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ASCOSPHEROSIS INCIDENCE IN BEES INVESTIGATED FOR MAJOR BACTERIOSIS IN THE BEEKEEPING YEAR 2016

Florentin Gheorghe MILEA[1], Ion RADOI[1], Agripina ŞAPCALIU[2], Vasilică SAVU[2], Ovidiu POPA[1]

[1]University of Agronomical Sciences and Veterinary Medicine, Bucharest, Romania
[2]Beekeeping Research and Development Institute of Bucharest, Romania

Corresponding author email: sapcaliuagripina@yahoo.com

Abstract

Ascospherosis is an invasive mycosis occurring in Apis melifera bees, caused by Ascosphera apis that affects the 1-5 days aged bee larvae of maximum receptiveness at the age of 1-2 days. From the total of 18 apiaries identified in the active-inactive season 2016 in which the evolution of mycotic diseases was diagnosed, the chalk brood was present as a morbid entity with unique evolution in 10 apiaries (55.55 %), stone brood evolved in 2 apiaries (11.11 %), and mycotic diseases of mixed evolution were registered in 3 apiaries (16.66 %), out of which suspicions of major bacterial diseases in one apiary (5.55% cases) and in 2 apiaries evolved together with internal and external parasitoses (11.11 %). Regional incidence of chalk brood places the south-east area on first place having over 2/3 of positive tests. Season incidence of the chalk brood shows that over 38.8 % cases presented it at the end of the beekeeping season, and in the inactive season months (January – February) the incidence is minimum (11.11 % cases). Complex laboratory tests in all the 18 apiaries diagnosed positively with mycotic diseases permitted identification of Ascosphera apis spores in the samples collected from live bees intestines, pollen, bee bread and brood combs. Bee colonies in the monitored apiaries (59.56 %), in which ascopherosis evolved and did not present clinical signs, may be deemed infestation sources.

Key words*: Apis mellifera carpatica, chalk brood, honey bee.*

INTRODUCTION

Ascospherosis is an invasive mycosis found in *Apis melifera* bees, caused by *Ascosphera apis*, which accompanies most often the disease episodes in major bacteriosis in bees (Savu & al, 2013).

The history of the incidence and geographical spread of the parasitosis in *Romania* is based on research carried out in the Beekeeping Research and Production Institute in Bucharest, which after 1971 made available for beekeepers the product *Micocidin*, especially for prophylactic purposes and to stimulate bee colonies (*Ogradă, 1986*).

The causal agent *Ascosphera apis* affects larvae aged 1-5 days, of maximum receptivity when aged 1-2 days.

After spores' germination on bee brood larvae, these will invade their organism so that the vegetative forms will invade the entire larva under the form of a mycelium (Yoshiyama Mikio, 2011). Mummified larvar forms end up being dark brown to black in color and remain an infestation source (Sarah A. Maxfield-Taylor*, 2015*).

Taxonomic classification of the etiologic agent *Ascosphera apis* is the following: *Ascomycota; Pezizomycotina; Eurotiomycetes; Eurotiomycetidae; Onygenales; Ascosphaeraceae; Ascosphaera apis* (Annette Bruun Jensen et al*., 2011*).

MATERIALS AND METHODS

Studies performed in the beekeeping year 2016, as part of the research project 157/2014, on a number of 287 apiaries belonging to all beekeeping regions of Romania (N, S, E, V), out of which 18 apiaries were selected for investigations regarding laboratory confirmation of the clinical evolution of mycotic diseases caused by *Ascosphera apis*.

The 18 apiaries diagnosed positively for mycoses totaled 1,625 bee colonies out of which 531 bee colonies were affected by the disease and 126 bee colonies presented mortality.

Laboratory tests were carried out on intestine samples collected from live bees, pollen and bee bread, from bee colonies diagnosed positively for mycoses. The methodology of

laboratory tests observed protocols O.I.E. 2008, for bee diseases diagnosis, also using an original method of investigating adult bee intestine content.

