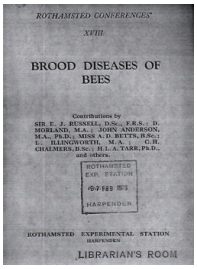
ROTHAMSTED RESEARCH

Brood Diseases of Bees

Full Table of Content



Recent Work at Leeds on Foul Brood Disease

C. H. Chalmers

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RECENT WORK ON FOUL BROOD OF THE HONEY BEE

By C. H. CHALMERS, B.Sc., The University, Leeds

THE experimental work on foul brood, carried out at Leeds and extending over a period of two seasons, was essentially of a preliminary character.

It was the increase in the number of cases of foul brood in Yorkshire which stimulated the experimental work at Leeds. The gradual eradication of acarine disease amongst bees has given way to a gradual increase in foul brood. This is probably due to the fact that the earlier treatment of acarine disease was the complete destruction of the hive. The use of fumigation and the consequent preservation of the stock has enabled the causal organism, once again, to obtain a foothold and become a serious problem and a menace to bee husbandry.

Enquiry showed that there is much confusion in the minds of authorities as to the predominant type of foul brood, the causal organism, the source of infection, the progress of the disease and the treatment. The work of Cheshire and Cheyne, carried out in 1885, is the only scientific investigation which has been made in this country. It is unfortunate that this meeting today could not have been held a year or so earlier, when we might have had the pleasure and honour of the company of Sir Watson Cheyne, who carried out the bacteriological work for Mr. Cheshire with such skill and meticulous care. He might have been able to enlighten us on some points which are not explained in his paper. Cheshire and Cheyne were of the opinion that an organism, which they named *B. alvei*, was responsible for the disease.

In 1912, G. F. White, working in America, took up the problem, and after some years of careful work, suggested that the type of foul brood predominant in his country was caused by a rod-shaped organism, slightly more slender than that of Cheshire's, but producing a spore similar in size and shape. This organism he named *B. larvae*. Although he isolated *B. alvei* from a number of diseased stocks, he was unable to reproduce a foul brood with this organism, thus casting some doubt on the work of Cheshire and Cheyne.

The first season at Leeds was confined to the isolation of *B. alvei* and the repetition of Cheshire and Cheyne's work. The isolation of *B. alvei* and its cultivation on the ordinary media of the laboratory is simple and straightforward. It is never found in pure culture is diseased material, being invariably associated with *B. subtilis* species and *Streptococcus apis*. It should be noted, however, that *B. alvei* in seldom, if ever, present in those cases of foul brood, which show the typical characters of the so-called American type, i.e. marked ropiness and the characteristic " glue-pot " odour. Throughout this work, much difficulty was experienced in obtaining frames showing typical

https://doi.org/10.23637/ERADOC-1-211

European foul brood, and consequently it was not possible to study the symptoms manifested by such a diseased brood. During the summer, attempts were made to produce disease by the use of *B. alvei*. Three methods of inoculation were employed, namely feeding the spores of the organism in a syrup solution, spraying the larvae with warm sterile milk containing the spores, and painting the larvae with the vegetative form of the organism. The season closed, however, and none of the infection experiments were successful; the stocks being as healthy at the end of the season as they were at the beginning.

In the spring of 1933, it was decided to repeat White's work. Several frames, showing typical American foul brood, were obtained. All the diseased frames came from beekeepers in Yorkshire, with the exception of two, which were obtained from the Quantock Hills. A stock of healthy bees were infected with foul brood by inserting one of the diseased frames in the centre of the hive. This stock was kept at a considerable distance from the healthy experimental stocks, and was used as a source of material. The progress of the disease in this hive was interesting. The bees cleaned up the diseased frame and, after some little time, the queen commenced to lay in it. After a period of about 18 days, disease appeared in one of the healthy frames and gradually spread to one side of the hive. Disease did not appear in the introduced frame to any very marked extent until the second brood of larvae were present. The disease then spread steadily to the other part of the hive. This suggests that the responsible organism was being carried by the nurse bees. The first symptom of the disease was an attack on the unsealed larvae just prior to capping. As the disease progressed in severity, this symptom disappeared and only sealed larvae were affected. Later, however, when the disease was at its height this symptom reappeared. This sequence of events was also observed in hives infected experimentally and is considered important. It may be the reason for some of the confusion, which has arisen when diagnosing European and American foul brood by microscopic characters only. At one time most of the diseased larvae were uncapped, suggesting European, whilst at another time all were capped, which suggested the American type.

When the disease had thoroughly established itself in this hive the queen was transferred to a healthy stock, but the stock did not, as a result, show at any time diseased larvae. This observation has, to some extent, been confirmed, for on three further separate occasions the queen of a diseased stock did not produce disease when transferred to a healthy stock. It is not concluded, however, from this observation that the queen does not carry the responsible organisms of disease. Our experience is that in an active healthy hive, where only a few larvae are attacked, a slight infection is rapidly and completely cleared out. It is to be expected that the queen, in her wanderings. over diseased frames, will carry organisms on her body and legs. From this infection a few larvae in the healthy hive may have been

https://doi.org/10.23637/ERADOC-1-211

attacked and rapidly cleaned out before they were observed. Moreover, Sturtevant has shown that a very considerable number of organisms had to be introduced to a stock before disease is produced. This experiment does, however, suggest that the organism is not pathogenic to the queen, that her eggs are healthy and that provided the stock is vigorous, there is little risk in introducing a queen from a diseased stock to a healthy one.

