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The investigation of honey from bee colonies for *Bacillus larvae*

Undersøgelse af bifamiliers honning for Bacillus larvae

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Summary

Results from an investigation of honey for spores of the foulbrood bacterium *Bacillus larvae* have been compared with those from a traditional investigation for American foulbrood in the bee colonies concerned.

11% of the honeys investigated were contaminated with *B. larvae*. 9% of the honeys investigated were contaminated without the colonies of bees showing any clinical symptoms of American foulbrood in the same year as the investigation or in the following year.

The number of *B. larvae* spores triggering the disease varies greatly. In some cases, disease was found when, in a representative sample of honeys from colonies of an apiary, there were between 30,000 and 60,000 spores per 5 g honey. In other instances, disease was only found to occur when there was a far greater concentration of spores. In one particular apiary, more than 15 million spores per 5 g honey were found without any clinical symptoms of American foulbrood appearing in any of the colonies of bees.

In most cases, spores of the *B. larvae* were found to occur in the honey the year before the outbreak of American foulbrood in the colonies of bees.

It is concluded that the investigation of honey for the presence of *B. larvae* can play a practical role in the preventive treatment of the disease.

Key words: Honey bees, *Bacillus larvae,* American foulbrood, honey.

Resumé

Undersøgelse af honning for sporer af bipestbakterien *Bacillus larvae* er sammenlignet med en traditionel undersøgelse af bifamilierne for ondartet bipest.

11% af de undersøgte honninger var inficeret med *B. larvae*. 9% af de undersøgte honninger var inficeret, uden at bifamilierne havde kliniske symptomer på ondartet bipest samme år eller året efter, prøven var udtaget.

Den mængde *B. larvae* sporer, som udløser ondartet bipest, kan være meget forskellig. Der er i nogle tilfælde fundet sygdom, når der i sammenstik af honning fra bigårdens bifamilier er mellem 30.000 og 60.000 sporer pr. 5 g honning. I andre tilfælde er der først fundet sygdom ved langt højere antal sporer. I en enkelt bigård er der fundet flere end 15 millioner sporer pr. 5 g honning, uden at der er kliniske symptomer på ondartet bipest i nogle af bifamilierne.

I de fleste tilfælde er der fundet *B. larvae* sporer i honningen året før udbrud af ondartet bipest i bifamilierne.

Det konkluderes, at undersøgelse af honning for *B. larvae* kan indgå som et forebyggende led i det praktiske sygdomsarbejde.

Nøgleord: Honningbier, *Bacillus larvae*, ondartet bipest, honning.

Introduction

American foulbrood is a disease affecting the brood of honey bees *(Apis mellifera)*. It is caused by the spore generating *Bacillus larvae* bacterium. In Denmark and many other countries, treatment for the disease is undertaken by the public authorities. Usually, treatment in Denmark is carried out by means of the shaking method, which is used on all the colonies of bees in apiaries where the disease has been detected. When using the shaking method, all the bee colonies are shaken onto strips of wax. After 3 to 4 days, they are shaken onto new comb foundation. Brood combs from diseased colonies are burnt. All other combs are melted down and the equipment cleaned. No drugs are used in this treatment.

Every year, colonies of bees are investigated for the presence of American foulbrood by the State Bee Disease Committee. Investigations are carried out in the environs of apiaries with American foulbrood and also in regions which have been selected for official inspection. Such regional inspections involve the investigation of, if possible, all colonies of bees in areas where there have at some stage been problems with the disease.

In earlier papers, investigations of honey for the incidence of *B. larvae* (4, 5) were described. In order to learn whether *B. larvae* spores are present in honey before any clinical symptoms of American foulbrood have been detected amongst the colonies of bees, honey from Danish colonies of bees was investigated. The results have been compared with traditional investigations of the bee colonies concerned carried out during regional inspection. This paper contains a description of the investigation.

Methods

In the early summer of 1979, a regional inspection of bee colonies was carried out in the east of Funen. As part of this inspection, beekeepers in the area were requested to send in samples of honey from the 1978 harvest. They were then requested to submit honey samples from the 1979 harvest. These later samples originate from extractions taken after the conclusion of the regional inspection. Each sample represents all colonies of bees in one apiary.

In the early summers of 1980 and 1981, regional inspections were carried out in a district in the East of Jutland. Beekeepers from this area were requested to submit honey from the 1979, 1980 and 1981 harvests. The honeys from 1980 and 1981 were extracted after the conclusion of the regional inspection. As in the previous case, each sample represents all colonies of bees in one apiary.

In one particular apiary in East Jutland with approximately 10 colonies of bees, investigations were carried out into honey from 1979 to 1984 and into colonies of bees from 1980 to 1985.