RESULTS AND DISCUSSION

The results of clinical tests confirmed increased regional incidence of chalk brood in Romania in the S-E area of the country (66.66 % positive sample).
This may be justified also through the presence of a large number of samples from this area collected while monitoring major bacterioses in bees.
The regional incidence of chalk brood in Romania in the beekeeping year 2016 is presented in table 1 and figure 1

Table 1. Regional incidence of chalk brood in Romania

Area of investigated apiaries	Number of apiaries
South	12 (66.66 %)
North	3 (16.66 %)
Esst	2 (11.11 %)
West	1 (5.56 %)

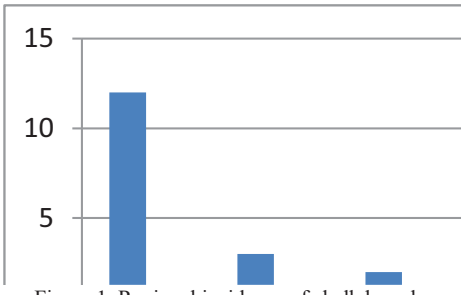

Figure 1. Regional incidence of chalk brood in Romania in the active-inactive season 2016

As regards the season incidence of this disease in bee brood, we noticed at the end of the active season an incidence in 38.88 % cases, as compared to the end of the inactive season (11.11 %), an indication of the dependence on the evolution of brood development in hives and, probably due to a protection effect of propolis with which hives are padded at the end of the active season, that has an antiseptic action during the inactivity period (November, December, January and February) (Vojvodic, S and al. 2011a., Vojvodic, S and al 2012.).

A slight increase in the number of cases occurs in sprin months (March, April, May) when the bee brood develops (22.22%) and progressively grows in the summer (June, July, August), at the flow peak. Season incidence of chalk brood throughout the entire beekeeping season 2016 is presented in table 2 and figure 2.

Table 2. Season incidence of chalk brood in the (active-inactive) beekeeping season 2016

Season	Number of apiaries
Winter (January - February)	2 (11.11 %)
Spring (March –April -May)	4 (22.22 %)
Summer (June – July - August)	5 (27.77 %)
Autumn (September - October)	7 (38.88 %)

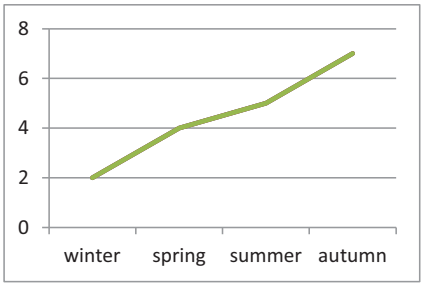

Figure 2. Status of season incidence of chalk brood in the 18 apiaries diagnosed positively in the (active-inactive) beekeeping season 2016

Of the 287 tested apiaries, 18 apiaries (6.27 %) were declared positive, having clinical signs typical of mycotic diseases.
The 18 apiaries diagnosed positively were made up of 1,625 bee colonies, out of which 531 bee colonies were affected by the disease, with mortality in 126 bee colonies) (Table 3, figure 3).

Table 3. Status of investigations in the apiaries examined in the beekeeping active-inactive season 2016

Number of examined apiaries	Number of apiaries diagnosed with mycotic diseases	Total number of bee colonies affected by mycotic diseases	Bee colonies affected by chalk brood	Mortality
287 (100 %)	18 (6.27 %)	1,625 (100 %)	531 (35 %)	126 (8.22 %)

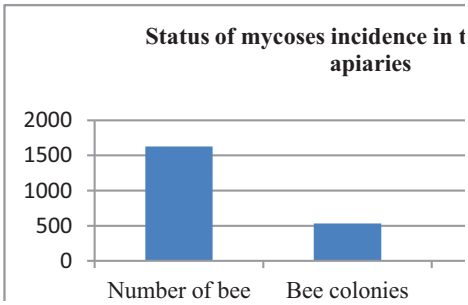

Figure 3. Status of investigations in the examined apiaries diagnosed with chalk brood in the beekeeping season 2016

Of the total of 18 apiaries in which mycotic diseases evolution was diagnosed, chalk brood was present as a unique morbid entity in 10 apiaries (55.55 %), stone brood evolved in 2 apiaries (11.11 %), and mycotic diseases with mixed evolution were registered in 3 apiaries (16.66 %).