Another interesting observation in connection with this diseased hive was that later the drone brood became attacked and finally the queen cells. By September, practically all the bees were dead, the queen being amongst the last to die.

The changes through which the larvae pass from the time of infection are interesting and throw some light on the methods of attack by the organism and the spread of the disease. Generally, the majority of the larvae die in capped cells, although, as has been explained, some may die before capping. Some of the caps may be removed from cells containing diseased larvae and portions of the larvae removed. It is only when the disease has been present in the hive for some time that sunken and punctured caps appear. The first symptom is that the bluish-white of the healthy larva changes to a very light brown, the surface markings being very similar to those of a healthy larva. The larva at this stage may, with care, be removed from the cells, but an examination shows that the internal tissue is disorganised. Soon, however, the diseased larva is easily ruptured and the decaying mass becomes viscid and adheres to the cell wall. The colour deepens and later becomes so viscid that the mass can be drawn out into thread-like strings. Eventually it dries out, leaving a dark tough scale. Microscopically diseased larvae, at first, show the presence of numerous slender streptobacilli, but by the time the cells are capped, these rods are replaced by spores. These observations suggest that the organism attacks the larva by piercing the gut and extending through the tissue. It will be seen later that it is improbable the organism attacks the larva externally through the body wall. Moreover, a slight attack of the disease probably does not gain a foothold readily, because the few diseased larvae can in the early stages be removed intact and thrown out of the hive, thus eliminating the infection. These conclusions are supported to some extent by later experiments.

The media used to isolate the organism were (1) White's Brood Agar, (2) Egg Yolk Nutrient Agar, (3) Chocolate Agar, (4) Red Blood Agar, (5) Inspissated Serum, and (6) Sturtevant's Egg Agar. *B. larvae* grew well on all of these media, but to a varying extent. Red Blood Agar and Sturtevant's gave the best results. The organism is easily recognised by its slender shape, variable size and motility, its smooth, greyish-white slightly viscid growth on the media mentioned and its inability to grow on any of the ordinary media of the laboratory. The spore is central, but not difficult to stain by ordinary spore staining methods. The organism does not spore readily on Sturte-

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vant's, unless the egg is omitted. Pasteurisation or desiccation does not induce spore production. The organism is present in diseased larvae in practically pure culture. By heating the spore containing material in aqueous suspension at 80°C. for ten minutes, the occasional contaminating organisms are eliminated and a pure culture usually obtained without plating. Several methods of inoculation with *B. larvae* were tried, vigorous healthy stocks of Italian and X-bred bees being used. The methods of inoculation were: (1) Feeding spores in syrup, (2) Inoculating frames, not containing larvae, by means of a capillary pipette, with both the spores and vegetative forms of the organism, (3) "Painting" uncapped full-grown larvae with spores and with active growth from an agar slope, (4) Spraying frames containing eggs and larvae with both the spores and vegetative form of the organism in warm sterile separated milk. The last method was by far the most successful—typical foul brood being produced in from fourteen to twenty-one days. "painting" the larvae and inoculating empty frames gave negative results, whilst the feeding of spores was disappointing. The results, however, are interesting and the following explanation is offered:

(*a*) *Painting full-grown larvae.*—The organism apparently does not gain entrance to the larva through the body wall, and since feeding at this stage is practically at an end, there is little opportunity for the organism to infect the larvae.

(*b*) *Inoculating empty frames.*—The cells of the frame are, as far as possible, thoroughly cleaned and polished by the nurse bees before the eggs are laid, and consequently any infection is cleaned out. It was interesting to note, however, that a few larvae took the disease, but these were soon removed, and the frame remained healthy.

(*c*) *Feeding infected syrup.*—This experiment was commenced in June, and negative results were obtained until about the end of August, when foul brood appeared. It is suggested, therefore, that the syrup was stored and not used as part of the food of the young larvae, until outside food was becoming scarce.

(*d*) *Spraying warm milk, containing spores and vegetative rods.*—This was successful, because the active form of the organism became mixed with the actual food of the young larva, and being absorbed into the gut almost immediately, set up an infection rapidly. The disease produced was typical American foul brood, and the organism was re-isolated from the diseased larvae with ease.

From these preliminary experiments, the following conclusions are suggested. It should be remembered, however, that much more work requires to be carried out before final conclusions can be drawn. The most frequently occurring type of foul brood is the American type. It was only with difficulty that specimens of the European type were obtained and these were not typical. *B. alvei* was isolated from cases of so-called foul brood, but infection of healthy stocks could not be produced, either by inoculation of the larvae, or by feeding. *B. larvae* was isolated in pure culture, from typical cases of

https://doi.org/10.23637/ERADOC-1-211

American foul brood, and the disease reproduced by feeding infected syrup and by spraying healthy larvae with the organism. It is probable that infection of the larva is through the wall of the intestine and is carried by the nurse bees. The difficulty encountered by the bees in cleaning out badly-diseased grubs and dried scales is responsible for the persistence of the disease in a stock. The trailing of pieces of badly-diseased larvae across the frame containing healthy brood no doubt produces some infection. Frames containing much diseased brood will cause the disease when placed in a hive containing healthy larvae. It is probable that in nature, a considerable period elapses from the time the hive is first infected until the disease obtains a foothold. Strong healthy stocks of vigorous bees will, in the early stages of the disease, eradicate it by the complete removal of diseased larvae. Slight infections of the disease may, therefore, disappear without treatment. All types of larvae are subject to disease but adult bees, including the queen, do not appear to suffer. Finally, there appears to be little risk of transmitting the disease by transferring the queen of a diseased stock to a healthy stock.

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