Where *B. larvae* spores were detected in the final investigation of honey from the apiaries in East Funen and East Jutland, traditional examinations of the colonies were carried out for disease in the brood the following year.

243 apiaries with approximately 1,700 bee colonies were included in the investigation. All honeys (532 samples) were examined for *B. larvae* by means of direct inoculation into Petri dishes containing medium (5). From each honey sample 5 g honey was taken and placed in each of 3 sterile 100 ml Erlenmeyer flasks. The flasks were heated in a water bath to a temperature of 88–92°C and kept at this temperature for 5 minutes. As a control, a sample of honey which was known to be contaminated with *B. larvae* was also submitted. After the heat treatment, honey from each flask was inoculated into 3 Petri dishes, each containing 10 ml freshly prepared J-agar. The J-agar was

made from 5 g tryptone, 15 g yeast extract, 3 g K_2PO_4, 20 g agar, 1000 ml demineralised water and 2 g glucose, which were separately sterilized and added after autoclaving (3). The 3 dishes were consecutively inoculated with the amount of honey contained in the opening of an inoculation loop which on average is 0.08 g honey. The dishes were incubated at a temperature of 36°C. Using this method, the presence of *B. larvae* can be established when, on average, there are more than 30,000 spores per 5 g honey.

Counts were taken from day 3 to day 5. For the counts, the colonies of bacteria were assessed microscopically, and bright field microscopy of gram stained preparations was carried out to detect rods. After approximately 7 days, microscopy of spores was performed by means of phase contrast. In cases of doubt, the possibility of further examination of catalase activity was exploited. At each count, comparison was made with the contaminated honey used as a control with all cultures.

Experience from earlier investigations (5) has shown that counts conducted along these lines are sufficient for routine examinations of honey.

To determine the degree of contamination in the honeys, the *B. larvae* colonies were counted after 5 days. Each colony represents on average 30,000 spores per 5 g honey (5). Normally only up to 20 colonies were counted. If the sample had more than 20 colonies, it was merely noted that it had more than 600,000 spores per 5 g honey. Only in one honey sample from East Jutland from 1984 were higher counts of colonies carried out.

Results

Table 1 gives the results of the investigation of 532 honeys. It shows that, in all, 56 of the honeys were contaminated with *B. larvae*, i.e. 11% of the honeys.

47 of the honeys, i.e. 9%, were contaminated without any sign of outbreak of American foulbrood amongst the colonies of bees either the same or the following year. In 9 apiaries with approximately 60 bee colonies where the honeys were contaminated, American foulbrood was detected the year after the extraction. In 2 cases, clinical symptoms of American foulbrood were detected in the colonies of bees without any *B. larvae* contamination of the honey the previous year.

Table 1: Distribution of honeys investigated.

	Number of honeys	Number of honeys with *B. larvae* contamination
Apiaries *without any clinical symptoms* the same year or the year following the extraction of the honey.	521	47
Apiaries *with clinical symptoms* the year after the extraction of the honey.	11	9
Total	532	56

Table 2 shows the degree of contamination in the honeys. It appears that of the contaminated honeys from the set for which no clinical symptoms of American foulbrood were detected the year following the sampling, 16 or about one third had more than 600,000 spores per 5 g honey. The other contaminated honeys from this set had less than 180,000 spores. From the set for which American foulbrood was detected the year following the extraction of the honey, just over half of the contaminated honeys contained more than 600,000 spores per 5 g honey. The remainder had less than 600,000 spores per 5 g honey.

The investigation was of too short a duration to provide any concrete evidence as to how long a colony of bees can carry *B. larvae* infection without an outbreak of American foulbrood. Nor is it known whether the disease will break out if no measures are taken. However, note needs to be taken of the fact that contamination has been found in honey from colonies of bees in one particular apiary over a period of 6 years but as yet

Table 2: Distribution of honeys investigated in relation to degree of contamination.

	Average number of *B. larvae* spores per 5 g honey				
	0	30,000 −60,000	90,000 −180,000	210,000 −600,000	above 600,000
Apiaries *without any clinical symptoms* the same year or the year following the extraction of the honey.	474	28	3	0	16
Apiaries *with clinical symptoms* the year after the extraction of the honey.	2	4	0	0	5

with no sign of the disease. The incidence of *B. larvae* spores in this particular apiary can be traced in Table 3. As can be seen, the honey from 1984 contains more than 15 million spores per 5 g honey, and yet at no time has there been an outbreak of American foulbrood amongst the colonies of bees.

Table 3: Degree of contamination in honey extracted over a period of 6 years from colonies of bees in one particular apiary where there has been no clinical symptoms of American foulbrood.

