

Detection of American Foulbrood in a Bee Family from Timiș County

Daniela Moț

University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Sciences and Biotechnologies,
300645 Timișoara, 119 Aradului Way, Romania

Abstract

It seems that after information media lately have been detected more cases of American foulbrood in our country, but this is not yet known by the beekeepers. In November 2013 a beekeeper brought laboratory six dead bees and portions of honeycomb, because bees dying and he did not know why. Following clinical examination of dead bees was not obvious a major change so bacteriological examination was used. After taking over samples by a single dead bee in liquid culture medium, in the second day, the bacteriological examination emphasized a pure culture, rich of bacilli. There are rare situations when one can observe a pure culture of bacteria obtained from a single sample. After cultivation on special mediums, like agar with egg, the developing of rough colonies was no longer any doubt that the isolated bacteria are *Paenibacillus larvae subsp. larvae* (*Bacillus larvae*), etiologic agent for American foulbrood.

Key words: bee, clinical examination, bacteriological examination, bacilli, agar, colonies.

1. Introduction

American foulbrood (AFB) is by the point of view of beekeepers from temperate, sub-tropical regions and around the world generally regarded as possibly the most destructive microbial disease affecting bee brood. This disease is widely distributed anywhere *Apis mellifera* are kept [1]. The disease is contagious and the pathogenic bacteria causing AFB can remain dormant for as much as more than fifty years. The etiologic agent of the disease is a spore-forming bacterium called *Paenibacillus larvae* (*Bacillus larvae*) which affects bee brood. This bacterium is isolated from brood combs with characteristic lesions for AFB, such as brown colour, caps stifled cells, content brown, sticky and philanthe. The etiologic agents are Gram positive bacilli with rounded or slightly bent extremities, mobile, enveloped, aerobic.

When the disease appears in a bee colony, if remedial actions are not taken, it can quickly spread to other colonies in the apiary, as a result of robbing, drifting workers or by beekeeper's hive manipulations. In the same way the pathogenic agent can spread to other apiaries. Often spores enter to bee colony through foreign honey. Honey for commercial purpose can be highly contaminated and a special attention should be paid near honey processing enterprises and waste disposal sites [2, 3]. American foulbrood was also identified in our country in the last years with economic losses for beekeepers, but there is not known yet where are more often appearances of disease. Last autumn were been reported twelve AFB outbreaks in the North-East part of our country, but it seems that there are more other zones where disease progresses without beekeepers to take any action against disease evolution.

* Moț, D., Phone 0256 277 192, Fax 0256 277 110,
Email dana_tm@animalsci-tm.ro

2. Materials and methods

In November 2013 a beginner beekeeper from the west side of the country brought to Microbiology laboratory six dead bees and small portions of honeycomb from a colony where observed fewer and fewer bees. The six dead bees and honeycomb were examined. Than from one of the dead bees were been taken samples, inoculated in a liquid culture medium and incubated 24 hours at 37°C. After incubation the liquid culture medium has a moderate turbidity and a surface ring adherent of the tube walls. From this medium of culture was made a microscopic preparation stained by Gram method. Also, material from the inoculated liquid medium of culture was cultivated on agar and Columbia agar medium, specific for family *Paenibacillaceae*, genre *Paenibacillus*, with horse blood on Petri plates [4, 5]. All cultivated Petri plates were than incubated 24 hours in 35-37°C.

3. Results and discussion

At the six dead bees examination was not been detected any special sign (photo 1, 2), but the comb has darker capped cells from which the brood has not emerged, but all these aspects were not easy to observe because there are only a few small portions of comb (photo3). The microscopic preparation stained by Gram method were been observed at microscope examination Gram positive bacilli with rounded extremities and some of them with central spores (photo 4). From the Petri plates cultivated and incubated were well developed in those with Columbia agar medium. In this medium were been observed round colonies with opaque edges as the center, with rough surface, regular edges and diameter less than 1 milimeter, specific to *Paenibacillus larvae subsp.larvae*.



Photo 1, 2. Died bees (workers) from diseased colony



Photo 3. Honey-comb with characteristic changes



Photo 4. *Paenibacillus larvae* subsp. *larvae*

The obtained results demonstrate that what is believed about the AFB incidence in our country it seems that does not correspond to reality. The simple fact that from a single dead bee from which samples collected was obtained a pure and rich culture of *Paenibacillus larvae* demonstrates that the etiologic agent of disease is more prevalent than beekeepers think. Judging by how samples look and after beekeeper assertions looks like disease started since spring evolving in the bee

family, which also spread to families and even apiaries in the neighbourhood, in many other cases of diseases described by another authors from another countries was happened [1]. Because at the end of November diseased bee family is in wintering period a treatment is not possible, the only solution that requires is the liquidation of disease outbreak. In the next spring it is necessary to control all families exposed to disease and to apply the treatment of this serious honey bee

disease. When the conditions requires drastic measures will be destroyed all contaminated families, apiaries and all hive materials belonging to the colony will be disinfected or destroyed by burning. Some beekeepers destroy all the hives and start over, massive restocking genetic material free from diseases other choice is the embodiment of the "artificial swarm" process is to eliminate all the combs in the hive and their destruction or processing authorized places wax recovery. Queen bees with another hive are written in disinfected by shaking and placed with a large cardboard funnel. The new hive only endows artificial honeycombs, bees and will be fed sugar syrup 1/1, if there collected in nature. Basically the method is to put the family in a state of artificial swarm. Romanian beekeepers must understand that they have to take preventive measures, changing the max. Two years of all combs from hives, disinfecting annual hives, frames, combs the book, disinfecting tools as needed; prevent pilferage, keep in apiary only powerful families (the weak unite or destroy) the procurement genetic material free of disease, limiting the pastoral as possible, maintaining the apiary as a territory "closed".

4. Conclusions

Although the situation of American foulbrood (AFB) incidence in our country is not known, a sample from a beekeeper originated in Timiș County demonstrate that this serious disease it seems to be more extensive than is believed in our country. The examination of six dead bees not reveals any important changes because adults are carriers of etiologic agents, the disease symptoms

being evident at bee brood and combs. The small portions of combs brought to the laboratory have darker capped cells from which the brood has not emerged. The samples taken from a single dead bee processed into microscopic preparation stained by Gram method revealed at microscope examination revealed Gram positive bacilli with rounded extremities and some of them with central spores. Samples cultured in special mediums (Columbia) after incubation formed round colonies with opaque edges as the centre, with rough surface, regular edges and diameter less than 1 millimeter, specific to *Paenibacillus larvae subsp.larvae* demonstrating that in the examined bees family evolves American foulbrood. It should be paid more attention to establish the incidence of the disease in areas in our country and taking measures to limit the evolution.

References

1. Gialbert F. White, American foulbrood, edit. Nabu Press, London, 2011, pp 21-35.
2. Kevan P. G., Elimination of American foulbrood, Ed. Enviroquest, 1995. Asiatic Hive Bee: Apiculture, Biology & Role in Sustainable Development in Tropical & Subtropical Asia. Ontario, pp. 315.
3. Matsuka, M., Verma, L.R., Wongsiri, S., Shrestha, Ed. S. K. & Partap, U. 2000. *Asian beekeeping*, Apicultural Association Conference, pp. 264.
4. Răpuncean G., Boldizsar E., *Practicum de bacteriologie specială*, Ed. Academicpres, 2001, Cluj-Napoca.
5. Târziu E., Cumpănășoiu C., Monica Șereș, Gros R., *Bacteriologie specială-Practicum-*, Ed. Waldpress, 2010, Timișoara.