

Diagnosis and control of American foulbrood disease of honey bees in the Netherlands

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American foulbrood disease (AFB), caused by the bacterium *Paenibacillus larvae*, is a disease of the European honey bee (*Apis mellifera*) and other honey bee species (*Apis* sp.). It is distributed world wide. Spores of the bacterium can infest larvae of the bees, ultimately leading to the death of the larva or pupa. Although often spores are present in bee colonies, it only sporadically leads to serious disease symptoms, and to the death of the colony.

In most countries the disease is under an obligation of notification, and control is often carried out by the government. Until 2005 this was also the situation in the Netherlands, where the disease was controlled using a 'stamping out' procedure. However, since the fall of 2005 the beekeepers are responsible for the control of AFB. Therefore PPO Bee Unit together with the Dutch beekeepers organizations has developed material for disease recognition, made a script for AFB control and organized training meetings for bee health officers.

In the new control script the strict 'stamping out' approach (destroying the whole apiary) has been abandoned. Now only the visibly diseased colonies will be eliminated, the other colonies of the apiary will be cleaned and restarted on foundation comb (limited stamping out).

Keywords: honey bee, *Apis mellifera*, American foulbrood, *Paenibacillus larvae*, swarm shook method

The disease

American foulbrood (AFB) is a disease of the brood (larvae & pupae) of the honey bee, caused by the bacterium *Paenibacillus larvae*, and distributed world wide (Ellis & Munn 2005). The larvae can be infected by one spore when it is 24 hours old, and the spore dose that kills 50% of the larvae (ID_{50}) is 8.5 spores per larvae for 24-48 hours old larvae. With increasing age of the larvae progressively more spores are needed to initiate an infection, and above 53 hours age from hatching a larva can not be infected (OIE Terrestrial Manual 2004). An infect-

ed larva is ultimately killed by the spores, generally in the pupation stage. Then the pupa changes into a glutinous mass containing up to a billion spores. These spores may be carried to young larvae by the nurse bees, and the infection can spread very quickly through the colony, finally leading to its collapse. Since an infected and diseased colony is weakened, bees from neighbouring colonies enter and rob the colony. By doing this they take many spores with them, and can spread the disease rapidly. Spores of *P. larvae* are very persistent and long lived (Hansen & Brødsgaard 1999).

Control of AFB in the Netherlands changes

Until 2005 the control of the disease was looked after by the Dutch Ministry of Agriculture, Nature and Food quality (LNV). When a break out of AFB was reported and confirmed, all colonies of the whole apiary were eliminated and all beekeeping material was destroyed. The beekeeper was financially compensated by the government. An area with a radius of 3 km around the apiary was established, in which no movement with bees, bee colonies and bee materials was allowed. In this area all bee colonies were checked for visual symptoms of AFB. When the bee colonies in the area were free of AFB symptoms, and no new case was reported within a month, the restrictions were alleviated and the area declared free of AFB.

In the new approach much remains the same, but a few things change. The most important changes are:

- the beekeepers organizations become responsible for the control
- only colonies with symptoms of the disease are eliminated
- the bees of not diseased colonies of the apiary are separated from their brood (which is destroyed), and restarted in clean hives on foundation combs
- beekeepers are partially compensated by their organizations
- PPO Bee Unit coordinates and takes care of the training of bee health officers

It is expected that this approach will be equally effective, but far more cost efficient.

Swarm shook method versus elimination and destruction

Since in more than 10% of the apiaries foulbrood spores are present, and in less than 1% AFB symptoms develop, it may be concluded that bees can cope with AFB. So stamping out a whole apiary might be a too drastic measure. On the other hand, when there is a diseased colony on an apiary, the spore load to the healthy colonies may be or become very high. Eliminating the source of the spores (the diseased colony) will strongly reduce the spore load to the healthy colonies, and ultimately the number of spores will decrease to zero in these colonies (Lindström 2005, Fries *et al.* 2006). Lindström (2005) showed that even in the diseased colonies the number of spores was reduced to almost zero when the colony was shaken into new hives with new foundation combs.

Shaking of a colony means the following procedure: the bees including the queen are shaken into a new hive or a cleaned and disinfested hive, with frames with foundation comb only, and fed with a sugar solution. All brood and combs are eliminated and destroyed. Several versions of the method are developed, and are applied in states of federal Germany. The hives can be kept closed for a day to reduce the chance of absconding. The shook swarm method can also be applied to control European foulbrood (Waite *et al.* 2003).

