CONTROL OF AMERICAN FOULBROOD DISEASE WITHOUT ANTIBIOTIC USE

Amerikan Yavru Çürüklüğü'nün Antibiyotik Kullanmadan Kontrolü

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Summary: With regard to the prohibited use of veterinary preparations containing antibiotics and sulfonamides in apicultural practice in the Republic of Bulgaria, attempts for healing apiaries affected by American Foulbrood without recourse to these drugs were performed. Three apiaries with a total of 119 bee families were healed. The healing of bee colonies was achieved via quarantine, destruction of affected bee families via burning and disinfection of hives with 4% sodium hydroxide. Following two negative examination at 15-day intervals for clinical signs of diseased bee families, a microbiological examination of bee honey, sampled from all families in the apiaries, for contamination with *Paenibacillus larvae ssp. larvae* spores was performed. The bee families, contaminated with spores, were moved as an "artificial swarm" in disinfected hives on new frames with wax foundation. Prior to moving, the bees were kept for 48 hours without food in a cool premise. In bee families, diagnosed as diseased at the end of the active season, the examination of honey for contamination with spores and the transfer of swarms were done in the spring of the next year. The old combs of these families were melted and the nests were broadened with new frames for building combs. The apiaries were considered as healed and the quarantine was removed one year after the last destruction of a bee colony and negative results in the examination of honey samples for contamination with spores.

Key words: American foulbrood (AFB), alternative control, honey bee, *Paenibacillus larvae larvae.*

ÖZET: Bulgaristan arıcılığında yasaklanan antibiotic ve sulfonamids içeren veteriner ilaçları kullanmadan amerikan yavru çürüklüğü hastalığının tedavi çalışması yapılmıştır. 3 arılıktan toplam 119 koloni tedavi edilmiştir. Kolonilerin tedavisi karantina, hastalıklı kolonilerin yakılması ve %4 sodyum hidroksit ile dezenfekte edilerek yapılmıştır. Klinik belirtileri olan hastalıklı kolonilerin 15 günlük aralıkla 2 kez negatif olmasının ardından bu arılıklardaki tüm kolonilerin balları *Paenibacillus larvae ssp. Larvae* sporları için mikrobiyolojik incelemeye alınmıştır. Sporlarla bulaşık arı kolonileri dezenfekte edilmişyeni çerçeve ve temel petek verilmiş ve yeni kovanalara yapay olarak aktarılmıştır. Aktarılmadan önce arılar serin bir yerde besin olmadan 48 saat tutulmuştur. Aktif sezonun sonunda hastalıklı olarak belirlenen kolonilerin baları gelecek yılın ilkbaharında sporlar açısından mikrobiyolojik incelemeye alınmış ve oğul transferi yapılmıştır. Bu kolonilerin eski petekleri ertilmiş ve yeni çerçevelerin kabartılması için kovanda yer açılmıştır. Kovanlardaki balların spor açısından mikrobiyolojik incelemesi negatif ve hastalıklı arı kolonilerinin imha edilmesinden bir yıl sonra karantina kaldırıldı ve kolonierin hastalıktan kurtulduğu sonucuna varılmıştır.

Anahtar Kelimeler: Amerikan yavru çürüklüğü (AmerYÇ), alternatif kontrol, bal arısı, *Paenibacillus larvae larvae.*

INTRODUCTION

American Foulbrood is the most severe brood disease (Otten, 2003) that, without proper treatment, results not only in death of affected bee colonies, but also in death of entire apiaries. The American Foulbrood pathogen is the spore-forming bacillus *Paenibacillus larvae larvae*, whose spores are exceptionally resistant to environmental influences (Brodsgaard & Hansen, 2003; Haklova M. 2003).

Lately, the disease is becoming a problem in the Republic of Bulgaria. The traditional methods of control through killing and burning of affected bee families that were in use until several years ago and the prophylactic treatments of the other bee families with antibiotics and sulfonamides were a real hazard with regard to the accumulation of drug residues in honey bee products. That is why, since 2003, the use of antibiotics and sulfonamides in Bulgarian apiculture, similarly to the practice in most European countries, is prohibited by the law (Law on Apiculture, 2003). It has, therefore, necessitated the development and implementation of alternative methods for prophylaxis and control of American Foulbrood that exclude the use of antibiotics.

