IMPORTANCE OF MICROSCOPIC TESTING OF HONEY AND POLLEN SAMPLES IN THE PROPHYLAXIS OF MAJOR BACTERIAL DISEASES IN *APIS MELLIFERA CARPATHICA* BEES

Vasilică SAVU[1], Agripina SAPCALIU[1], Petruţ TANASE[2], Victor CALIN[2], Ion RADOI[3], Cristina Ştefania PÎRVULEŢ[4]

[1]Beekeeping Research and Development Institute Bucharest, Romania
[2]Spiru Haret University Bucharest
[3]University of Agronomical Sciences and Veterinary Medicine Bucharest, Romania
[4]Academy of Agricultural and Forestry Sciences*"Gheorghe Ionescu-Sisesti"*
E-mail address:drsilesavu@yahoo.com

Abstract

The purpose of the study was to monitor the presence of bacilli in the honey and pollen samples in correlation to the positive diagnosis of these major bacterial diseases in bees. The study took 3 years, and approximately 156 samples of honey and bee bread from reserve honeycombs and 156 live bee intestine samples were processed. To identify the bacilli in honey, bee bread (pollen) reserve and live bees intestine , we used our own method, and the confirmation of their presence was done through methodology OIE/2008. Of the total tested samples, the bacilli were found present in 63 samples from reserve honeycombs and in 67 samples from live bees' intestine. The bee colonies that did not test bacilli in the samples examined for the duration of the monitoring, did not present a disease episode and did not register mortality of pathologic nature. The mortality registered in the apiaries under study throughout the 3 year-period was 30-100 % for the apiares from which samples testing positive for bacilli had been received. The study confirms that a correlation exists between the presence of bacilli in samples of honey and bee bread from reserve honeycombs, and their presence in adult bees' intestine. The microscopic testing of honey and pollen samples, as well as of bee intestine, may constitute an important prophylactic method in the management of major bacterial diseases in bees (American and European foulbrood).

Keywords: *honeycombs, major bacterial diseases, live bee intestine*

Introduction

Major bacterial diseases (American foulbrood and European foulbrood) are infectious-contagious diseases present in almost all countries (including Romania) that exist as devastating diseases, affecting the larva stage of *Apis mellifera* and other species of *Apis* bees. (4, 5)

During the inactive period (winter), when the reserve food is insufficient to raise the brood and feed the bee families, in reserve honey and pollen in active season and in the form of solid nutrients in inactive season. Supplementary food is necessary when bees do not have sufficient reserve honey in the winter, thus avoiding losses from starvation (1, 2).

The purpose of the study was to monitor the presence of bacilli in the honey and pollen samples in correlation to the positive diagnosis of these major bacterial diseases in bees. (8)

Materials and methods

The study took 3 years from 8 apiaries, and approximately 156 samples of honey and bee bread from reserve honeycombs and 156 live bee samples were processed, compared with control lot (2 apiary). (Table 1)

Table 1

Total tested samples of reserve honeycombs (honey and bee bread) and adult live bees' intestine

Total tested samples	**Honey and bee bread from reserve honeycombs**	**Adult live bees' intestine**
312 samples	156 samples	156 samples

To identify the bacilli in honey, bee bread (pollen) and live bee intestine, we used our own method, and the confirmation of their presence was done through methodology *from* Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. (3, 6, 7).

Of the total tested samples, the bacilli were found present in 63 samples from reserve honeycombs, in 67 samples from live bees' intestine and 182 negative samples (absent bacilli) (Table 2) (Fig. 1).

Table 2

Total tested positive and samples negative from honey, pollen and live bees' intestine

Honey and pollen from reserve honeycombs (present bacilli)	**Honey and pollen from reserve honeycombs (absent bacilli)**	**Live bees' intestine (present bacilli)**	**Live bees' intestine (absent bacilli)**
63 positive samples (40.4 %)	93 negative samples (59.6 %)	67 positive samples (42.9 %)	89 negative samples (57.1 %)

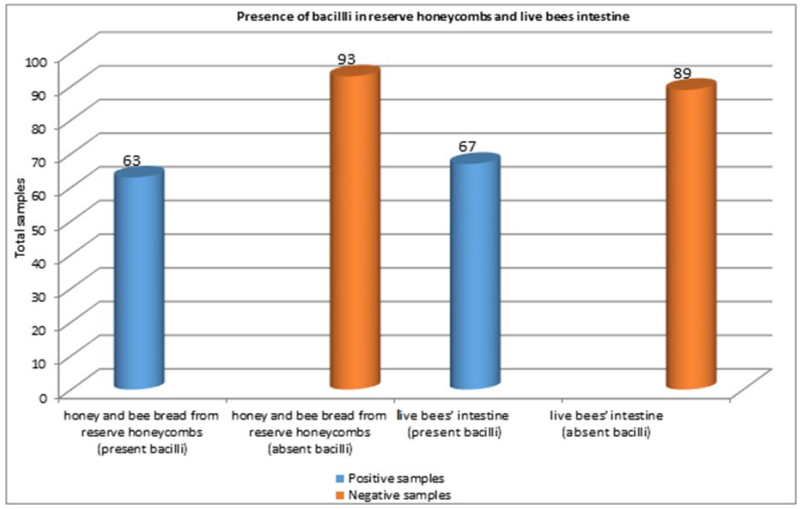


Fig. 1 Total tested positive and samples negative from honey, pollen and live bees' intestine

