American Foulbrood Disease Part II: Subclinical Infections

By R.M. Goodwin, J.H. Perry, & H.M. Haine
Apicultural Research Unit, Ruakura

The disease control strategy that most beekeepers in New Zealand use for their own beekeeping outfits, either knowingly or unknowingly, has been traditionally based on the following assumption;

If you inspect a colony and don't find larvae with obvious American foulbrood disease symptoms then the colony does not have American foulbrood disease.

If no disease is found, the next step is often to perform hive manipulations that could spread the disease to another colony if it was present. There are obvious problems with this scenario.

Firstly, most beekeepers do not usually perform complete brood checks (i.e. every brood frame in a hive is not examined for larvae exhibiting disease symptoms). The reasons for this are obvious considering the large amount of time that a complete brood check takes. It is common practice to inspect only three brood frames (often in the top super) with some beekeepers only inspecting a single frame. If there is only one diseased larvae in a hive with 12 frames of brood there is a 75% chance of it being missed if only three frames are checked. There are therefore problems in assuming that a hive is disease free based on an incomplete brood inspection. The simple solution to this problem would be to inspect every brood frame, but in most cases this is simply not practical.

However, even a complete brood inspection cannot guarantee a colony is free of American foulbrood disease. Colonies can contain American foulbrood spores but not exhibit any visual symptoms of the disease, so that even if you inspected every brood frame carefully you would not identify such a colony as having American foulbrood disease. When we conducted a survey of commercial beekeepers with Amercian foulbrood problems, we tested a large number of colonies for the presence of *Bacillus larvae* spores (Table 1). The colonies that tested positive received a complete brood check either by the beekeeper (Beekeepers C, D, E and G) or by us (Beekeepers A, B, and F). Only 26.4% of the colonies that tested positive for the presence of *B. larvae* spores contained larvae exhibiting American foulbrood symptoms. Therefore, most colonies that contained *B. larvae* spores did not exhibit visual symptoms of American foulbrood disease.

TABLE 1

Number of colonies tested, percentage (%) that tested positive and the percentage found to contain larvae exhibiting symptoms of American foulbrood disease.

Beekeeper	Hives	% Positive culture tests	% of positive hives with diseased larvae
A	400	9.3	35.1
B	422	81.8	28.9
C	200	10.0	5.0
D	200	6.5	15.3
E	200	24.5	18.3
F	200	0.5	0.0
G	200	6.0	8.3
Total	**1822**	**26.2**	**26.4**

Before the waters get too muddy we have to answer the question of what constitutes a diseased colony or larva. At this point we will ignore legal definitions or definitions of convenience and use a dictionary definition. This is important as the definition needs to assist in the control of American foulbrood disease.

The presence of B. larvae spores in the gut of larva does not necessarily mean that a larva is diseased. A larva is not diseased until the B. larvae bacteria are

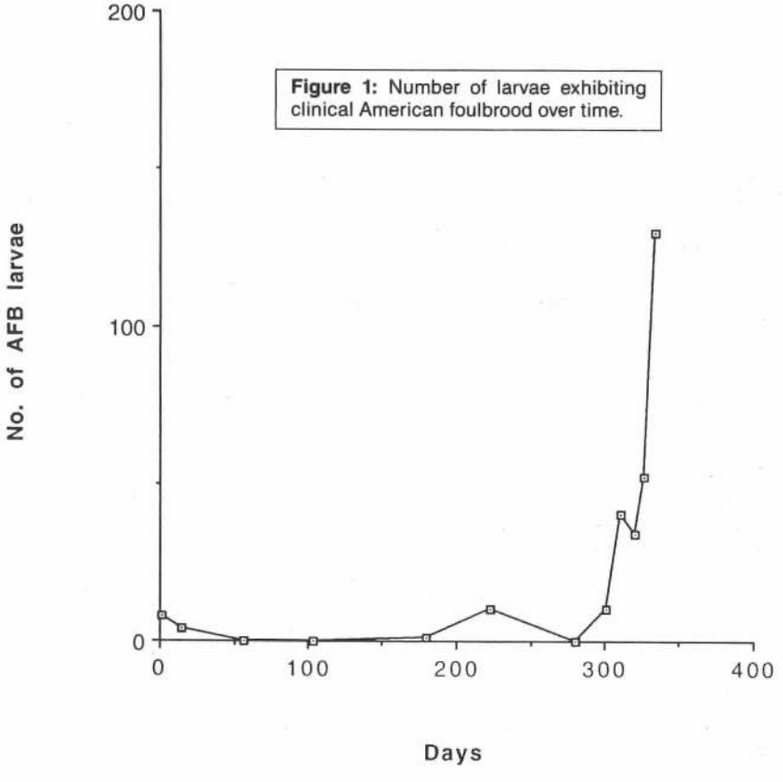

Figure 1: Number of larvae exhibiting clinical American foulbrood over time.

having adverse effects on it. Likewise the presence of spores inside a hive does not mean the colony is diseased. The colony is not diseased until it contains a diseased larva.

