# DECONTAMINATION OF BEEHIVES CONTAINING SPORES OF THE FOULBROOD BACTERIUM PAENIBACILLUS LARVAE LARVAE

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#### Abstract

Wooden multiple storey hives from colonies with clinical symptoms of American foulbrood (AFB) were examined for the presence of Paenibacillus larvae larvae spores. Furthermore, it was examined if these contaminated hives constituted an infection risk if they were re-used for housing uninfected bee colonies. The results showed contamination with P.I. larvae of the hives and subsequent contamination in the honey of the transferred colonies.

Part of the contaminated hives were cleaned using different methods. The efficacy was measured by inoculation of samples from the hive sides on J-agar before and after the cleaning procedure. The following treatments were examined: either A) scorching with a blowtorch, B) scrubbing with stiff pads and hot soapy water, C) high-pressure flushing with cold water, D) treatment with Virkon<sup>R</sup> S, or E) treatment with steam for 15 min., thereafter, immersing in a boiling solution of 3% NaOH and 2% soft soap for 3 min., thereafter, rinsing in a vessel with cold water, and finally, high-pressure flushing with cold water. The results show, that treatment A), B) and C) have efficacies in removing P.I. larvae spores of approximately 80%. Treatment E) has an efficacy of 99% that is significantly different from the former.

After the cleaning, bees and brood combs from colonies without infection of P.I. larvae were introduced into the four hives from each of the treatments A), B) and C). In the following two years, these 12 colonies were examined for clinical symptoms of AFB. Honey samples from the colonies were examined for P.I. larvae spores. Disease or infection was not found.

Keywords: Apis mellifera, Honeybee, Paenibacillus larvae larvae, American Foulbrood control, decontamination.

### Introduction

American foulbrood (AFB) is a serious brood disease of the honeybee (*Apis mellifera* L.). It has been considered the most destructive of brood diseases (BURNSIDE and STURTEVANT, 1949). Normally, colonies with clinical symptoms of AFB will die if treatment is not conducted (HANSEN and BRØDSGAARD, 1997). Therefore, the disease can cause substantial economic losses for both beekeepers and seed producers. In spite of its common name the disease is spread world-wide (MATHESON, 1996).

AFB is caused by the sporeforming bacterium *Paenibacillus larvae larvae* (WHITE). The bacterium is very resistant to heat. Only temperatures above 60  $^{\circ}$ C for 15 minutes ensure that vegetative cells of *P.I. larvae* are killed (ROSE, 1969). Spores of the bacterium are not destroyed until they have been heated for 30 minutes or more at 100  $^{\circ}$ C (WHITE 1920). Furthermore, after treatment of *P.I. larvae* contaminated wooden hives by scorching with a blowtorch a substantial number of spores is still viable (HANSEN and RASMUSSEN, 1990). The bacterium is also very resistant to different chemicals. WHITE (1920) showed that the spores could persist in a dilution of carbolic acid for months and a 10% formalin dilution for hours.

Beekeeping equipment from infected and diseased colonies can be contaminated with *P.I. larvae* spores. GOCHNAUER (1981) found  $3.5 \times 10^5$  *P.I. larvae* spores per 100 cm<sup>2</sup> hive scrapings from bee colonies heavily infected with AFB. This indicates that reuse of contaminated bee hives probably can transmit the spores to non-infected colonies.

A biotechnical control method of AFB (the shaking method) is used in Germany (RITTER, 1994) and Denmark (HANSEN and RASMUSSEN, 1986). The advantage of this method is that it saves bee colonies and there are no residues from drugs in honey and wax after the treatment. The shaking method was originally proposed by SCHIRACH in 1769 (WHITE, 1906) and rediscovered by W. McEVOY (HOWARD, 1907). The method involves transfer of adult bees onto frames fitted with strips of wax. After 3 to 4 days, the bees are shaken onto frames with new foundation. Normally, the shaking method is combined with hive cleaning and comb melting from diseased colonies and store rooms (HANSEN and RASMUSSEN, 1986).

