CONTROL OF AMERICAN FOULBROOD BY THE SHAKING METHOD

Henrik Hansen & Camilla J. Brødsgaard

Danish Institute of Agricultural Sciences, Research Group Entomology, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark, Tel.: +45 58 11 34 31, Fax: + 45 58 11 33 05, E-mail:henrik.hansen@agrsci.dk

Abstract

In a Danish apiary 15 bee colonies were fed honey containing 1.0×10^9 P. l. larvae spores and examined for two beekeeping seasons. 43 days after the spore feeding, 12 of the colonies showed clinical symptoms of American foulbrood (AFB). Only these 12 colonies were then treated with the shaking method. After the shaking method was carried out a major reduction in the number of spores in the honey of the treated colonies was seen. The number of spores were reduced to a level at which they did not provoke further clinical symptoms of AFB. Even though all colonies were fed the same number of spores 20% of the colonies never showed clinical symptoms. These colonies were able to reduce the number of spores in the honey within one season to a level that was equal to the treated colonies. In Denmark, the shaking method has been used successfully for the past 90 years.

***Key words: American foulbrood* / Paenibacillus larvae larvae / *shaking method* / *honey bees* / Apis mellifera**

Introduction

American foulbrood (AFB) is a serious disease of the honey bee brood. The disease is caused by the spore-forming bacterium *Paenibacillus larvae larvae* [13]. The spores can survive for many years in scales, hive products and equipment [23], and they are very resistant to heat and chemicals [11]. Normally, colonies with clinical symptoms of AFB in capped brood cells will die if treatment is not carried out (9).

In many areas of the world, AFB is controlled by antibiotics. However, residues of oxytetracyclin (OTC) have been found in honey from the brood nest of colonies fed antibiotic extender patties [21]. Furthermore strains of *P. l. larvae* may develop resistance to sulfathiazole and OTC under continous use of the drugs [17] and recently increasing problems with resistance of *P. l. larvae* to OTC have been reported [1,20] . OTC is also used in treatment of human bacterial infections, and thus this method of AFB control is not advisable. The use of these antibiotics are therefore not registered for use on bees in Scandinavia and Germany.

In Denmark, in order to control AFB without use of antibiotics, a variation of the shaking method has been used for the past 90 years [3]. Originally the shaking method was proposed by Schirach in 1769 [22] and later rediscovered by McEvoy [14]. The method involves transferring the adult bees to a disease-free hive without drawn combs and destroying the brood combs of the infected colony. The contaminated honey in the honey stomach of the transferred bees is then consumed while the bees build new combs.

The bees can also be shaken into a screened box and kept there for several hours at outdoor temperature [16] or a few days in a cool cellar [19] to ensure the consumption

of the contaminated honey. Afterwards the bees are hived on frames with new foundation. In Denmark, the adult bees from colonies with clinical symptoms are shaken onto frames fitted with strips of wax. After 3 to 4 days, the bees are shaken onto frames with new foundation. Colonies in the apiary without clinical symptoms are not treated. If the nectar flow is weak the colonies can be fed just after they are shaken onto these new foundations [10].

In 1998 and 1999, field experiments were carried out on a small Danish island. The aim of the experiment was to elucidate the infection course in bee colonies in an apiary with clinical symptoms of AFB before and after treatment of the diseased colonies with the shaking method.

Material and methods

In early summer 1998, an apiary with a experimental group of 15 Danish *Apis mellifera ligustica* bee colonies with inseminated queens of same origin were established. Each colony was inoculated by feeding 100 g honey to which a sterile physiological saline solution containing 1.0×10^9 *P. l. larvae* spores was added. The spores originated from foulbrood scales of a Danish *P. l. larvae* strain (JT-79) [5]. The scales were supspended in sterile physiological saline, cultured, harvested, diluted, and counted as described by Brødsgaard *et al.* [5]. The counts were repeated at least four times until the standard error not exceed 5%. The spore concentration in the dilution was calculated on the basis of the mean value of the counts. Furthermore, four uninfected bee colonies placed in another apiary served as a control group.

Before the inoculation honey samples were taken from each colony and examined for *P. l. larvae* spores. The examination of the honey for *P. l. larvae* spores was done using direct inoculation on J-agar plates as described by Hansen [8] with the modification that nine plates were used and the honey solution used contained 0.24 g honey per three plates. The detection level was 2,000 spores per g honey. After the inoculation, honey samples were taken from each colony regularly and examined for *P. l. larvae* (totally 12 samplings). At the days of sampling two to four honey samples were taken depending on the amount of honey in the colonies. At each sampling, the colonies were also examined for clinical symptoms of AFB. The colonies were observed for two beekeping seasons (16 months).

At the third sampling, 43 days after the inoculation, 12 of the colonies in the experimental group showed clinical symptoms. Only these 12 colonies were treated with the following modification of the shaking method. The colonies were shaken onto frames fitted with strips of wax placed in decontaminated wooden boxes. The decontamination included scraping wax with a hive tool followed by treatment with Virkon® S which inactivates approximately 80% of the spores [2]. Queen excluders were placed under the boxes to prevent the colonies from swarming. The boxes were placed on bricks to allow the bees to fly. After 4 days, the bees were again shaken onto frames with new foundation in decontaminated boxes, and all colonies (including the three non-treated colonies) were fed with sugar.

