

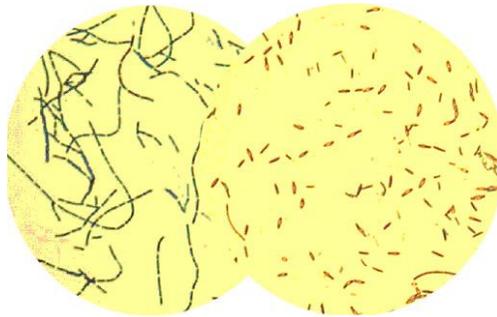
American Foulbrood Bee Disease

Khalil Hamdan, Apeldoorn, The Netherlands

Characteristics of the disease

Honeybees are plagued by the American foulbrood disease (AFB), which is considered to be the most fatal of honeybee brood diseases. The disease attacks only the very young larvae. Larvae older than 48 hours are not susceptible. Adult bees are not affected by the disease.

American foulbrood disease is caused by the spore-forming bacterium known as *Paenibacillus larvae*. The bacterium exists in two forms: the spore stage and the vegetative stage, which consists of a slender rod-shaped bacterial cells. Only the spore stage is contagious to bees.



LEFT *Paenibacillus larvae* in the vegetative stage. RIGHT *Paenibacillus larvae* spores without appendages.
Image credit: Baylor College of Medicine www.hgsc.bcm.tmc.edu

The disease disseminates rapidly through a colony and can result in significant losses in colony production and the death of the colony. The beekeeper should ensure his colonies are free of AFB to prevent reduced production and death of colonies.

It is of paramount importance that every beekeeper should know the symptoms and means of transmission of AFB and be able to recognize it in its early stages. It is vitally important to detect the disease as early as possible. Early detection will prevent the disease spreading to healthy colonies.

Points to note about AFB:

- AFB is a highly contagious brood disease.
- The causative organism can produce as many as 2.5 billion spores in each larva.
- Adult bees can carry spores without themselves becoming diseased.

- As few as 10 spores being sufficient to cause mortality to a larva one day old after egg hatching.
- The larvae die after the cell has been sealed.
- The spores are extremely resistant to extremes heat, cold and chemicals that kill most bacteria. They can remain dormant for at least 40 years in old combs, honey, wax, or in derelict hives.
- Once a colony is infected the disease will progress until most of the brood is affected, depriving the colony of new adult bees, causing it to dwindle and then to die out.
- AFB can spread quickly within an infected bee colony and is easily transmitted to healthy colonies.
- Spores can contaminate both honey and pollen.
- Once a colony is infected, it cannot be cured.

Spread of the disease

- (i) Spread within colony: The American foulbrood disease is readily spread within a colony by the spores remaining at the base of brood cells and by house bees that clean these cells, and those that feed larvae. When the house bees clean out the cells containing the dead larvae these spores are distributed throughout the hive and more and more larvae become infected.
- (ii) Spread between colonies: AFB spores can be spread from diseased to healthy colony by:
 - Transferring brood combs from one colony to another.
 - Feeding honey or pollen from infected colonies.
 - Bees robbing honey from infected hives.
 - Drifting bees.
 - Installing packaged bees from colonies infected with the disease.
 - Artificial swarms from infected colonies.
 - Swarms issuing from an infected hive may carry spores with them.
 - The introduction of queen bees from infected colonies.
 - Purchasing or using contaminated beehives and equipment.
 - Unsterilized tools may also be a source of contamination.

Pathogenesis

Infection occurs when bee larvae ingest *P. Larvae* spores in contaminated food given to them by nurse bees. The spores germinate in the larval midgut into the vegetative forms (rod stage) a day after ingestion by the larvae, becoming bacteria. The rods penetrate the gut wall entering the tissues where they proliferate rapidly and at an enormous rate, feeding at the expense of the tissues, and continuing to proliferate until larval death. New spores form after the larva dies. The infected larvae die after their cell is sealed over. When this occurs the nourishment supply of the bacteria is no longer maintained, and their growth and proliferation cease. Each bacterium then transforms itself into

spore stage. After death, the white larvae become dark brown and decay into a glue-like mass, which will rope. The decaying mass has a foul smell, hence the name foulbrood. At the final stage, within a month or so, a dead larva or pupa dries to a dark brown scale that adheres tightly to the lower side of the cell and cannot be removed by the bees. Each scale contains millions of infective spores. These spores are a potential source of infection. Once inside the larval gut again, the cycle will repeat.