The aims of this study were to examine if infection with the foulbrood bacterium can be transferred to bee colonies by re-using hives from colonies with clinical symptoms of AFB, to examine the efficacy of different cleaning methods of wooden multiple storey hives from colonies with clinical symptoms of the disease, and to examine weather these cleaned hives can transmit any left-over infection to new bee colonies.

### Materials and methods

#### Transmission of infection

Three multiple storey hives each containing two boxes from colonies with clinical symptoms of AFB (WHITE, 1920) were examined for contamination of *P.I. larvae* spores (experimental hives). Samples were taken by sweeping the inside of the hive with a sterile cotton stick moistened with sterile demineralised water using the procedure shown in figure 1. Hereafter, the cotton stick was placed in a sterile tube with 500 µl

sterilized demineralised water and placed in a water bath at 90  $^{\circ}$ C for 10 min in order to kill non sporeforming bacteria (HANSEN, 1984b). After the thermal treatment the tube with the suspension was placed on a shaker for 30 sec. 200 µl of the solution were transferred to one petridish and 100 µ to each of two petridishes with a sterile micropipette (figure 2). The petridishes each contained 10 ml J-agar prepared as described by GORDON et. al. (1973). The petridishes were placed in an incubator at 35  $^{\circ}$ C. On days 3-4 and 6-7, the bacteria colonies of *P.I. larvae* were identified (HANSEN, 1984b) and counted.

The test bee colonies used in this experiment had naturally mated *Apis mellifera* L. queens. They were checked for clinical symptoms of AFB and contamination of *P.I. larvae* in the honey by direct inoculation on J-agar as described by HANSEN (1984a) and found negative. Three colonies were transferred to the experimental hives. Four other bee colonies placed in an apiary about 300 m from the experimental colonies served as controls. The experiment was carried out on a small Danish island without other apiaries. 71 days after start of the experiment all colonies were examined for clinical symptoms of AFB (WHITE, 1920). Furthermore, three honey samples from different combs were taken from each colony. The number of *P.I. larvae* spores in these samples were determined by direct inoculation (HANSEN, 1984a).



Figure 1 – Before cleaning or re-use and after the cleaning of the boxes, samples were taken by sweeping the interior hive side with a sterile stick moistened with sterile demineralised water



Figure 2 – The sample taken from the interior hive sides with a sterile cotton stick was placed in a sterile tube with 500 μl sterilized demineralised water in a water bath at 90 OC for then min. Thereafter, the tube was placed on a shaker for 30 sec., 200 μl of the solution were transferred to J-agar in one petridish, and 100 μl to each of two petridishes

## Decontamination efficacy

From another set of colonies, also with clinical symptoms of AFB wooden multiple storey hives were decontaminated. Before the decontamination, all interior sides of the hives were scraped with a hive tool. After the scraping, samples were taken and cultured as described above (figure 1 and 2).

After scraping the hives, the following treatments were conducted: either A) scorching with a blowtorch until the surface was brownish black, B) scrubbing with stiff pads hot soapy water solution containing 0.15 I powder soap for automatic dishwasher (Neophos<sup>R</sup>) dissolved into 10 I boiling water, C) high-pressure flushing with cold water, E) treatment with Virkon<sup>R</sup> S) which is a biodegradable disinfectant), or D) treatment with steam for 15 min, thereafter, immersing in a boiling solution of 3% NaOH and 2% soft soap for 3 min, thereafter, rinsing in a vessel with cold water and finally, high-pressure flushing with cold water (this treatment was carried out in a rendering plant. The number of treated hive sides were 18 for each of the treatments A, B and C, 15 for E and 76 for D.

After the treatment samples were taken from the interior hive sides (figure 1), inoculated on J-agar (figure 2) and the *P. I. larvae* colonies were counted as described above. The efficacies of treatments were calculated on basis of the number of *P.I. larvae* colonies before and after the cleaning. The results were analysed by KRUSKAL-WALLIS multiple comparisons (SIEGEL and CASTELLAN, 1988).

