

MONITORING BEE COLONIES' HEALTH BY DIAGNOSING AGENTS OF MAJOR BACTERIAL DISEASES IN SAMPLES

MONITORIZAREA SĂNĂTĂȚII FAMILIILOR DE ALBINE PRIN DIAGNOSTICAREA BACTERIOZELOR MAJORE DIN PROBELE RECOLTATE

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ABSTRACT | REZUMAT

The major bacterial diseases (American foulbrood and European foulbrood) are infectious and contagious diseases, that are spread in almost all countries, inclusive Romania. They are a devastating diseases that affect a larvar stage of bee *Apis mellifera* and other bee species of Genus *Apis*.

The purpose of study consisted of monitoring of the presence of bacilli in samples from honey and bee bread (pollen) reserve hives and in bee intestines as vegetative forms of major bacterial diseases' pathogenic agents in bees (American foulbrood and European foulbrood) and this correlation with the positive diagnosis of these diseases in bee colonies. During a 3-year period, 30 samples from 15 apiaries in the South of Romania were collected and examined, out of which 15 samples came from honey and bee bread reserve hives and 15 intestines samples were taken from live bees. In order to identify the bacilli in honey and bee bread hives and in bee intestines, we used our own method and for confirmation of the presence of etiological agents of major bacterial diseases we used OIE/2008 methodology.

Results indicated the presence of bacilli in 6 samples from reserve hives and in 6 intestine samples taken from live bees in the experimental lot. In the witness lot, no bacilli or etiological agents of major bacterial diseases were found in the *Apis mellifera carpatica* bees for which growth and exploiting technologies requirements had been observed.

Microscopic examination of honey and pollen hive as well as bee intestine samples may constitute an important prophylactic measure in the management of major bacterial diseases in bees.

Keywords: honey, pollen, major bacterial diseases, bee intestines

Bolile bacteriene majore (loca americană și loca europeană) sunt afecțiuni infectocontagioase prezente în aproape toate țările, inclusiv România, evoluând ca boli devastatoare ce afectează stadiul larvar al albinei *Apis mellifera* și al altor specii ale genului *Apis*.

Scopul studiului a constat în monitorizarea prezenței bacililor în probele de miere și polen, corelată cu diagnosticul pozitiv al acestor afecțiuni bacteriene majore la albine. Studiul a fost derulat pe o perioadă de 3 ani și au fost prelucrate și analizate 15 probe provenite din fagurii de rezervă cu miere și păstură, și 15 probe de albine vii (intestin de albine). Pentru identificarea bacililor din miere și păstură (polen) s-a utilizat o metodă proprie, iar confirmarea prezenței acestora s-a efectuat prin metodologia OIE/2008.

Rezultatele obținute au arătat că din totalul probelor analizate s-a semnalat prezența bacililor în 6 de probe provenite din fagurii de rezervă și 6 probe din intestinul albinelor vii. Nu a fost depistată prezența altor agenți etiologici ai bolilor la *Apis mellifera carpatica* pentru familiile luate în studiu (lot martor) la care au fost respectate cerințele tehnologice de creștere și exploatare. Examenul microscopic a probelor de miere și polen, precum și al intestinului de albine, poate prezenta o măsură profilactică importantă în managementul bolilor bacteriene majore la albine.

Cuvinte cheie: miere, polen, boli bacteriene majore, intestin de albine

The major bacterial diseases (American foulbrood and European foulbrood) are infectious and contagious diseases that affect a larvar stage of bee *Apis mellifera* and other bee species of Genus *Apis*, that are spread in almost all countries, inclusive Romania [5].

The American foulbrood is the most devastating disease affecting the larval stage of bees, causing substantial economic losses, practically every colony showing clinical signs of American foulbrood is doomed to die if corrective measures are not taken in the first stage of symptoms [1, 5].

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The European foulbrood normally affects uncapped brood aged 4-6 days, as well as older larvae, causing important losses in bee colonies [2, 5].

The purpose of study consisted of monitoring of the presence of bacilli in samples from honey and bee bread (pollen) reserve hives and in bee intestines and this correlation with the positive diagnosis of these diseases in bee colonies [3,4].

MATERIAL AND METHOD

During a 3-year period, 30 samples from 15 apiaries in the South of Romania were collected and examined, out of which 15 samples came from honey and bee bread reserve hives and 15 intestines samples were taken from live bees which represented the experimental lot. The witness lot was made of two clinically healthy apiaries, from which a total of 4 samples were collected (Table 1).

