Danish Research Service for Plant and Soil Science **Report no. 1923**
Administration
Department of Bee Diseases
Kongevejen 83
2800 Lyngby

A preliminary experiment involving induced infection from *Bacillus larvae*

Indledende smitteforsøg med Bacillus larvae

HENRIK HANSEN, BENTE RASMUSSEN and FINN CHRISTENSEN

Summary

In this paper an experiment involving induced infection from *Bacillus larvae* is described.

Eleven bee colonies were used in the experiment. Of these, six colonies were fed with infected honey whilst five colonies were used as a control.

The experiment showed that colonies fed with 50 g honey containing 2,900 million *B. larvae* spores and with 800 g honey containing 46,400 million spores could give rise to clinical symptoms of American foulbrood in some colonies of bees. In other colonies no clinical symptoms presented themselves, and in some the symptoms disappeared again.

Whether or not a bee colony is affected by the disease does not only depend on the quantity of spores introduced or the level of spores present in their own honey. The environment, the hereditary resistance of the bees to the disease and possibly other factors must also play a part.

Forty days into the experiment, the infection had spread from the six colonies fed with infected honey to four out of five control colonies. The spread of the infection must be due to the bees robbing each others food and drifting to the wrong hives.

As infection spreads so rapidly within an apiary, it is wise to treat all colonies for the disease even though clinical symptoms of American foulbrood appear in only a few colonies in the apiary.

Key words: Honey bees, *Apis mellifera, Bacillus larvae,* American foulbrood, induced infection, feeding with honey.

Resumé

Der er indledt smitteforsøg med *Bacillus larvae.* Elleve bifamilier indgik i forsøget. Seks familier blev fodret med inficeret honning, mens fem familier var kontrolfamilier.

Forsøget viste, at fodring med 50 g honning, som indeholdt 2.900 mio. *B. larvae* sporer, og med 800 g honning, som indeholdt 46.400 mio. sporer, kunne give kliniske symptomer på ondartet bipest i nogle bifamilier. I andre familier kom der ingen kliniske symptomer, og i nogle forsvandt symptomerne igen.

Det afhænger ikke kun af den tilførte mængde sporer eller antallet af sporer i honningen, om bifamilien bliver syg. Miljøet, biernes arveligt betingede resistens mod sygdommen og eventuelle andre faktorer må spille en stor rolle.

Efter 40 dage var infektionen spredt fra de seks honningfodrede familier til fire ud af fem kontrolfamilier. Spredningen må være sket på grund af biernes fejlflyvning og røveri.

Da smitstoffet spredes så hurtigt inden for bigården, kan det være fornuftigt at behandle alle familier, selv om der kun findes kliniske symptomer på ondartet bipest i enkelte familier i bigården.

Nøgleord: Honningbier, *Apis mellifera, Bacillus larvae,* ondartet bipest, smitteforsøg, honningfodring.

Introduction

American foulbrood is caused by the spore-generating bacterium *B. larvae.* The disease affects the brood of honey bees (*Apis mellifera*).

In an earlier paper (1), it was shown that in many instances the honey of bee colonies can contain spores of the American foulbrood bacterium *B. larvae* without the colonies showing any clinical symptoms of American foulbrood. In addition, it was shown that generally the spores of the American foulbrood bacterium can be detected at least a year before the outbreak of the disease.

Further, it was found that the number of spores able to trigger American foulbrood can be highly variable.

In the literature, there is a variety of data indicating how many *B. larvae* spores are necessary to trigger American foulbrood in a bee colony (2,4). In these experiments, the bee colonies were fed with sugar water into which *B. larvae* spores had been introduced. To the best of our knowledge, experiments under controlled conditions involving the induced infection of bee colonies by means of infected honey have not previously been undertaken.

It would be desirable to know the circumstances which determine whether or not infected bee colonies fall victim to the disease. For this reason, an experiment involving induced infection with *B. larvae* was mounted.

For the first year of the experiment, the aim was to try to infect colonies of bees by means of infected honey. That is the experiment described in this paper.

Method

The experiment was carried out on Drejø, an island approximately 5 km long and with an area of c. 4 km^2. It has a varied flora of both cultivated and wild plants.

Eleven colonies of bees were involved in the experiment. The apiary was established at the beginning of the main period of nectar flow, namely towards the end of June 1986. To the bee colonies, which at this time were of uniform size, were introduced Italian queen bees of a closely related type *(Apis mellifera ligustica),* which had been pure bred on the island of Bågø. There were no other bee colonies on Drejø.

To reduce the risk of the bees drifting to the wrong hives the bee colonies were placed in four separate groups in a hedgerow sheltered by a farmhouse. There was approximately 10 m between each group. The first three groups were made up of three bee colonies each, while the last group, which was purely a control group, consisted of two colonies.