Disease effects on the colony

If left alone, the disease will spread throughout the colony killing much brood. The number of young bees diminishes; and the colony becomes unable to produce offspring. Eventually, there is a decline in adult bees and the colony becomes weak and shows abnormal bee flight and dies. The death of the colony may occur at any time of the year. The disease may advance rapidly and seriously weaken then kill the colony or it may not develop to this critical fatal stage until the following year.

Signs of Infection

- Combs from an infected colony may show spotty brood (unevenly scattered empty cells). These cells, on inspection, contain scales that are a major source of infection.
- Cappings over the cells containing diseased larvae are generally moist, discoloured and sunken; some may be punctured with tiny holes where the house-cleaning bees have attempted to open them. When these cappings are removed you can expect to see the diseased larvae or pupae.
- Decaying larvae are soft, sticky and ropy when drawn out with a matchstick, which is one of its identifying characteristics.
- Dead larvae are brown or black coloured.
- Larvae that die from AFB lay in upright position after capping.
- Scales on bottom walls of open cells.
- AFB has a characteristic smell in the advanced stage.
- A pupa that has died in capped cell shows a fine threadlike tongue or mouthparts projecting in the centre of the cell.
- Honey and pollen may be stored within the brood area.

Precautions Against the Spread of American Foulbrood

Beekeepers must adapt precautionary measures to prevent their hives from contracting this disease and reduce further spread. The disease incidence can be kept as low as possible in a number of ways:

- Do not transfer brood combs between one colony and other, or divide colonies without first checking carefully for AFB symptoms.
- Do not feed bees honey or pollen from an unknown source.
- Check for AFB signs twice a year during spring and autumn when brood is present in the hive.
- Prevent robbing at all time.

- Arrange the hives in the apiary in such a way that drifting of bees is reduced to minimum.
- Replace old brood combs (3-4 per brood box) every year with frames fitted with foundation to maintain a clean and healthy brood.
- Hive swarms of unknown origin on to foundation and do not feed for 3 days. The honey contaminated carried by the bees is then consumed while the bees build new combs. Keep them isolated at a distance from other colonies and inspect them for signs of AFB at least 3 months before incorporating them into the apiary.
- Remove frames with diseased brood or scale, and burn.
- Do not purchase old combs or used beekeeping equipment as they may harbour spores for many years.
- Always disinfect second-hand hives by thoroughly scorching with a blowtorch before use.
- Do not put out extracted (wet) supers for bees to feed from.
- Sterilise the hive tool, smoker, gloves or hands with alcohol or boiling water after working on a hive you may suspect to be infected, so as not to spread the infection from colony to colony.

Diagnosis

There are two approaches to diagnosing American foulbrood in the field:

(i) The matchstick test or ropiness test: Use a matchstick or small stick and thrust it into the suspect larva or pupa, stir then withdraw it slowly with a twisting motion. If AFB is present, the larva tissues will draw out in a brown, sticky-like thread longer than 2.5 cm (an inch).

