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STUDIES ON THE DIAGNOSIS OF BEE ASCOSPHEROSIS ON LIVE BEES SAMPLES AND BROOD COMB THROUGH MORPHO-CLINICAL TESTING AND LABORATORY EXAMINATION

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Abstract

The purpose of this work is to monitor bee health through the morpho-clinical testing and laboratory examination of live bee samples and brood comb for the prophylaxis and control of bee ascospherosis. We investigated in the active year 2020 a number of 68 samples (34 samples of live bees and 34 samples of brood combs). Samples were collected in the beginning of the inactive season and examined morpho-clinically and in the laboratory. The laboratory method employed was in accordance with OIE regulations (2008) and adapted to an original methodology in the Pathology Laboratory of ICDA. Laboratory test results emphasized the presence of Ascosphera apis fructification bodies and hyphae in 20 live bee intestines (58.82%) the remaining 14 samples being negative (41.18%). The morpho-clinical testing of the 34 brood comb samples proved the existence of chalk brood in 10 samples (29.41%), the respective samples being correlated to the existence of Ascosphaera in the live bee intestines sample, and the remaining 24 samples were negative (70.59%).

Key words: *bees, chalkbrood, Ascosphera apis, fructification bodies.*

INTRODUCTION

Ascospherosis is the most important mycotic disease that affects the health of bees in apiaries (*Apis mellifera carpathica*) throughout the year, it evolves in both beekeeping seasons (active and inactive), it weakens the bee colony and is critical in the development of other contagious diseases. (Savu & Sapcaliu, 2013; Ansari et al., 2016). Ascospherosis affects the larvae of the *Apis* melliferous bee (Liang et al., 2000), as well as the larvae of solitary wild bees, like *Megachile* (Castagnino et al., 2020). In *Apis mellifera*, the disease is caused by the *Ascosphera apis* fungus, in the *Fungi* genus, the *Ascomycota* family, the *Eurotiomycetes* class, *Ascospherales* order; being characterized by dead larvae coverage, as a mantle, in the fungal mycelium, and by dehydration and dead larvae transformation into white rugous mummies (Gilliam et al., 1978), looking like chalk-brood or black in color (Savu & Sapcaliu, 2013; Asiminei et al., 2016). There are also significant losses as result of diminished number of bees as well as decreased bee colony productivity, and lower honey yield by 5-37% (Ansari et al., 2016). The damages caused by mycoses in bee colonies and in the hive economy, respectively, (Aronstein et al., 2010), are the more important as they evolve together with other parasitoses (varroasis, nosemosis) and major bacterial infections (American loca, European loca) (Ansari et al., 2016). There is data that proves a rise in incidence in the recent years. In Europe, in 1913, Maassen described the ascospherosis for the first time, and in the second half of the 20th century it was diagnosed in Germany (Aronstein et al., 2010), Russia and Great Britain. By 1977, ascospherosis was recognized as the most serious infectious disease in bees in Norway (Heath, 1985). In 1957, ascospherosis started evolving beyond Europe, being identified in New Zeeland (Reid M., 1988),

Central America, Japan (Yoshiyama et al., 2011), North America and Canada (Aronstein & Murray, 2010), Australia (Sheba et al., 2020) and China (Zhi et al., 2018).

MATERIALS AND METHODS

Bee colonies' health monitoring through morpho-clinical and laboratory tests on samples of bees (Milea, 2017; 2019) and brood comb was performed with purposes of prophylaxis and control of ascospherosis in bees (Radoi, 2018).

In 2020, investigations were carried out on 68 bee colonies in 4 apiaries (17 bee colonies per each apiary), through morpho-clinical examinations, a number of 68 samples being collected at the beginning of the inactive season for laboratory tests, consisting in 34 live bee samples and 34 brood comb samples (Table 1).

Table 1. Number of samples collected per bee colony

Examined apiary	Number of bee colonies (experimental lot)	Samples of collected live bees	Samples of collected brood comb
Apiary 1	17	8	8
Apiary 2	17	8	8
Apiary 3	17	9	9
Apiary 4	17	9	9
Total	68	34	34

The morpho-clinical examination of the collected samples was followed by the laboratory test which was performed through an original method in the Pathology Laboratory of ICDA Bucharest, adapted for intestine samples collected from live bees according to OIE regulations (OIE, 2018) to identify bee diseases (Jensen et. al., 2013). Some descriptions of the ascosphaera and observations on the ascospores were also made by use of a NIKON ELIPSE E_{400} microscope and a morphometrics software.