Results

Before inoculation no contamination in the honey was found in any of the colonies.

Figure 1 show the average number of *P. l. larvae* spores per g honey in the colonies in 1998 and 1999 respectively. At the second sampling, the mean spore level in the honey

was $3.5\times10^6 \pm 1.6\times10^6$ spores per g honey. In spite of the high spore levels none of the colonies showed clinical symptoms of AFB. At the third sampling, 43 days after the spore feeding, 12 of the colonies in the experimental group showed clinical symptoms. The mean spore level in this group was then $1.7\times10^6 \pm 5.1\times10^5$ spores per g honey. These 12 colonies (dotted curve) were treated with the shaking method. Though the spore level was equal to the colonies with clinical symptoms (Komogorov-Smirnov, $P>0.05$) the remaining three colonies did not show clinical symptoms at any of the samplings in the rest of the observation period either (dashed curve) and remained untreated.

At the first sampling after the shaking procedure was carried out the spore level in the honey samples from the treated colonies were significantly reduced (Wilcoxon, $P<0.05$) with 99.95% to a theoretic mean of 1000 ± 726 spores per g honey (this actually is under the detection level of the method). Hereafter, the level increased slightly during the remaining observation period of the first year. At the first sampling in the second year the spore level had increased to a mean of 22,963 ± 8,847 spores per g honey. At the end of the observation period of the second year the spore level was decreased to the same level as just after the treatment. No clinical symptoms were observed at any of the samplings in the observation period after the treatment.

The spore levels in the honey from the non-treated colonies in the experimental group were reduced throughout the observation period in the first year and at the end of the first year the level was slightly below the level of the treated colonies. At the first sampling in the second year and at the end of the observation period the spore levels were the same as in the treated group (Komogorov-Smirnov, $P>0.05$).

The control colonies did not have detectable spore contents in the honey or show clinical symptoms at any sampling point in the observation period.

Discussion

The results of the present experiment with induced infection by inoculation show that even though all colonies were fed the same number of spores and spore levels in the honey reached the same high level 20% of the colonies never showed clinical symptoms of AFB. Furthermore, these colonies were able to reduce the number of spores in the honey to very low levels towards the end of the first season and clinical symptoms did not occur in the following season. This corresponds to earlier studies [9, 12]. Hence, even though the bees of the colonies in this experiment were very closely related a major difference in the ability to control the disease was seen.

Furthermore, the results shows that after the shaking method has been carried out, a major reduction in the number of spores in the treated colonies was seen. The spores were reduced to a level at which they did not provoke further clinical symptoms of AFB.

On the basis of four samplings after the shaking method was carried out (one bee keeping season's examinations) Oehring [18] states that not only does the shaking method control AFB but it also eliminates the spores from the colony. However, approx. 38% of the colonies in her experiment still had detectable spore levels at the end of the examination. This latter finding corresponds to our results showing that, though spore level was reduced significantly during the observation period, spores could still be detected at very low levels in the samples the following year. In addition, Oehring's conclusion that after the shaking method was carried out the spores are eliminated to an extend where no clinical symptoms are found and the bee colonies are healthy corresponds to our findings.

Oehring [18] treated all the colonies by the shaking method if the spore level in the honey was high (whether or not they showed clinical symptoms). We only treated the colonies showing clinical symptoms and our results indicate that even though the queens were closely related there were still differences in tolerance against AFB. The colonies without clinical symptoms overcame the infection even though the spore levels in these colonies were equal to the colonies showing clinical symptoms.

After many years experience with statutory control of AFB in Denmark using the shaking method, it is our experience that this method is sufficient to control the disease providing that it is combined with hive decontamination [2] and comb melting from diseased colonies and store rooms. However, some Danish Buckfast strains recently have lost a part of their natural tolerance mechanisms against AFB, making these strains are very sensitive to the disease [4]. Experience from the statutory control suggest that the shaking method is not effective on these strains because the method seems to fail in controlling AFB. Therefore, colonies of these Buckfast strains are killed when the have clinical symptoms of AFB. The results of the present experiment indicates that all of the colonies from the tested bee strain do possess a certain degree of tolerance since no clinical symptoms or increase of spore levels in honey was seen the season following the treatment.

It has been demonstrated that both shaking followed by a single treatment of oxytetracycline hydrochloride (OTC) and shaking without OTC are effective in eliminating AFB [7, 15]. Consequently OTC treatment is not necessary. Other authors [6, 7] also report that infected honey bee colonies treated only with the shaking method showed a significant reduction of the spore levels in honey, and in spite of the spores remaining in the colonies, no clinical symptoms were seen in the one season observation period. As shown also in our experiment, the shaking method will not eradicate all the foulbrood spores. Therefore, a precondition for an effective control using the shaking method may be that the treated bee colonies have a natural tolerance against American foulbrood e.g. larval resistance, hygienic behaviour and/or food inhibition of bacterial growth.

The advantages of the shaking method are that it saves the bee colonies and that there are no residues from drugs in honey and wax after the treatment. Another reason why the shaking method is a viable control option is that strains of *P. l. larvae* has developed resistance to antibiotics. The disadvantages may be that the method is labour-intensive and a certain tolerance of the hoeny bees against AFB is necessary.

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