Stamping out offers the advantage that it selects for the least virulent strains of the bacterium on colony level (Fries *et al.* 2006). This advantage is highly needed, since common practices in beekeeping tend to facilitate AFB (no swarming, no brood less periods). Feral colonies, especially in areas where few apicultural colonies are held, rarely contain detectable numbers of spores (Hornitzky *et al.* 1996). This advantage of stamping out is maintained with a limited stamping out, i.e. the swarm shook method, but at far lower costs.

Recognition and diagnosis by beekeepers

AFB clinical symptoms are recognized by a patchy pattern of the closed brood, with sunken and perforated cappings of some cells. Inside these cells the remains of a larva or pupa are slimy and brown in colour. With a matchstick these remains can be drawn out in a brown, slimy rope. This rope discriminates the symptoms from those of European foulbrood (EFB), which can easily be confused with AFB. These symptoms until now labelled the colony as suspect of AFB, and the approval of the presence of AFB spores by laboratory cultures led to the confirmation of an AFB casualty.

Recently lateral flow device test kits based on immunology came available for conclusive confirmation of AFB and EFB (Vita Europe Ltd.), which makes reliable diagnosis in the field possible, and which reduces the time needed for a conclusive diagnosis. As soon as these test kits are accepted by the government they will be routinely included in the AFB control script.

Training of bee health officers and beekeepers

To diagnose and control AFB correctly, without professional officers, beekeepers have to be instructed and certified. The training program of bee health officers, which availability will cover the whole country, is now in progress. It is conducted by PPO Bee Unit, and concentrates on diagnosis, script, the check of all colonies in the 3 km area, and the measures of eliminating (stamping out) and cleaning of the apiary, including the shaking of not diseased colonies.

The printed diagnosis material, which includes the diagnosis of EFB, will be sent to all beekeepers in the Netherlands, as well as the outline of the AFB control script.

Script

Suspicion. A beekeeper discovers alarming symptoms in his colonies, and consults PPO Bee Unit (telephone, E-mail). PPO Bee Unit gathers as much infor-

mation as is needed and takes notes. If suspicion is not excluded:

Notification. The beekeeper has to mention the suspicion to the Veterinary Notification Desk. From PPO Bee Unit he receives the address of the local bee health officer.

Confirmation. The beekeeper and bee health officer check the colonies for visible signs of AFB (including matchstick ropiness test). They can test a sample from the brood with the Vita Test kits for AFB and EFB. If AFB is confirmed, this is notified to PPO Bee Unit, and forwarded to the Food Authority.

Establishing of a 3 km embargo zone. The zone is established, and published (government and local beekeepers). The bee health officer locates all apiaries and colonies in the 3 km area. No movement of bees and bee materials are allowed from and within the zone.

Tracing. Recent movements with bee colonies by the concerned beekeeper and to and from the 3 km area are traced, in order to confine the problem to this area.

Elimination of diseased colonies and cleaning of the apiary. The diseased colonies are killed by burning of sulphur and destroyed by burning. The other colonies are shaken into new clean hives on foundation comb and fed with sugar. The apiary is cleaned by using warm solution of soda (6%), and by flaming (hives).

Screening of the 3 km environment. All colonies are checked visually for symptoms, immediately and for a second time after 4 weeks. On a form the beekeepers declare that their apiaries are free from AFB.

Check of the original diseased apiary. After 4 weeks the shaken colonies of the diseased apiary are checked for symptoms by the beekeeper and the bee health officer. They declare on a form that the apiary is free of AFB.

Declaration that the 3 km zone is free of AFB. Now the whole AFB casualty is finished, and the reports and declarations are sent by the bee health officer to PPO Bee Unit.

Final report to Food Authority. PPO Bee Unit compiles a final report, and sends the dossier to the Food Authority.

Alleviation of embargo. The Dutch Ministry of Agriculture, Nature and Food Quality alleviates the movement and trade embargo.

Perspective

The approach seems to be less strict than the formerly applied method. However, it may be expected that limited stamping out is as effective as full stamping out. The screening of the environment is stricter in the new approach, since it is done twice. Because beekeepers have to take responsibility now, it might also help to increase the awareness of the health situation of the bees, since many cases of EFB, a disease increasing in importance, are nowadays neglected. The increased speed of diagnosis in the field by the test kits is also an improvement. In the new approach every beekeeper can consult an instructed

bee health officer in its vicinity. Giving the responsibility of AFB control, which was a governmental task, to the beekeepers resulted in a strong decrease in the number of AFB casualties in New Zealand (Goodwin 2005).

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