Distribution of samples and hives mortality percentages in the experimental lot is presented in table no. 3

Table 3

Distribution of samples and hives mortality percentages in the experimental lot

	Apiary no. 1	**Apiary no. 2**	**Apiary no. 3**	**Apiary no.4**	**Apiary no.5**	**Apiary no. 6**	**Apiary no. 7**	**Apiary no. 8**
Experimental lot (no. samples / no. colony)	13	27	17	10	32	24	15	18
Mortality in colonies (%)	13 (100%)	9 (35%)	10 (60%)	3 (30%)	16 (50%)	10 (40%)	5 (30%)	18 (100%)

According to Table no. 3, the distribution of samples in the control lot in the 8 apiaries studied for three years show that 10- 32 samples (pollen, honey, live bees' intestine) were collected for laboratory tests. Mortality percentages in the studied apiaries oscillated between 30-100% depending on the seriousness of the bacterial infestation and colony size. There is no correlation between colony size and mortality percentage in the beehives belonging to the control lot.

Mortality percentage in the control lot is presented in table no. 4

Table 4

Distribution of bee colonies and hives mortality percentages in the control lot

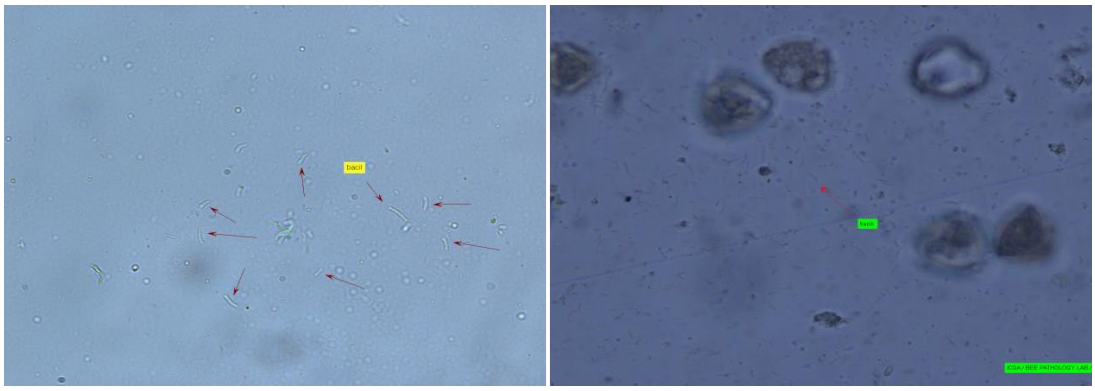
	Apiary lot no. 1	Apiary lot no. 2
No. beehives (control lot)	35	20
Mortality in colonies (%)	0	0

Bee colonies in the witness lot were distributed in the 2 apiaries as follows: 35 colonies in apiary no. 1 and 20 bee colonies in apiary no. 2, the mortality percentage being zero in both apiaries.

Results and discussion

Direct microscopic investigations made on the honey and live bees' intestine samples showed the presence of vegetative forms of etiological agents suspected of major bacterial diseases (Fig. 2 a, b).

The presence of bacilli in reserve honeycombs and in intestine (Fig. 3 a, b) was correlated to the bacterioscopic identification of etiological agents for the American foulbrood and for the European foulbrood (Fig 4, a, b), and subsequently with the evolution of disease episodes clinically manifested, of high morbidity and mortality in adult bees.



a b

Fig. 2 Vegetative forms of etiological agents suspected of European and /or American foulbrood from honeycombs (a) and live bees' intestine (b) (microscopic test directly from the intestine) x 400

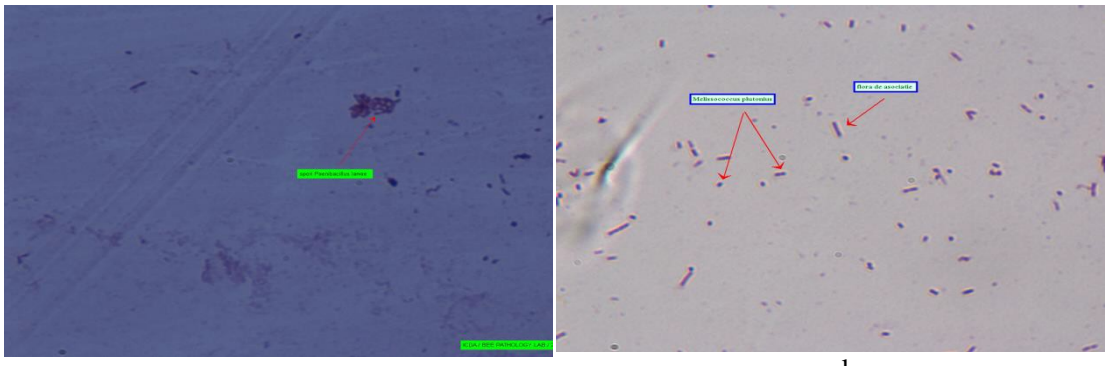


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Fig. 3 Highlighting the etiological agent suspected of American foulbrood (a) and European foulbrood from *live bees' intestine* (b) (Gram colored smear) x 1000