RESULTS AND DISCUSSIONS

As result of the morpho-clinical examination and laboratory tests in the 4 apiaries, samples from 30 bee colonies were found positive for *Ascosphera apis*, out of which 20 samples of live bees (58.82%) and 10 samples of brood comb (29.41%), the remaining samples being negative (Table 2, Figure 1).

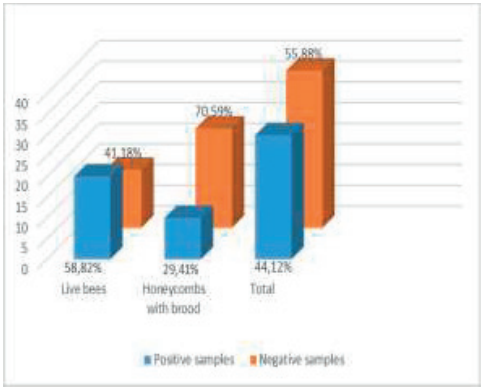

Figure 1. Proportion of positive and negative samples for ascospherosis in bee colonies examined in the 2020 season

Table 2. Samples diagnosed positive and negative for *Ascosphera apis* in live bees and brood comb

Bee colonies examined	Live bees	Brood combs	Total samples
Positive	20 (58.82%)	10 (29.41%)	30 (44.12%)
Negative	14 (41.18%)	24 (70.59%)	38 (55.88%)
Total	34	34	68

As noticed in Table 2 and Figure 1, the morpho-clinical examinations of hive brood samples highlighted the existence of chalk-brood in 10 samples (29.41%). Interestingly, some brood larvae that seemed unaffected presented initially only small white spots under the dermis and later (3-5 days) these larvae turned into chalk-brood (Figures 2, 3). This sign (small white spots under dermis in bee brood larvae), noticed while examining the larvae in the brood comb samples, may constitute a presumptive diagnosis of chalk-brood.

Figure 2. Comb with larvae affected by chalk-brood

Figure 3. Larva affected by chalk-brood (left) and larva with white spots susceptible of developing chalk-brood

The laboratory examination of intestine samples from live bees showed the existence of fructification bodies and hyphae of *Ascosphera apis* in 20 samples (58.82%), the remaining samples being negative (41.18%). The 20 samples of intestines from live bees that were found positive through microscopic examination can be deemed suspicion of ascospherosis in the examined bee colony (Figures 4, 5, 6, 7). To confirm this funding, all intestine samples from the live bees found positive for fructification bodies and hyphae of *Ascosphera apis* (Chorbinski et al., 2003) were correlated with samples of positive brood combs for ascospherosis that had been collected from the same bee colony.

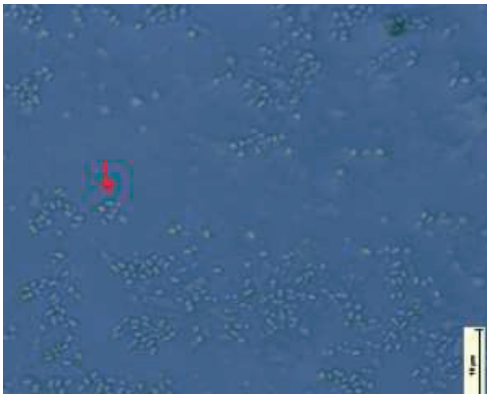

Figure 4. Spores of *Ascosphera apis* during examination of intestine in live bees. Prepared directly x 400

Figure 5. Hyphae and fructification bodies (ascospheres) of *A. apis*. Prepared directly from live bee intestine x 400

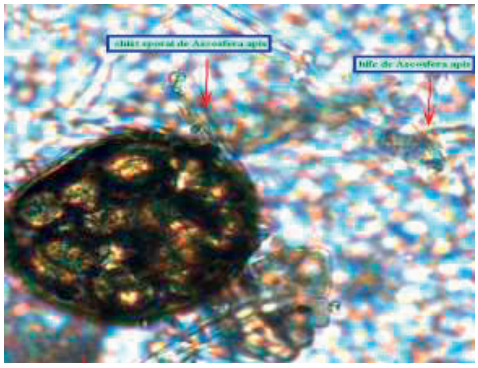

Figure 6 Ascosphaera with ascospores and hyphae of *A. apis* from live bees. Prepared directly, x 400