Year	Average number of *B. larvae* spores per 5 g honey
1979	60,000
1980	30,000
1981	above 600,000
1982	above 600,000
1983	above 600,000
1984	above 15,000,000

In 7 cases, investigations were carried out on honey from apiaries where there had been treatment for American foulbrood. In all these apiaries, the disease was treated by the shaking method, as described in the introduction. The honeys were extracted in the year after treatment in all cases save 2, where they were extracted in the same year (approximately 2 months after treatment). In these 2 cases as well as in 3 of the other cases, there were 30,000 spores per 5 g honey. In 2 of the honeys no spores could be detected.

Discussion and conclusion

Gochnauer (2) investigated honey from 7 colonies of bees which were badly infected with American foulbrood. In that investigation, the honeys were dissolved in water and then centrifuged. *B. larvae* spores found in the sediment were then counted by means of a counting chamber. On average, 24.3 million *B. larvae* spores were found per 1 g honey. In 7 samples of honey from colonies of bees with no clinical symptoms of American foulbrood, *B. larvae* spores were not detected.

There is a variety of data as to how many *B. larvae* spores are necessary to trigger American foulbrood. *Woodrow* (8) demonstrated that bee larvae less than 2 days old are most susceptible to the disease. Building upon *Woodrow*'s results, *Bucher* (1) showed that one half of a batch of one-day-old bee larvae died when each one was fed with 35 *B. larvae* spores. *Sturtevant* (7) conducted some experiments with the feeding of colonies of bees and showed that American foulbrood was only triggered if at least 50 million spores were given to each colony of bees. Other studies have demonstrated that as many as 10,000 million spores are required before an outbreak of American foulbrood occurs in a colony of bees (6).

In the present investigation, the honey examined was a mixture of honey from all the colonies of an apiary. The investigation showed (Tables 1, 2 and 3) that honey can be considerably contaminated with *B. larvae* without any outbreak of American foulbrood occurring.

Furthermore, the investigation showed that there can be substantial differences in the number of *B. larvae* spores present before American foulbrood is triggered (Tables 2 and 3). In some cases, huge numbers of spores may be present in the honey stock of bee colonies without any outbreak of the disease (Table 3).

There are several reasons explaining why there is such variation in the number of spores triggering the disease. Different types of bee have different levels of hereditary resistance to American foulbrood (e.g. in respect of their capacity to evacuate and the functioning of the valve flaps in the mouth of their stomachs). In addition, American foulbrood is a slow, progressive disease, which means that a comparatively long time elapses between the time when the colony is infected and when the disease manifests itself, if indeed it ever does. Various external factors, such as the nectar flow, climate and apicultural intervention, probably also play some role in determining how great the timespan is.

In this investigation, there were only 11 cases of honey deriving from colonies of bees which had an outbreak of American foulbrood the following year. In 9 of these, *B. larvae* were detected in the honey. This result taken with other results of the investigation demonstrates that in most cases *B. larvae* spores are present in the honey the year before any clinical signs appear.

There are probably several reasons why, in 2 instances, no spores could be detected in the honey in the year prior to the outbreak of the disease. It may be that the sample investigated was not representative of all the colonies of the apiary, that the infection was too small to be discovered using our detection technique, or that the colonies were infected after the extraction of the honey.

The investigation further demonstrated that not all *B. larvae* spores can be removed from colonies with American foulbrood by the method of treatment employed. Yet, only low degrees of contamination were detected in the colonies that had been treated, which is in line with what might be expected from the method of treatment where all contaminated material was removed from the bees. However, even after treatment by the shaking method the bees may still have some bacterial spores in their coat. It should be said that in practical terms this method has proved very effective.

The results that have been obtained seem to demonstrate that the investigation of honey for the presence of *B. larvae* can play a practical role in the prevention of bee disease. Where spores are present in the honey, a traditional investigation can be carried out on the bee colonies in the apiaries concerned to determine the presence of disease amongst the brood of honey bees. If the investigation reveals American foulbrood in the colonies, they must be treated. If disease is not found, the beekeeper can be given advice on how to prevent an outbreak of disease by using special apicultural techniques. These involve such things as making exclusive use of new comb foundation, thoroughly cleaning reused materials, changing queen bees, feeding bees during periods when there is no nectar flow and generally providing a good environment for the bees.

It would be desirable to know more about the extent to which American foulbrood in a colony of bees is affected by hereditary conditions and different external factors. To extend our knowledge in this area, we need to trace the development of *B. larvae* infections in particular colonies of bees, for example in experiments with induced infection.

Translation into English by *Bodil Sampson.*

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