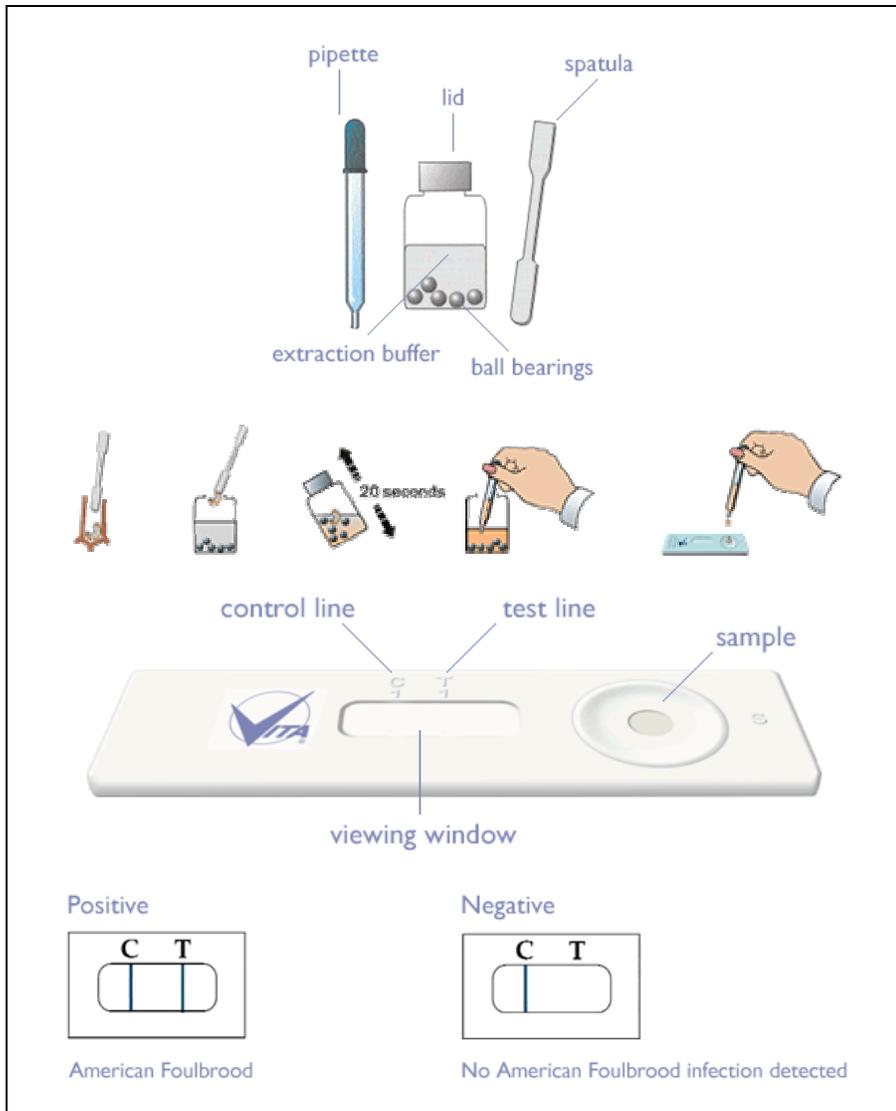
The matchstick used for the test should be burned in the smoker, as it could be heavily contaminated with AFB spores.



American foulbrood showing ropiness of dead larva.

Photo credit: Apitalia net

(ii) Vita AFB Diagnostic Kit: A sample of suspect infected larva material is placed in the buffer bottle and shaken for about 20 seconds. 2-3 drops of the resulting suspension are placed on the Test Device. After 3 minutes the control line should be visible in the viewing window of the device. The two blue lines at the C (control) and T (test) indicate a positive infection. The single line at C only indicates a negative result.



Step by step procedure for using AFA diagnostic kit to detect American foulbrood infections in honeybee larvae.

Diagram courtesy of Vita Europe Ltd

Hive inspection for AFB

If American foulbrood occurs and is not detected the infection will spread rapidly. Early detection and intervention is necessary to avoid further spread. All brood frames are examined in spring and autumn for AFB, although remains of diseased brood may be found throughout the year.