Table 1

The number of examined samples from reserve (honey and bee bread) hives and intestine samples from live adult bees

Total number of examined samples		34
EXPERIMENTAL LOT	Samples from honey and bee bread reserve hives	15
	Samples of intestines from live bees	15
WITNESS LOT	Samples from honey and bee bread reserve hives	2
	Samples Of intestines from live bees	2

In order to identify the bacilli in honey and bee bread hives and in bee intestines, we used our own method and for confirmation of the presence of etiological agents of major bacterial diseases, we used *OIE/2008* methodology. (*OIE - Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*) [3, 4].

RESULTS AND DISCUSSIONS

Direct microscopic examination of samples from honey and bee bread hives and from live bee intestines revealed the presence of bacilli in the experimental lot in 6 samples of honey and bee bread reserve hives and in 6 samples of intestines from live bees, while the remaining 9 samples in the experimental lot and in the witness lot were negative (Table 2).

Table 2

The number of positive samples (present bacilli) in the honey and bee bread reserve hives and in live bee intestines in the experimental lot

Positive samples of honey and bee bread in the reserve hives (present bacilli)	6	(40,4 %)
Negative samples of honey and bee bread in the reserve hives (absent bacilli)	9	(59,6 %)
Positive samples of intestines from live bees (present bacilli)	6	(42,9 %)
Negative samples of intestines from live bees (absent bacilli)	9	(57,1 %)

The repartition of samples and mortality percentage at the level of experimental lot is presented in Table 3.

Table 3

The repartition of samples and mortality percentage at the level of experimental lot

	Apiary 1	Apiary 2
Experimental lot (samples number)	10	15

In conformity with Table 3, the repartition of samples from experimental lot of 2 apiaries investigated during a 3-year period showed the existence of dead bee families by 5-10 samples (honey, bee bread reserve hives and 15 intestines samples were taken from live bees. In order to identify the bacilli in honey and bee bread hives and in bee intestines) used for laboratory exam. The percentage of mortality in investigated apiaries was 30-100% in function of bacterial infection gravity and colony dimension. There wasn't any correlation between colony dimension and the percentage of mortality in families from experimental lot. The repartition of bee families and percentage of mortality in control lot is presented in Table 4.

Table 4

The repartition of bee families and percentage of mortality in control lot

	Apiary 1	Apiary 2
Number of bee families	5	5
Number of dead bee families	0	0

The presence of bacilli as vegetative forms of ma-

for bacterial diseases in bees, in samples from honey and bee bread hives and from live bees' intestines is also presented in figures 1A and 1B.

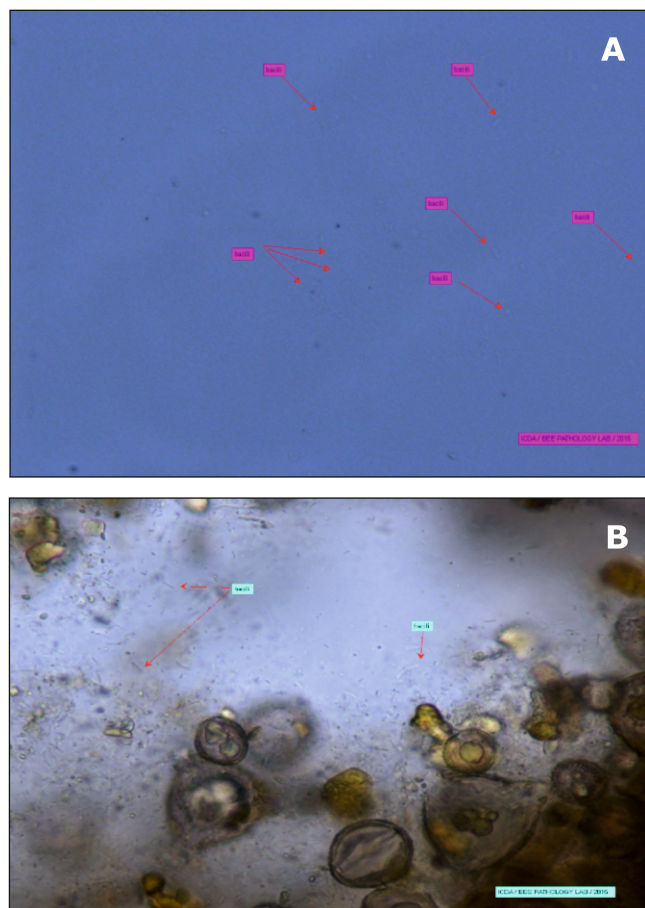


Fig. 1. The presence of bacilli in samples of honey and bee bread hives (A) and of live bees' intestines (B) x 400 (original)

The presence of bacilli in samples of honey and bee bread from reserve hives, as well as in the intestines of live bees from experimental lot was correlated with the expression of clinical signs typical of major bacterial diseases (American foulbrood and European foulbrood) in brood, and also with increased mortality that oscillated between 30% and 100%. In the witness lot, no clinical signs typical of major bacterial disease were found and neither was mortality in the bee colonies of this lot. Also, the existence of bacilli in the honey and bee bread reserve hives and in the intestine of live bees was correlated to the bacterioscopic identification of etiological agents for the American foulbrood and the European foulbrood (Fig. 2A and 2B as well as Fig. 3A and 3B) and subsequently to the clinically manifested evolution of disease episodes, to morbidity and increased mortality.