The evolution of mycotic diseases with bacterial diseases was registered in one apiary (5.55%), and the evolution of mycotic diseases together with internal and/or external parasitoses was registered in 2 apiaries (11.11 %).

The evolution of mycoses alone or in association with other diseases is presented in table 4.

Table 4. Incidence of apiaries affected by mycotic diseases in the active season 2016

Apiaries with					
mycotic diseases	chalk brood	stone brood	mixed mycotic diseases	mycotic diseases + suspicion of major bacterial diseases	mycotic diseases + parasitoses (internal, external)
18 (100 %)	10 (55.55 %)	2 (11.11 %)	3 (16.66%)	1 (5.55 %)	2 (11.11 %)

Following the laboratory tests, 287 test bulletins were issued (for each apiary), according to work protocols as part of project 157/2014 (stage 3/2016). Clinical tests allowed identifying in the monitored apiaries mycoses caused by *Ascosphera apis* in 18 apiaries, based on the aspect of brood combs in which the presence of the typical chalk brood was found (Fig. 5, 6, 7).

Complex laboratory testing of all the 18 apiaries positively diagnosed with mycotic diseases permitted the identification of *Ascosphera apis* spores in the samples collected from live bees intestine, pollen, bee bread and brood combs (Figure 8). According to the guidelines of good practice in beekeeping, easily accessible to beekeepers, the use of uncontrolled pollen for supplementary feeding of bees in Romania is avoided (Beekeepers' Association in Romania, 2011).

Figure 5. Diagnosed suspicion of major bacterial diseases accompanied by ascospherosis (Pathology Laboratory, ICDA, Nikon D 200)

Figure 6. Aspect of an affected chalk brood larva (Pathology Laboratory, ICDA, Nikon D 200)

Figure 7. Brood larva affected by ascospherosis (white color) compared to a normal larva (Pathology Laboratory, ICDA, Nikon D 200)

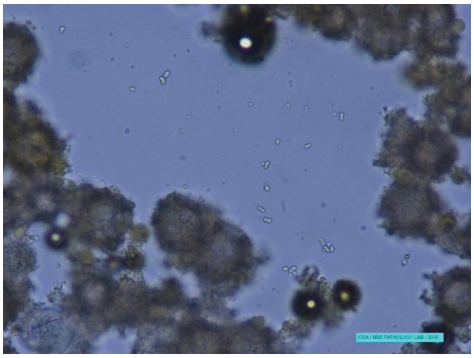

Figure 8. Identifying *Ascosphera apis* spores in the pollen from an apiary in which only ascospherosis developed (infestation source) (Pathology Laboratory, ICDA, Nikon ob x 40)

Microscopic examination of brood comb and samples of live bee intestine content showed various evolving forms of *Ascosphera apis* fungus, presented in fig. 9, 10, 11, 12, 13. (Aronstein K.A. and K.D. Murray, 2010.)

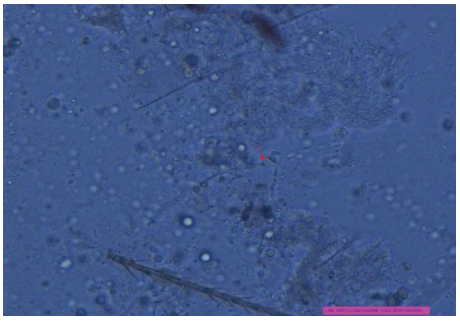

Figure 9. Development of *Ascosphera apis* ascogonia (Pathology Laboratory, ICDA, Nikon ob x 40)