A fortnight after the establishment of the bee colonies, the honey of each colony was examined for the presence of *B. larvae.* The investigation was carried out according to the method described by *Hansen* and *Rasmussen* (1) which is based on plating on agar medium. Using this method, *B. larvae* can be detected when there are an average 6,000 spores per 1 g honey. The investigation showed no infection of the honey.

On 7 August 1986, i.e. approximately 1 1/2 months after the establishment of the bee colonies, the experiment with induced infection was begun. At this point in time, the main nectar flow had ceased. The bee colonies each had c. 7 brood combs.

The bee colonies were fed with the same honey containing on average 58 million *B. larvae* spores/ 1 g honey.

One colony of bees in each of the three first groups was fed with 50 g honey containing 2,900 million *B. larvae* spores. Another colony in each of these groups was fed with 800 g honey containing 46,400 million spores. Finally, the third colony in each of these three groups was used as a control

and was not given any honey. Thus each group consisted of one colony receiving a substantial quantity of honey, one colony receiving a very limited quantity and a control colony receiving none.

All the colonies in the three groups as well as the two colonies in the control group were given 22 1/2 kg of solid food (Apifonda) for wintering.

The honey with which the bee colonies were fed originated from an apiary containing colonies which showed clinical symptoms of American foulbrood. The amount of spores present in the honey had been determined by the method described in an earlier paper (1).

After eleven days (18 August 1986) and 40 days (16 September 1986), samples of honey were taken from each colony of bees. In all, four samples of 15 g were taken from each colony. The colonies each had two boxes containing nine combs, and from these boxes the samples were collected from the rearmost honey comb and from the uppermost feeding edge on the comb in the middle of the brood nest.

The honey samples were investigated for *B. larvae* and the spores counted using the technique indicated earlier.

On the two dates specified, when the honey samples were collected, the bee colonies were also examined for clinical symptoms of American foulbrood in the traditional way.

In order to restrict the spread of infection, the extraction of honey samples and the examination of bee colonies were carried out with the control colonies first of all, then with the colonies that had been given 50 g honey and finally with the colonies that had been fed with the large amount of honey (800 g). In addition, while taking the honey samples and examining the bee colonies, ordinary hygienic precautions were taken.

Results

The results of the experiment with induced infection are shown in Table 1.

From the table it can be observed that on the day of the first examination, namely 18 August i.e. ten days after the feeding of the bees, infection was present in the honey of all the colonies which had been fed with spore-infected honey. In both the spore-fed groups under investigation, two colonies also had clinical symptoms of the disease. Neither infection nor clinical symptoms could be discovered in the control colonies.

At the second examination, on 16 September, i.e. 40 days after the feeding, infection was still present in all the colonies that had been fed with spores. Four out of five control colonies were discovered to have infected honey. Thus, in the course of 40 days, infection had spread to ten out of eleven bee colonies. The control colony in which no infection could be detected was grouped

Table 1. Incidence of disease in bee colonies fed with honey infected with *B. larvae*.

No. of bee colonies	7 August	18 August		16 September	
	No. of *B. larvae* spores introduced	No. of bee colonies infected with *B. larvae*	No. of bee colonies showing clinical symptoms of American foulbrood	No. of bee colonies infected with *B. larvae*	No. of bee colonies showing clinical symptoms of American foulbrood
5 (3+2 control colonies)	0	0	0	4	0
3	2,900 million (50 g honey)	3	2	3	0
3	46,400 million (800 g honey)	3	2	3	2
11		6	4	10	2

Table 2. The average total of *B. larvae* spores found to be present per 1 g honey.

	18 August	16 September
Control colonies	0	$0 - 6\times10^3 - 6\times10^3$ $6\times10^3 - 3\times10^4$
Colonies fed with 2,900 million spores	$1.2\times10^4 - 6.9\times10^4 - 1.8\times10^6$	$16.8\times10^4 - 21.1\times10^4 - 35.5\times10^4$
Colonies fed with 46,400 million spores	$1.7\times10^6 - 5.1\times10^6 - 5.2\times10^6$	$2.8\times10^6 - 4.0\times10^6 - 4.0\times10^6$

together with two honeyfed bee colonies of which both had infected honey and one of which presented clinical symptoms.

No clinical symptoms presented themselves in the control colonies, nor in the colonies which had been fed with the smaller amount of infected honey. Indeed, the symptoms had disappeared in the two colonies which were sick at the first examination.

In the colonies fed with the larger amount of honey, there were still clinical symptoms in the same two colonies, as shown in Table 1.

Table 2 shows the number of spores found in the different groups at the different stages of the experiment.

The table shows that the number of *B. larvae* spores found on 18 August in the honeys of the two honey-fed groups of colonies is apparently not determined by the number of spores initially given to the two groups. However, in the samples of 16 September the number of *B. larvae* spores present seems to be directly so determined.

In the examination on 18 August, there were clinical symptoms in two colonies belonging to the group that had been given 2,900 million spores. In these colonies, 69,000 and 1.8 million *B. larvae* spores per 1 g honey were found respectively. The colony with 12,000 spores per 1 g honey had no clinical symptoms of the disease.