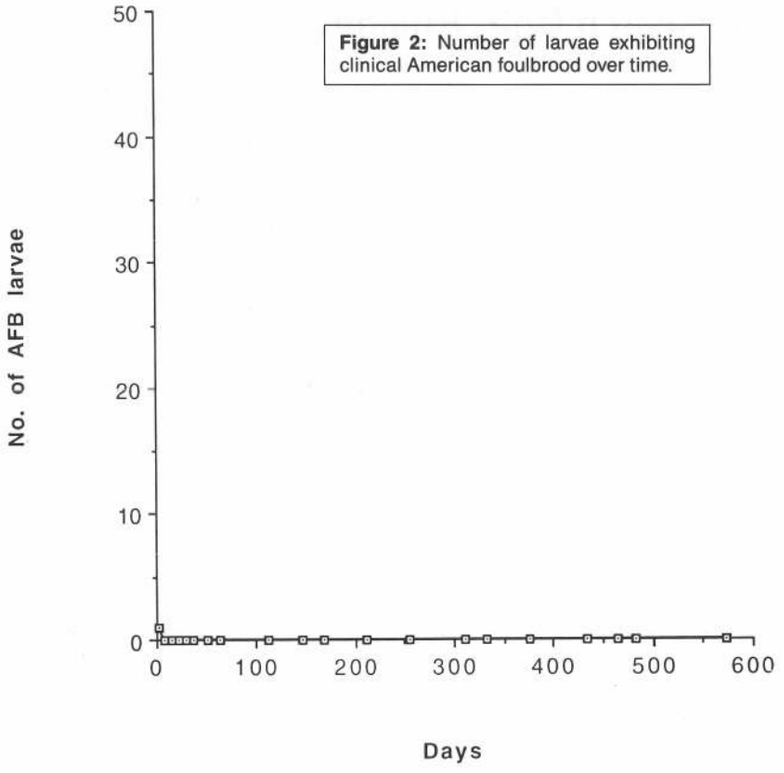

Figure 2: Number of larvae exhibiting clinical American foulbrood over time.

There are therefore three possible states:

1. *B. larvae* is present in a hive but causing no ill effects on any of the larvae (contamination).
2. *B. larvae* is adversely affecting at least one larva but the disease is not apparent to an observer (a subclinical infection).
3. *B larvae* is adversely affecting larvae and producing visible symptoms of American foulbrood disease (a clinical infection).

The presence of *B. larvae* spores in a contaminated colony, or one with a subclinical infection, can only be detected by allowing the bacteria to multiply on culture plates until the colonies are large enough to be identified. If spores are present in a hive and there is no clinical infection it will probably be for one of two reasons. The first is that the bees are not coming into contact with the spores and are not feeding them to larvae e.g. the spores may be sealed under the cappings of a frame of honey. However, more likely, the spores are being fed to larvae but not in sufficient quantities to cause the disease. *B. larvae,* like many pathogens, often needs more than one spore to be fed to a larva to cause the disease. The more spores that are fed the greater the possiblity that an infection will occur[1].

The presence of spores in a colony is, however probably indicative that either the colony is diseased, or a colony in the vicinity is diseased.

Subclinical infections occur at an individual larval level and at a whole colony level. Infected larvae do not show clinical symptoms of American foulbrood disease till they are an average of 12.5 days old. Therefore, the disease will remain subclinical for the first 12.5 days.

Many beekeepers are probably familiar with the symptoms of subclinical infections that occur on a whole colony level. Having found a colony with only a couple of diseased larvae, beekeepers are sometimes tempted to check through the colony again when they are going to destroy it. At this stage they are often unable to find any symptoms of the disease. The disappearance of the disease symptoms is probably due to the bees' hygenic behaviour. House bees will remove diseased larvae. In one trial it was demonstrated the 50% of the diseased larvae were removed before the larvae were 11 days old[2]. A colony may be diseased, but the larvae may be removed fast enough so that, when the beekeeper looks into the hive, all he sees is the empty cells where the diseased larvae used to be. Thus American foulbrood may give rise a patchy brood pattern. However inbreeding, failing queens or removal of larvae killed by other diseases such as sacbrood or chalkbrood can also give rise to a patchy brood pattern.