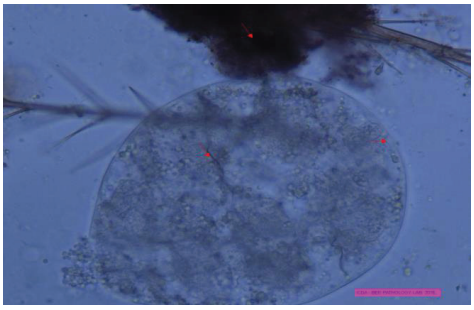

Figure 10. Green-brown ascocysts of *Ascosphera apis*. Detail of an ascocyst partially opened, containing spore-balls (Pathology Laboratory, ICDA, Nikon ob x 40)

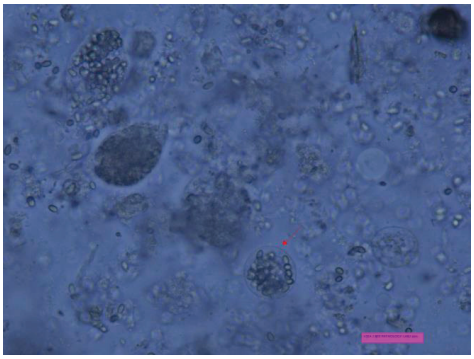

Figure 11. *Ascosphaera apis* spore cyst surrounded by fungal mycelia; B) inside the spore cyst there are numerous spore-balls; each spore-ball has millions of oval shaped spores. (Pathology Laboratory, ICDA, Nikon ob x 40)

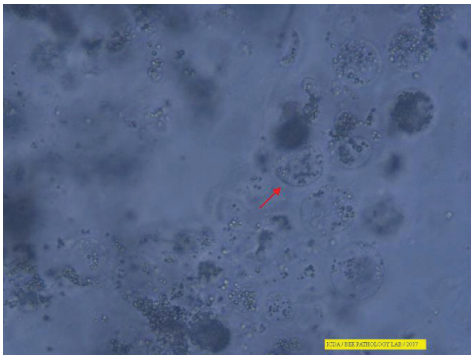

Figure 12. Asci resulting from the ascoma break that contain the fungal ascosphere identified in the intestine of a live bee collected from an apiary in which only ascospherosis evolved (Pathology Laboratory, ICDA, Nikon ob x 40)

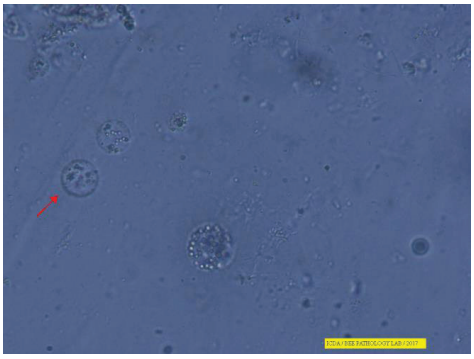

Figure 13. Showcasing ascospores identified in live bee intestine (Pathology Laboratory, ICDA, Nikon ob x 40)

We mention that the research conducted in the project 157/2014 has no connection with the activity of official territorial or central laboratories nominated for the monitoring and control of bee diseases.

CONCLUSIONS

Monitoring bee colonies as regards the evolution of major bacterioses indicated the presence of chalk in 55.55 % of the tested samples, a unique infestation (5.55 % associated with suspicion of major bacterioses).

The regional incidence of chalk brood places the South-East area on the first place with over 2/3 of the positive tests.

Season incidence of chalk brood shows that in over 38.8 % of the cases are at the end of the beekeeping season, and in the inactive season months (January-February) the incidence was minimum in 11.11 % cases.

Food (additional and stimulation feeding), temperature, humidity, seem to play an important part in the season incidence of chalk brood, thus 66 % cases are found in the hot season.

The presence of chalk brood in 55.55% cases, together with major bacterial diseases, shows that the common element of morbid bacterial and fungal entities is in a deficit of the immune system.

In 2016, out of the 287 tested apiaries, 18 apiaries (6.27 %) were declared positive for brood mycoses, having clinical signs typical of mycotic diseseas.

Bee colonies from the monitored apiaries (59.56 %), in which ascospherosis evolved and did not manifest sings of clinical disease may be considered infestation sources.

It is recommended to test live bees and inspect the main infestation sources (pollen, honey).

ACKNOWLEDGEMENTS

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