Bees and brood combs from noninfected colonies (examined as described above) were introduced into the cleaned hives of treatment A), B) and C). These treatments were chosen to represent widely used methods with an efficacy of approximately 80% (see results). Totally 4 colonies were made from each. Thereafter, the colonies were examined for clinical symptoms (WHITE, 1920) after 24 days, 68 days, 1 year 20 days, and 1 year 67 days. At the days of examination two to four honey samples were taken from each colony depending upon the amount of honey in the combs and examined for presence of *P. I. larvae* spores by direct inoculation (HANSEN, 1984a).

# Results

All the experimental boxes were contaminated with *P.I. larvae* before the start of the experiments. The average number of *P.I. larvae* colonies per hive side was  $2.2 \times 10^3 \pm 2.0 \times 10^2$ . Contamination with *P.I. larvae* spores was found in the honey from two of the three originally non-infected colonies after they had been transferred to the boxes from colonies with clinical symptoms (Table 1). Clinical symptoms of the disease were not found in any of the these colonies. The four control colonies did not show clinical symptoms or had contamination of the honey.

Table 1

The number of *P.I. larvae* spores in honey from originally non-infected bee colonies transferred to contaminated hives without previous cleaning. The hives originated from colonies with clinical symptoms of AFB

Bee colony	Average number of spores per 1 kg honey	s.e.	n
I	3.3x10 <sup>3</sup>	3.3 x 10 <sup>3</sup>	3
II	0	0	3
III	$4.4 \times 10^5$	1.6 x 10 <sup>5</sup>	3

In table 2 the results of the different cleaning methods are shown. Treatment E was most effective and significantly different from the other treatments (KRUSKALL-WALLIS, p<0.0001) but the treatment did not remove or kill all the *P.I. larvae* spores. The efficacy of treatment A, B, C and D was not significantly different from each other and had efficacies of approximately 80%.

Table 2

Mean efficacy of different decontamination methods against P.I. larvae spores				
Treatment	n	Mean effect %*	s.e.	
A. Scorching	17	83.984 <sup>b</sup>	622.910	
B. Scrubbing	17	75.729 <sup>b</sup>	635.168	
C. High-pressure	15	81.152 <sup>b</sup>	728.544	
D. Virkon <sup>R</sup> S	15	83.527 <sup>b</sup>	805.603	
E. Steam	84	99.997 <sup>a</sup>	160	

\* Means with the same latter are not significantly different

Clinical symptoms of AFB were not found in any of the bee colonies transferred to hives cleaned by treatment A, B or C. Furthermore, contamination with *P.I. larvae* was not found in any of the honey samples from these colonies.

## Discussions

The results suggest that hives from colonies with clinical symptoms of AFB are contaminated with foulbrood spores. These findings correspond with the experiment of GOCHNAUER (1981) in which five hives diseased colonies were examined and found contaminated with foulbrood spores. Furthermore, the results

suggest that hives from bee colonies with clinical symptoms of AFB without preceding cleaning can transfer infection of the foulbrood bacterium to non-infected colonies. Differences in tolerance and behaviour of the bees can be the reason why contamination of the foulbrood bacterium was found only in the honey of two of the three tested colonies. The hypothesis that infection can spread from contaminated hives has been widely accepted for many years. Therefore control programs in many countries contain regulations concerning burning, scorching or scrubbing of contaminated hive parts.

Experiments with induced infection of *Apis mellifera ligustica* Spinola bee colonies with *P.I. larvae* spores suggest that there is no simple correlation between the number of foulbrood spores in the honey of the colony and outbreak of American foulbrood (HANSEN and BRØDSGAARD, 1997). On the other hand, in colonies with clinical symptoms of AFB, the symptoms were observed for the first time when the number of foulbrood spores in one gram honey ranged from  $3 \times 10^3$  to  $4 \times 10^5$  (HANSEN and BRØDSGAARD, 1997). In the present study the level of contamination in one gram honey from the two contaminated colonies ranged from  $3.3 \times 10^3$  to  $4.4 \times 10^5$ . Therefore, it can be expected that re-use of hives from colonies with clinical symptoms of AFB without preceding cleaning can provoke outbreak of the disease.