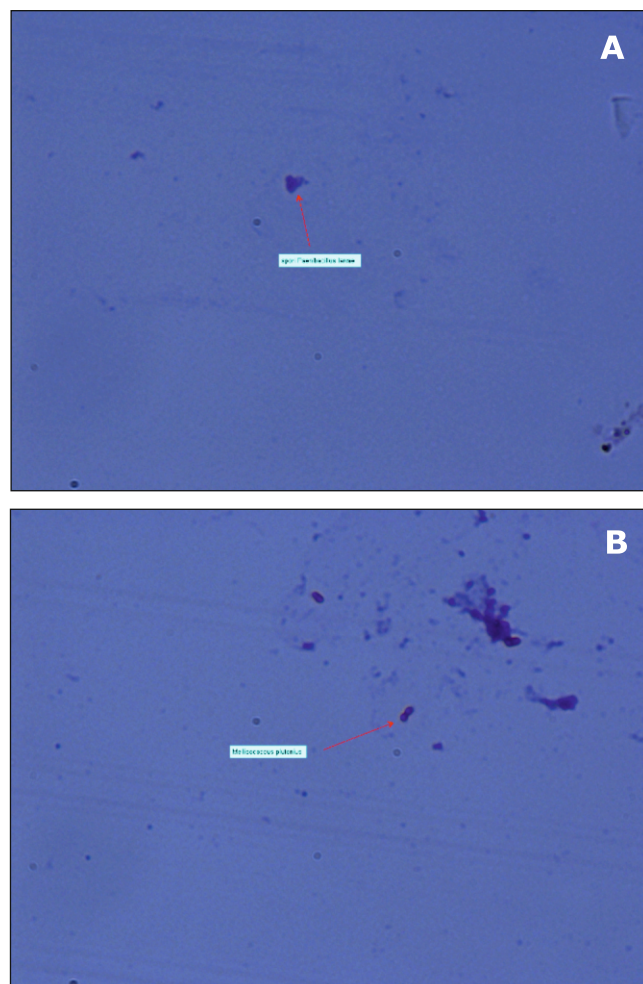


Fig. 2. Bacterioscopic highlighting of the etiological agent of the American foulbrood (A) and of the European foulbrood (B) in the reserve hives (Gram coloration) x 1000 (original)

The tests for bacterioscopic highlighting (Gram coloration) of etiological agents of major bacterial diseases in bees (American foulbrood and European foulbrood) in the witness lot, in samples from reserve hives (Fig. 4A) and from live bees' intestines (Fig. 4B), was negative in all examined samples.

Bee colonies in the witness lot were not diagnosed positive for bacilli in the samples examined throughout the entire duration of the monitoring, they didn't present episodes of manifest disease and didn't register pathologic mortality.

The presumptive diagnosis of major bacterial disease in the reserve hives and in bee intestines, by bacterioscopic highlighting of etiological agents for the American foulbrood and European foulbrood, was subsequently the base of the diagnosis for the bacteriologic and/or molecular confirmation of disease in the monitored bee colonies.