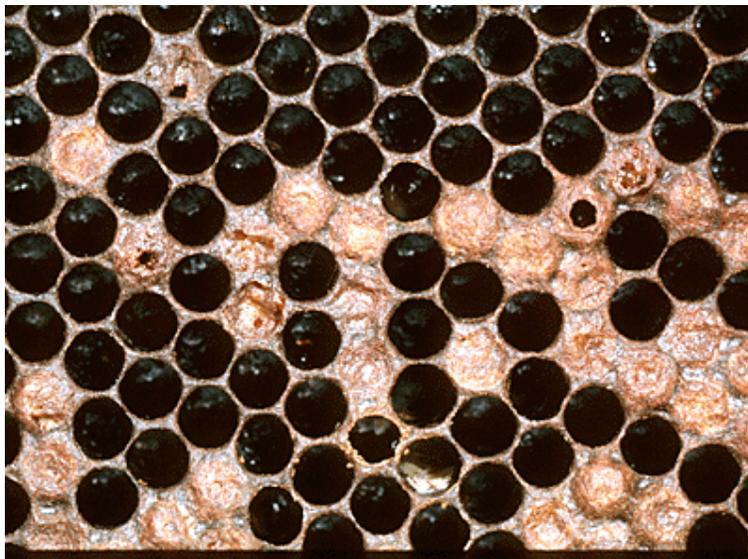
To look for AFB, shake all the bees off the brood comb to allow an unimpaired view of brood cells. Any abnormality is then easily spotted. Examine closely each comb and ensure that you examine the whole area of brood, both sealed and unsealed for brood patterns, discoloured larvae, and sunken or punctured cappings.

Open any sealed cells with abnormal cappings and examine the consistency

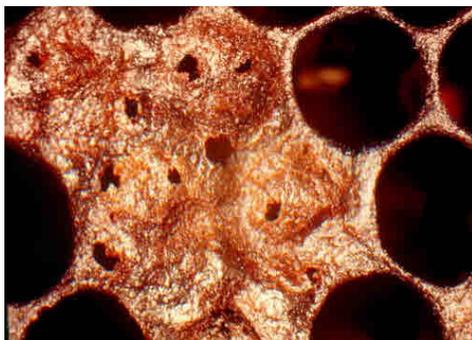
of any dead larvae by probing with a matchstick or small stick. If the soft decaying remains of the dead brood will stretch out the cell with the matchstick to a thin elastic thread between 3-4 cm (1.2 -1.6 in) long, this is almost certainly AFB.

Look for scales on the cell walls by holding the brood frame at approximately 15-20 degree angle facing the light so that the light illuminates the base of the brood cells being examined.

In a colony with light infection, only few cells may show signs of disease and the colony will appear normal. Inexperienced beekeeper may fail to notice it because the larvae die after they are sealed over. In advanced stages brood combs may show many uncapped cells mixed with capped cells, discoloured, sunken or punctured cappings.



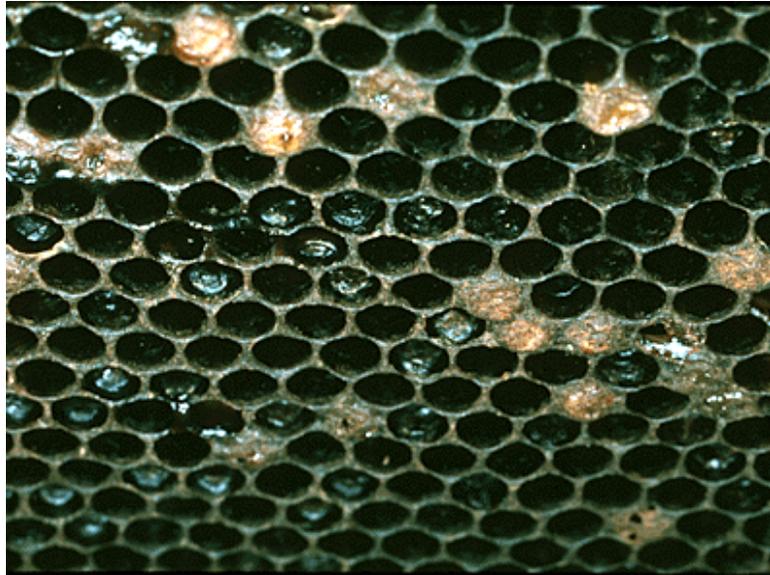
An American Foulbrood comb showing a scattered (irregular) brood pattern and sunken (concave) cell cappings. Photo credit: Maarec. Psu. edu



Punctured cell caps in AFB infected comb.
Photo credit: Prof. M.V. Smith



Dead pupa showing the prominent tongue. Photo credit: Vcely. sk



AFB scales. Photo credit: Maarec. Psu. edu

How to distinguish between AFB and EFB (European foulbrood) infected larvae

- EFB kills young larvae while they are in the coiled stage (before the cell is sealed). Thus the diseased larvae can be seen.
- Larvae infested by EFB are creamy-white or brown.
- The tracheal system is easily visible in diseased larva in the unsealed cells.
- Dead larvae have a soft and watery consistency.
- Larvae dead from EFB are twisted or melted in appearance.
- Scales do not adhere to the cell as in AFB, and larvae are easily removed from the cells.
- The dead viscous mass can be drawn out, but not as far as AFB.
- The ropiness test is the key distinguishing characteristics between EFB and AFB.