Similarly, on 18 August, clinical symptoms were found in two colonies belonging to the group fed with the larger amount of spores, namely 46,400 million spores. These colonies were found to have 1.7 million spores and 5.1 million spores per 1 g honey respectively. On the other hand, the third colony in this group showed no clinical symptoms despite the fact that it had the largest amount of spores of all the colonies namely 5.2 million spores per 1 g honey. In the examination on 16 September the same two colonies, showed clinical symptoms of the disease. At this point, they both had 4.0 million spores per 1 g honey. The third colony, which still presented no clinical symptoms, had 2.8 million spores per 1 g honey.

Discussion and conclusion

Sturtevant (4) has described how bee colonies were fed with sugar water to which *B. larvae* spores had been added. He discovered that in order for American foulbrood to be triggered at least 50 million spores had to be given to each bee colony. Other investigations using the same technique have been carried out by *L'Arrivee* (2), which showed that as many as 10,000 million spores had to be given in order to trigger American foulbrood in a colony.

In earlier Danish experiments (3), bee colonies were fed with thirteen different foreign honeys in order to investigate whether they could trigger American foulbrood. The disease broke out in colonies that had been fed with three of the honeys. No attempt was made to discover whether the colonies had been infected with *B. larvae* beforehand, nor whether the relevant honeys were *B. larvae* infected at all or how many spores were present in the honeys.

In the present experiment, bee colonies were fed with honey containing 2,900 million spores and 46,400 million spores respectively. Thus the smaller amount of spores was slightly less than the amount used by *L'Arrivee*. The larger amount of spores was nearly five times larger. Before the start of the experiment with induced infection no infection of *B. larvae* in the honeys of the bee colonies could be detected. The threshold for detection is on average 6,000 spores per 1 g honey. The

present results derive from the first year of the experiment and involve eleven bee colonies.

This experiment shows that feeding with honey containing either the larger or the smaller amount of spores may cause clinical symptoms of American foulbrood. However, the spores introduced do not necessarily cause clinical symptoms, and in some instances the clinical symptoms will disappear again.

In one colony, which had been fed with the smaller amount of spores and which presented clinical symptoms, there were 69,000 *B. larvae* spores per 1 g honey. On the other hand, another colony which had been fed with the larger amount of spores and which had 5.2 million spores per 1 g honey presented no clinical symptoms during the period of the investigation.

When all the data of the investigation are taken into account, the conclusion to be drawn must be that it is not just the quantity of spores introduced to a colony of bees which determines whether it falls victim to American foulbrood. And indeed, with certain reservations, it seems that the amount of spores present in the honey of the bee colony does not determine whether the disease breaks out either.

The findings concur with earlier investigations of 1,700 Danish colonies of bees (1). Here it was shown that the amount of *B. larvae* spores able to trigger American foulbrood can vary greatly. In some instances, disease was found to be present when in a representative sample of honey from a colony of bees of an apiary there were between 6,000 and 12,000 spores per 1 g honey. In other instances, disease was only found to occur when the number of spores was far greater. There was one apiary where 3 million spores per 1 g honey were detected without any clinical symptoms of American foulbrood appearing.

There are many reasons why varying amounts of *B. larvae* spores can trigger American foulbrood. General conditions, techniques used by the apiarists and the resistance of the bees to disease can play a part. In the present experiment, the general conditions and techniques used were made as uniform as possible.

The varying levels of susceptibility to the disease are presumably due to differences in the inherited resistance of the bee colonies.

The experiment showed that the spread of infection amongst the bee colonies happened very rapidly. In the examiniation 40 days after the feeding, it was found that the infection had spread to four out of five control colonies. The explanation for this doubtless lays in the bees drifting to the wrong hives and robbing honey from each other, but other unknown factors may also be involved. The spread of disease to the group of bees consisting of two control colonies must be entirely due to robbing as there was practically no chance of drifting.

As infection is spread at such speed, it seems sensible to treat all the colonies in an apiary although clinical symptoms may occur only in a few of the colonies.

That this is so is confirmed by our own practical experience in treating American foulbrood.

Translation into English by *Bodil Sampson.*

References

1. *Hansen, H. & Rasmussen, B.* 1986. The investigation of honey from bee colonies for *Bacillus larvae.* Tidsskr. Planteavl 90, 81–86.
2. *L'Arrivee, J. C. M.* 1958. Survival of honey bee larvae following colony inoculation with various dosages of *Bacillus larvae.* W. Iowa State College, Ph. D. Thesis. Consulted from Dissertation Abstracts 19, 195–196.
3. *Statens Bisygdomsnævn* 1971. Beretning for 1969 og 1970. Tidsskrift for Biavl 105, 105–109. (State Bee Disease Committee: Report for 1969 and 1970).
4. *Sturtevant, A. P.* 1932. Relation of commercial honey to the spread of American foulbrood. J. Agric. Res. 45, 257–285.

Manuscript received 30 November 1987.