THE WORLD EXPERIENCE

According to the world experience, the control of American Foulbrood only via killing the affected bee families without paying attention to the other families in the apiary and the region, is incorrect and not working (von der Ohe, W. 2003). Alternative methods of control are essentially related to the development and implementation in the practice of methods for early diagnostics of the disease via detection of Paenibacillus larvae larvae spores in the bee honey and bee wax (Hansen & Rasmussen, 1986; Ritter, W. 1996; Ritter, W. 2003).

A widely used alternative method is the artificial swarm method, used in several modifications–with restrain of bees in a dark premise, without restrain, with interchanging the places of combs in the hive (Ritter, 2004).

The method allows preserving of adult bees from diseased bee families. During the starvation phase of the "artificial swarm" that lasts for 1.5–2 days, the bees are grooming each other and later, the pathogenic spores are excreted in the environment with faeces. The combs with affected brood are then burned, and the equipment is disinfected with NaOH.

In Denmark, the shaking method is successfully used (Hansen & Brodsgaard, 2003). The method involves the transfer of adult bees in non-infected combs on frames with mounted wax foundation or strips and burning the brood combs from clinically ill families. Transferred bees consume the contaminated honey while building the new combs. The results showed that the shaking method reduced considerably the number of Paenibacillus larvae larvae spores to safe levels, and according to some German investigators, a complete decontamination could be achieved (Oehring, M. 1998 **)**

Since 1986 in Iran and since 2000 in Canada, the method for AFB control using caspian solution – a mixture of pollen, pheromones and other natural components combined with small amounts of antibiotics has been introduced (Yeganehrad, H. 2003).

In Germany, Prof. J. Dustmann and Werner von der Ohe proposed a method of AFB control through destroying diseased bee families by burning, disinfection of hives and the equipment and transfer of all conditionally healthy families as "artificial swarms". The outcome is controlled by analysis of bee honey for detection of contamination with spores.

Our experience evidences that in Bulgaria, due to the more rapid and more severe course of the disease, the healing of apiaries without destruction of diseased bee families is very hazardous and there is a risk for considerable economical losses. That is why, the normative documents valid in the country stipulate the obligatory destruction of AF-affected bee colonies and healing of diseased apiaries by the "artificial swarm" method.

MATERIALS AND METHODS

During the past two years, 3 naturally infected apiaries with a total of 119 bee families were healed. The apiaries were located over the Stara Zagora region.

The healing of affected apiaries was done via:

– Quarantine;

– Destruction of affected bee families via burning;

– Disinfection of hives and beekeeping equipment;

– Two examinations at 15-day interval for detection of clinically diseased families;

– Analysis of bee honey obtained from all bee families for contamination with *Paenibacillus larvae larvae* spores;

– Transfer of bee families contaminated with spores as "artificial swarms" in disinfected hives on frames with wax foundations following a preliminary 48-h stay in a cool place without food;

– The apiaries were considered as healed and the quarantine was removed one year after the last destruction of a bee colony and negative results in the examination of honey samples from all bee families for contamination with spores.

The American Foulbrood was diagnosed by clinical examination (pictures 1,2) microscopic study of a smear made of putrid mass and cultivation of the specimen in BHIA medium for 3 days at 35 C˚.

The analysis of bee honey samples for contamination with *Paenibacillus larvae larvae* spores was performed according to the Manual of Standards for Diagnostic Tests and Vaccines, 2000,OIE.

RESULTS AND DISCUSSION

The results of studies and the stages of apiary healing are presented in Table 1.**Table 1.**

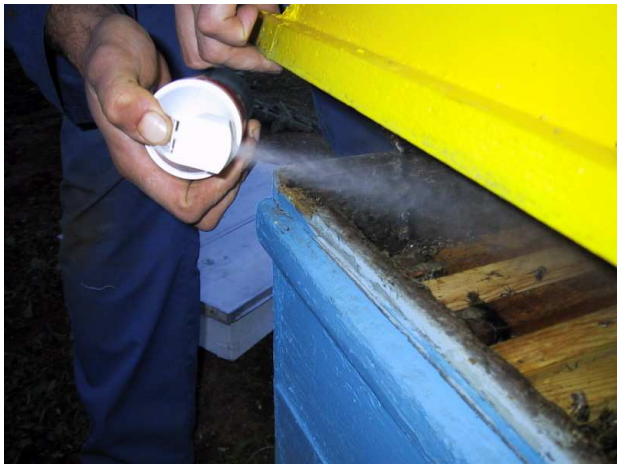
Apiaries	Number of bee families	Destroyed diseases bee families	Number of studied honey bee samples	Contaminated honey bee samples	Moved as "artificial swarm"	Number of contaminated at the end of the period
1	43	7	36	7	7	-
2	64	8	56	18	18	-
3	12	5	7	3	3	-