In many countries, it is common praxis to disinfect contaminated hives and other beekeeping equipment made of wood by scorching them with a blowtorch. It is advised that the equipment is scraped with a hive tool before scorching. The results of the present study support the results of SMIRNOV (1982) and HANSEN and RASMUSSEN (1990) in which it was shown that contamination of hives with *P.I. larvae* is not always eliminated by using this method. On the other hand, control programs of AFB have shown that scorching reduces the number of spores to a level at which they will not normally provoke clinical symptoms.

Our investigation shows that cleaning of wooden bee hives by scorching with a blowtorch Å) will only remove approximately 80% of the *P. I. larvae* spores. The results from our field test with bee colonies placed in these hives after disinfection with a blow torch suggest that this cleaning method normally will be sufficient to decrease the number of spores to a level at which clinical symptoms of American foulbrood will not be provoked. Furthermore, spores were not found in the honey of these colonies. These findings corresponds with the above mentioned recommendations in control programs of AFB. Each of the methods: scrubbing with stiff pads and hot soapy water B), high-pressure flushing with cold water C) and treatment with Virkon ® S D) had the same efficacy as the scorching method. HORNITZKY (1992) showed that even the use of 90°C hot water in high pressure flushing of contaminated hive boxes leaves spores. Nevertheless, our field test indicates that the cleaning methods B), C) and D) will also be sufficient to decrease the number of spores to a level at wich clinical symptoms of the disease will not be provoked.

Our investigation shows that it is necessary to decontaminate hives in controlling AFB. On the other hand, several studies report that colonies without clinical symptoms of AFB may contain honey contaminated with *P. I. larvae* spores (HANSEN and RASMUSSEN, 1986; HAGEN and HETLAND, 1988; HANSEN et. al., 1988; RITTER, 1990; HORNITZKY and CLARK, 1991; STEINKRAUS and MORSE, 1992; GRIMM and MOOSBECKHOFER, 1993; RITTER, 1993; DERAKSHIFAR, 1995; FERRARO, 1996). During several years (1978-1981 and 1985-1990) examination of Danish bee colonies showed that in average 9.7% of the colonies contained honey contaminated with *P.I. larvae* spores while only 3.7% of all colonies showed clinical symptoms of AFB (HANSEN, 1992). Therefore, it is concluded that the cleaning methods A, B, C and D have a sufficient efficacy and can be recommended for practical use in areas with a frequent outbreak of AFB and even in areas where outbreak of the disease occurs sporadically. Method E has a very high efficacy. But the method is very laborious and care should be taken when using the lye. Therefore, this method is only recommended for use in special cases when eradication is a realistic possibility.

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#### REFERENCES

- BURNSIDE, C.E.; A.P. STURTEVANT (1949) Diagnosis bee diseases in the apiary. USDA Circ., 392
- CANTWELL, G.E. (1970) The feasibility of using irradiation to control AFB. Amer. Bee J., 8, 310
- CANTWELL, G.E.; T. LEHNERT; R.S. TRAVERS (1975) USDA research on fumigation for control of bee diseases and pests of the honeybee. Amer. Bee. J., 115, 96-97
- DERAKSHIFAR, I. (1995) Das Auftreten von Faulrutsporen in Österreichischen Honigen als diagostische Methode zur Früherkennung von Faulbrutherden. *Bienev.*, **11**, 464-469
- FERRARO, R. (1996) Faulbrutsporen in Honig Situation in der Schweiz. Schweiz. Bienenztg. 119, 513-515
- GOCHNAUER, T.A. (1981) The distribution of *Bacillus larvae* Spores in the environments of colonies infected with American foulbrood disease. *Amer. Bee J.*, **121**, 332-335
- GORDON, R.E.; W.C. HAYNES; C.H. PANG (1973) The Genus *Bacillus*. USDA. Washington, 283 pp
  GRIMM M.; R. MOOSBECKHOFER (1993) Untersuchung Österreichischer Honige auf das Vorhandsein von *Bacillus Iarvae*
- Sporen. Bienenv., 4, 167-171
  HAGEN, A.; A. HETLAND (1988) Forekomst av yngelråtebakterien (Bacillus larvae) I honning. Aktuelt fra Statens Fagtjeneste for Landbruket, 1, 389-392