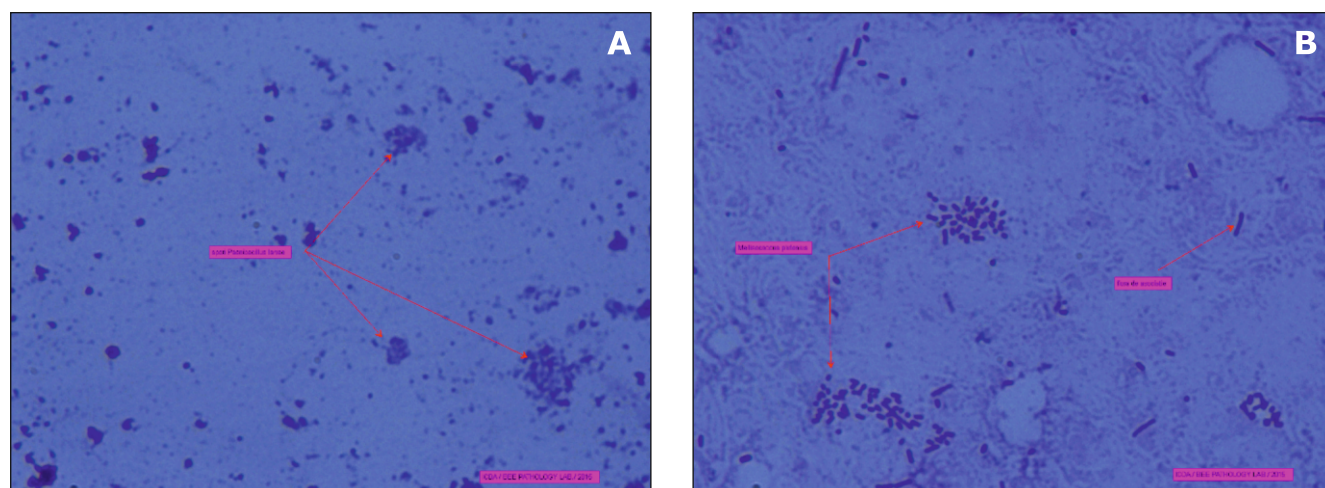


Fig. 3. Bacterioscopic highlighting of the etiological agent of the American foulbrood (A) and of the European foulbrood (B) in bee intestine (Gram coloration) x 1000 (original)

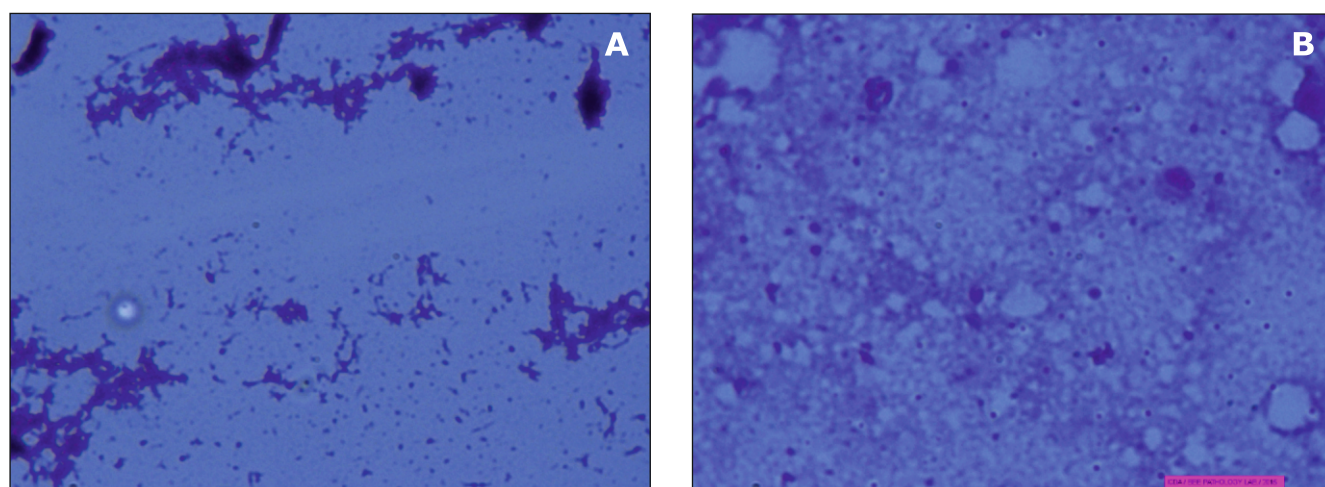


Fig. 4. Negative bacterioscopic diagnosis in samples from reserve hives (A) and live bee intestines (B) in the witness lot (Gram coloration) x 1000 (original)

CONCLUSIONS

Studies concerned with monitoring bee colonies' health by diagnosing agents of major bacterial diseases in the samples from honey and pollen hives and live bee intestines allowed setting the following conclusions:

- 1) Direct microscopic examination of samples from honey and bee bread hives and from live bee intestines revealed in certain samples the presence of bacilli;
- 2) There is a correlation between the presence of bacilli in samples of honey and bee bread in reserve hives and the presence of bacilli in adult bee intestines;
- 3) The presence of bacilli in the samples of honey and bee bread in reserve hives and in live bees' in-

testines as potential vegetative forms of major bacterial diseases in bees, may constitute a form of presumptive diagnosis of major bacterial diseases in bee colonies;

- 4) The presence of bacilli in the samples of honey and bee bread in reserve hives as well as in live bees' intestines was also correlated to expressions of clinical signs typical of major bacterial diseases (American foulbrood and European foulbrood) in the brood, as also to increased mortality in the respective bee colonies;

- 5) Direct microscopic examination and subsequently bacterioscopic examination of honey and bee bread samples from hives, as well as of samples from bee intestines may constitute an important method in the management of major bacterial diseases in bees.

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