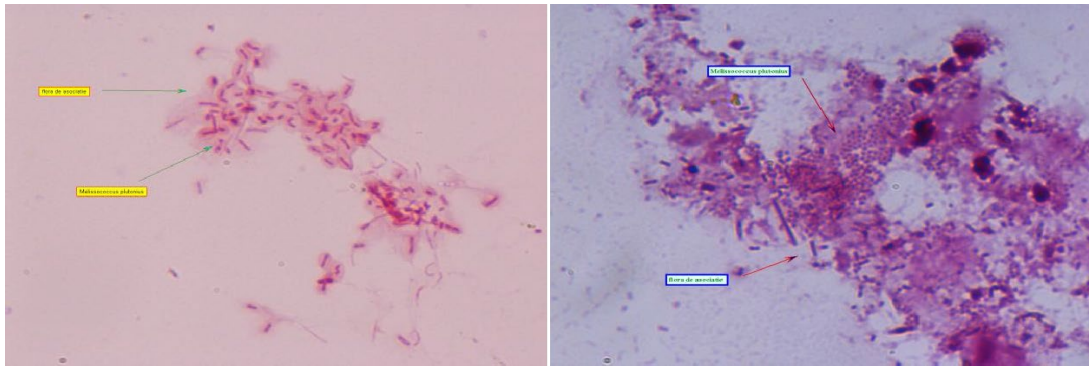

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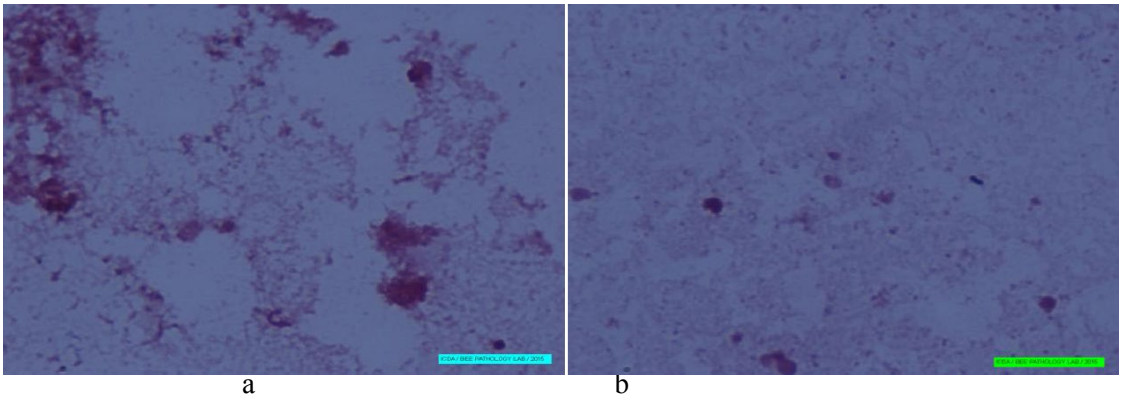
Fig. 4 Highlighting the etiological agent suspected of American foulbrood (a) and European foulbrood from *honeycomb*s (b) (Gram colored smear) x 1000

The identification of etiological agents suspected of major bacterial diseases by bacterioascopic examination, after Gram colouring, of reserve honey and live bees' intestine samples constituted the base for the confirmation diagnosis.

Confirmation examination on the witness lot were negative for all tested samples, correlated to the zero mortality percentage in these lots (Fig. 5 a, b).



a b

Fig. 5 Negative samples of reserve *honey combs* (a) and *live bees' intestine* (absent bacilli) (b) (Gram colored smear) x 1000

The bee colonies that did not diagnostic tested bacilli in the samples examined for the duration of the monitoring, did not present a disease episode and did not register mortality of pathologic nature. The mortality registered in the apiaries under study throughout the 3 year-period was 30-100 % for the apiaries from which samples testing positive for bacilli had been received.

Conclusion

The study confirms that a correlation exists between the presence of bacilli in samples of honey and bee bread from reserve honeycombs, and their presence in adult bees' intestine.

The microscopic testing of honey and pollen samples, as well as of bee intestine, may constitute *an important prophylactic method in the management of major bacterial diseases* in bees (American and European foulbrood).

Acknowledgements

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