the percentage of mortality of honey bees during the winter period.

CONCLUSIONS

1. Performing the bacteriological research in bee families after the winter period would allow us to determine the bacteriocenosis in bee families during the cold period of the year. This, in turn, enable us to intervene with some actions to reduce the risk factors for the occurrence of some bacterial or fungal diseases in bee families before and during the winter period.
2. The bacterial flora, consisting of bacillary, coccoid and fungal forms, constitutes a permanent risk of triggering some diseases in adult bees., It also presents an increased danger for the brood of bees that will appear during the active foraging period in the bee families.
3. Bacteriological monitoring of bee hives can highlight and minimize the risks of infectious diseases in bee families. It also helps in reducing or eliminating factors that might trigger infectious diseases in bees.

CONFLICT OF INTEREST

There is no conflict of interest regarding the material presented in the paper, both between the authors and the material basis for the research.

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