EFB-Infected larvae are twisted in various positions in the cell.

Photo credit: ent.uga.edu/bees



EFB-infected larvae showing yellow/brown discoloration and prominent tracheae.

Photo credit: melliferabees. net

Treatment and control measure

Medicating - The antibiotic terramycin (oxy-tetracycline) and currently tylosin (tylosin tartrate) are used as preventive treatment against American foulbrood. The treatment is carried out in early spring and must stop 4 weeks before the main honey flow to avoid contamination of the honey crop, and again in autumn after the honey crop is removed. Follow the label instructions on the product package for preparation of the medication.

Treatment with antibiotics does not kill the spores but serves to prevent their development when present in the larvae. The bees are then able to develop and mature normally and the colony can produce honey, but the disease will reappear when the treatment is stopped. A diseased hive being treated with antibiotic should be considered contaminated with AFB spores for good and should be therefore treated with antibiotics forever. Since antibiotic treatment is not totally effective it cannot be recommended. The repeated use of antibiotics as a preventive treatment may lead to the emergence of antibiotic-resistant bacteria, and results in residues of antibiotics in hive products.

In the UK, New Zealand and the Netherlands no drug is permitted for the treatment of AFB. The remedy is the eradication of the diseased hives by burning. This measure helps to reduce the incidence of AFB dramatically.

Shaking bees treatment - This method aims at treating an infected colony without use of antibiotics. The bees are shaken into a disease-free hive fitted with new foundation with no drawn combs. All brood and honeycombs are destroyed by fire. The contaminated honey in the transferred bees will be metabolised when they build new combs. If the nectar source is poor in the field the bees can be fed with 1:1 or 2:1 sugar syrup after 3 to 4 days, by which time they will have consumed the food in their bodies.

This method saves the bee colonies and is effective at reducing the level of spores and drug residues in honey and wax.

Control measure - The most effective way of dealing with AFB disease is to destroy any hives that are infected by burning because it can spread from that single hive to hives all around. All the combs, frames and bees are burnt.

Before burning, the bees should be killed. In the evening when all the bees have returned, the entrance of the hive should be closed securely with wads of newspaper or adhesive tape and a piece of wire screen placed over the hole in the inner cover so that no bees can escape into the space under the outer cover. About a half of a litre of petrol (1 litre for 3-4 box hives) is poured through the wire screen over the hole and the outer cover is placed. The bees will be dead after half an hour.

Note: If a ventilated floor is used, it must be replaced with a solid wood floor or closed off with a board.

A pit should be dug in the ground about 1 meter (3 ft) across and ½ meter (1.5 ft) deep. Bring the hive close to the pit and start a fire in the pit, place all the

contents of the hive - bees, combs and brood in the fire. When all has been burnt, it should be well buried with soil.

The boxes, inner cover, outer cover and bottom board are disinfected and re-used. Any wax or propolis should be scraped clean with the hive-tool and the inner surfaces scorched with a blowtorch. Scorch the surface until it is light brown and ensure that the flame reaches all the corners.

Queen excluders, feeder, the hive-tool and smoker are scrubbed with a stiff brush and hot soapy water prepared by mixing 500 g (1 pound) washing soda (sodium carbonate) with 4.5 litres (1 gallon) hot water. After the tools have been scrubbed, they should be rinsed in water before drying.

More information

Elimination of American foulbrood without the use of drugs: A Practical Manual for Beekeepers by Mark Goodwin & Cliff Van Eaton. Published by the National Beekeepers Association of New Zealand. A good reference about AFB. The book has sections on life history, symptoms, spread, inspection and diagnosis, and dealing with infected equipment and hives.