The presence of subclinical infections can be demonstrated dramatically if you look at the disease history of individual colonies that were kept after American foulbrood disease was first detected (Fig 1). This colony was diagnosed as having American foulbrood disease by a visual inspection. The length of time that it was diseased before the diseased was first diagnosed is unknown. As you can see it exhibited no further visual symptoms of the disease for a considerable period of time after American foulbrood disease was first diagnosed. If you had inspected this colony during that period you would have failed to recognise it as having American foulbrood disease. Adult bees from the colony tested positive for the presence of *B. larvae* spores during the period of time that it did not exhibit any clinical symptoms. Whether the colony had a subclinical infection during the time that it exhibited no clinical symptoms of the disease or was reinfected with spores stored in the hive is unknown.

Contrary to what is suggested in some quarters, colonies will not necessarily die out if they become infected with American foulbrood disease. Some colonies will recover from the disease completely (Fig 2). We will never know how many colonies become lightly infected (a couple of diseased larvae) and recover without ever being diagnosed as having American foulbrood disease.

It is possible to get an idea from the number of spores being carried by adult bees as to whether a colony is diseased or will become diseased in the near future. We have tested samples of 30 adult bees from approximately 3,000 colonies for the presence of *B. larvae* spores over the last 2 years. Where spores were present, we counted the number of bacterial colonies growing on the plates to gain an indication of the number of spores the bees were carrying. All of the colonies that tested positive and nearly 400 of those testing negative had every frame checked for diseased larvae. We then related the number of *B. Larvae* colonies growing

on the plates to the occurrence of visual disease symptoms within the colonies (Fig. 3). From this you can see that the more spores that the bees are carrying, the greater the likelihood that the colony will exibit clinical symptoms. Several of the colonies that tested negative were later diagnosed as having American foulbrood disease by further visual examinations. Whether this was due to errors in the plating or the colonies becoming infected in what was often two to three months between sampling bees and the colonies receiving a visual examination, is unknown.

Conclusions

Not only is the assumption of freedom from disease incorrect if you do not conduct a complete brood check, even a complete brood check is no guarantee of the absence of American foulbrood spores or diseased larvae. You need to consider this when you are about to take a frame of brood or honey from one colony to place in another. You may be spreading American foulbrood disease.

References

1. Woodrow, A.W. 1943: Susceptibility of honeybee larvae to individual inoculations with spores of *Bacillus larvae*. *Journal of Economic Entomology 35:* 892-895

2. Woodrow, A.W.; Holst, E.C. 1942: The mechanism of colony resistance to American Foulbrood. *Journal of Economic Entomology 35:* 327-330

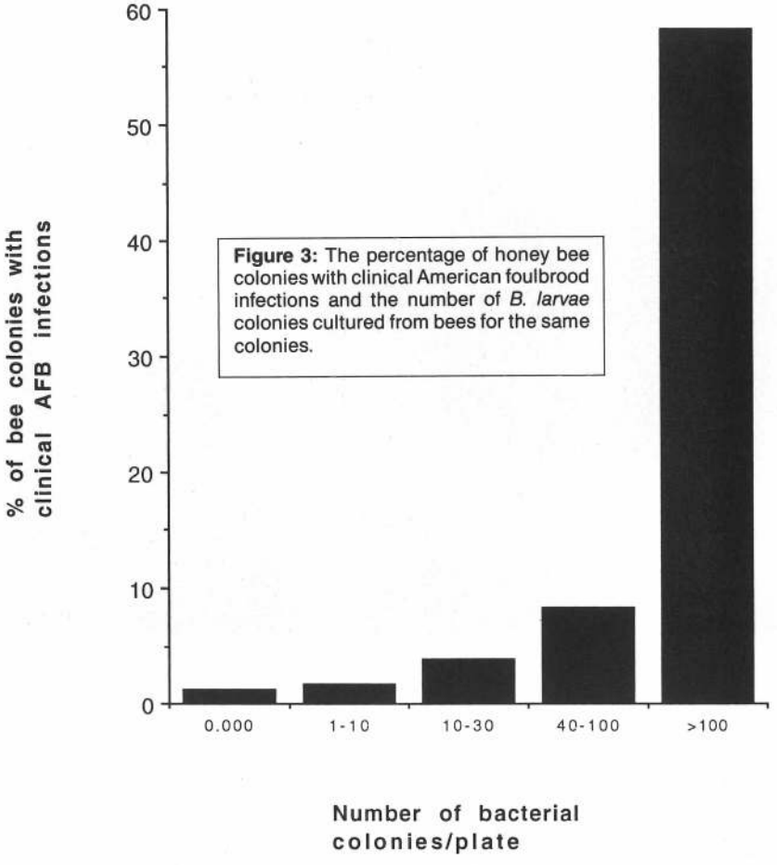

Figure 3: The percentage of honey bee colonies with clinical American foulbrood infections and the number of *B. larvae* colonies cultured from bees for the same colonies.