The presence of American Foulbrood in apiaries No 1 and 2 was detected in May and June 2003 whereas in
apiary No 3 – in August 2003. Quarantine was instituted in apiaries in order not to allow the distribution of the disease. The source of infection was eliminated through burning affected bee families (pictures 3,4,5) and disinfection of hives and equipment by boiling in 4% NaOH for 20 min (picture 6).

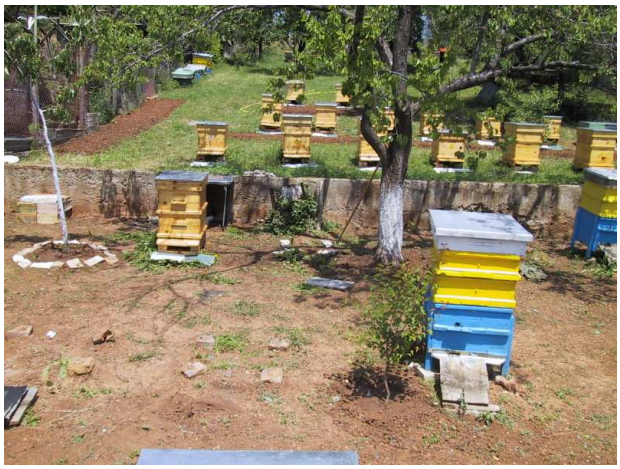
The clinical examinations performed at 15-day interval revealed that only in apiary No 3, another 2 diseased bee families were present. The epizootological study of the area within a radius of 3 km from affected apiaries did not find out other clinically ill bee families and showed that diseased families in apiary No 2 have been bought the year before from another region and in the areas of the other two apiaries, the disease has appeared during the previous years as well.


Picture 1.


Picture 2.


Picture 3.


Picture4


Picture 5.


Picture 6.


Picture 7.


Picture 8.


Picture 9.


Picture 10


Picture 11.


Picture 12.

The microbiological analysis of bee honey from all conditionally healthy bee families showed that in apiary No 1, 7 honey samples were contaminated with Paenibacillus larvae larvae spores, in apiary No: 2–18, and in apiary No: 3–3 samples. Those results confirmed the opinion of other authors (Hansen & Rasmussen, 1986) that in bee honey, pathogenic spores could be detected long before the appearance of clinical symptoms of AFB and that the clinical manifestation of the disease depended on the degree of contamination with spores in the hive, the resistance of the bee family, its hygienic behaviour etc.
For elimination of the contamination with spores, all contaminated bee families were transferred as an “artificial swarm” in mesh cages (pictures 7,8) that resided in a dark and cold place for 48 h. (picture 9). A longer exposition was not possible because after the end of the 48-h period, starved to death bees were found on cage bottoms. The transfer as “artificial swarm” of bee families that evidenced a spore contamination reduced significantly the operating costs of the beekeeper.
The artificial swarms were colonized in disinfected hives on frames with wax foundations (picture 10) and fed up with 2–3 L sugar syrup. Up to the end of the active season, the bee families built up their nests and were wintered as usually (pictures 11,12).
The later detection of AFB in apiary No 3 (in August) did not permit the building of new nests until the end of the active season. Therefore, the study for contamination of honey samples with spores and the transfer of “artificial swarms” were done in the spring of 2004.

With regard to the optimal decontamination of bee nests, we observed the consumption of winter food supplies in all bee families. The old combs were melted, and the nests were renewed and broadened with new frames.
As a result of these procedures, the honey of bee families from healed apiaries was free from spores of *Paenibacillus larvae larvae* at the end of the quarantine, suggesting that this method could be successfully used for control of American Foulbrood in Bulgaria.

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