- HANSEN, H. (1984a) Methods for determining the presence of the foulbrood bacterium *Bacillus larvae* in honey. *Tidsskr. Planteavl,* **88**, 325-328
- HANSEN, H. (1984b) The incidence of the foulbrood bacterium *Bacillus larvae* in honeys retailed in Denmark. *Tidsskr. Planteavl,* **88**, 329-336
- HANSEN, H. (1992) Forekomst af ondartet bipest og bipest-bakterien in Danmark. Tidsskr. Biavl, 126, 125-128
- HANSEN, H.; C.J. BRØDSGAARD (1997) Der Verlauf der Amerikanischen (Bösartigen) Faulbrut in künstlich infizierten Völkern. *Allg. Dtsch. Imkerztg / die Biene*, **3**, 11-14
- HANSEN, H.; B. RASMUSSEN (1986) The investigation of honey from bee colonies for *Bacillus larvae*. *Tidsskr. Planteavl*, **90**, 81-86
- HANSEN, H.; B. RASMUSSE (1990) The sensitiveness of the foulbrood bacterium *Bacillus larvae* to heat treatment. Proceedings of the International Symposium on Recent Research on Bee Pathology, Gent, 146-148
- HANSEN, H.; B. RASMUSSEN; F. CHRISTENSEN (1988) A preliminary experiment involving induced infestion from *Bacillus larvae*. *Tidsskr. Planteavl*, **92**, 11-15
- HORNITZKY, M.A.Z. (1992) Salvage of American brood disease infected hive boxes by the use of high pressure water sprays. NSW Apiarists' Association Inc. News, November-December, 3
- HORNITZKY, M.A.Z. (1994) Commercial use of gamma radiation in the beekeeping industr. Bee Wid., 75, 135-142
- HORNITZKY, M.A.Z.; S. CLARK (1991) Culture of *Bacillus larvae* from bulk honey samples for the detection of American foulbrood. *J. of Apic. Res.*, **30**, 13-16
- HOWARD, L.O. (1907) Report of the meeting of inspectors of apiaries. San Antonio, Texas, November 1906. USDA, Bureau of Entomology, Bulletin 70
- MATHESON, A. (1992) Strategies for prevention and control of American foulbrood. Amer. Bee J., 132, 399-402, 471-475, 534-537, 547
- MATHESON, A. (1996) World bee health update 1996. Bee Wld., 77, 45-51
- MICHAEL, A.S. (1964) Ethylene oxide, a fumigant for control of pests and parasites of the honeybee. Glean. Bee Cult., 98, 102-104
- MORSE, R.A., H. SHIMANUKI (1990) Summary of control methods. In: (Ed. Morse, R.A.) Honeybee, Pests, Predators and Diseases, Cornell University Press, Ithaca, 342-361
- RITTER, W (1990) Bösartige Faulbrut: Wie ist das Vorkommen von Sporen der bösartigen Faulbrut im Honig zu bewerten. Allg. Dtsch. Imkerzth., 9, 13-16
- RITTER, W. (1993) Eignet sich die Untersuchung von Honigproben yum erkennen der Amerikanischen (Bösartigen) Faulbrut? Allg. Dtsch. Imkerztg., 1, 13-16
- RITTER, W. (1994) Bienenkrankheiten. Ulmer; Stuttgart, Germany, 128 ppo
- ROSE, R.I. (1969) Bacillus larvae isolation, culturing and vegetative death point. J. Invert. Pathol., 14, 411-414
- SIEGEL, S.; N.J. CASTELLAN (1988) Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Company, 399 pp
- SMIROV, A.M. (1982) Study of microbial contamination of hives and combs, and methods of disinfection. Apiacta, 17, 100-104, 119
- STEINKRAUS, K.H.; R.A. MORSE (992) American foulbrood incidence in some US and Canadian honeys. *Apidologie*, 23, 497-501
   WHITE, G.F. (1906) The bacteria of the apiary with special reference to bee disease. USDA, Bureau of Entomology, Technical
- Series, No. 14, 50 pp
- WHITE, G.F. (1920) American foulbrood. USDA, Bureau of Entomology, Bulletin 809, 54 pp