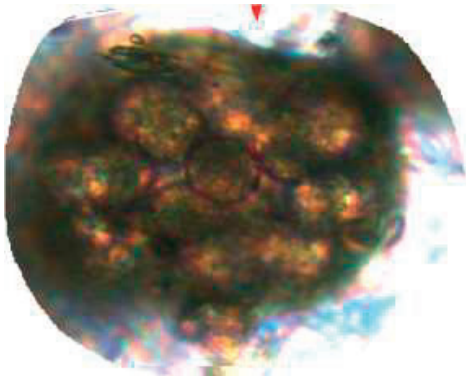

Figure 7. Ascosphaera with ascospores of *A. apis* from live bee intestine. Prepared directly, x 400

This demonstrates that the examination of live bee intestines may be introduced as an simple laboratory examination for the suspicion of ascospherosis in the bee colony, without samples of brood comb. Testing bees before the inactive season for *A. apis* in samples of intestines, correlated with the morpho-clinical examination of combs, represents an important

prophylactic method to diagnose chalkbrood in bees (Savu, 2017; Sapcaliu, 2017; Radoi, 2018; 2019).

The laboratory examination of live bee intestines also included measuring dimensions of ascosphaera, puffballs and ascospores with the morphometric microscope. The results we have obtained show a dimension of the ascosphaera' diameters of 110-190 µm (average 155 µm), the dimension of puffballs being 16-41 µm (average 32 µm), and the dimension of ascospores (length/width) was 2.7-4/1.3-1.9 µm (Figure 8). Our results concur with the results obtained by Wynns et al., 2013; Aronstein et al., 2009; Anderson et al., 1998.

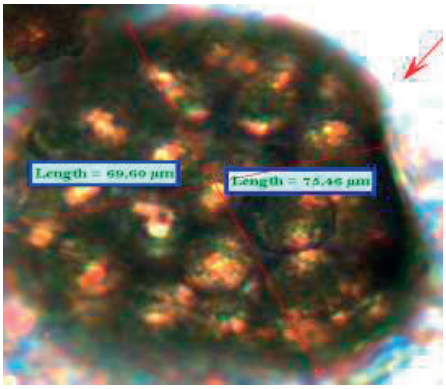

Figure 8. Morphometry of ascosphaera and puffballs in the intestine of live bees

Correlating the results of laboratory tests on live bee intestines with the morpho-clinical examination of larvae in the brood combs (Heath, 1982a), even in the visible absence of chalk-brood larvae, we may suspicion an ascospherosis diagnosis. Thus, the examination of live bee intestine, when we notice the existence of ascospheres, puffballs and ascospores, may become an instrument for early diagnosis of ascospherosis in bee colonies.

CONCLUSIONS

The morpho-clinical and the laboratory examinations of live bee intestines in 2020 on 68 bee colonies revealed ascopherosis in 30 bee colonies (40.12%).

Of the 30 bee colonies diagnosed with ascopherosis, in 20 samples the ascopherosis was suspicioned in the microscopic examination of live bee intestines (58.82%), while in 10 samples the ascopherosis was shown by morpho-clinical examination of brood combs (29.41%).

All live bee intestine samples found positive for the existence of fructification bodies and hyphae of *Ascosphera apis* were correlated with samples of brood combs found positive for ascospherosis collected from the same bee colonies.

The morphometrics of ascosphaera, puffballs and ascospores showed dimensions of 110-190 µm, 16-41 µm and 2.7-4/1.3-1.9 µm, respectively, for ascospores, values that concur with studies by other authors.

Testing bees before the inactive season for *A. apis* on intestine samples correlated with the morpho-clinical examination of combs represents an important prophylactic method to diagnose chalk-brood in bees.

The examination of live bee intestines, where we notice the existence of ascosphaera, puffballs and ascospores, may suspicion early diagnosis of ascospherosis in bee colonies.

Compliance with ethical standards. The research does not involve human and/or animal experimentation.

Conflict of interest. The authors declare that they have no conflict of interest. We mention that the research conducted has no connection with the activity of official territorial or central laboratories nominated for the monitoring and control of bee diseases.

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