



ATTTA

Atlantic Tech Transfer
Team for Apiculture

American foulbrood (*Paenibacillus* larvae)

What is American foulbrood (AFB)?

American foulbrood (AFB) is a disease caused by the spore forming gram-positive bacterium *Paenibacillus* larvae which exists in both a spore and vegetative state^{1,2}. The disease typically affects the immature stages of honey bees (brood) and is highly contagious¹. It can infect the strongest to the weakest colony in an apiary³. Infected brood usually die at the pre-pupal or pupal stage^{1,3} with clinical symptoms seen after cell capping of the larva (> day 8). Heavy infections can affect most of the brood, severely weakening the colony and eventually killing it¹. There is no treatment for the disease, meaning that destruction of infected colonies and equipment by burning or irradiation becomes necessary^{1,2}.

Transmission

The disease can rapidly spread from colony to colony and from apiary to apiary¹. The main ways the disease spreads are through transmission of the AFB spores through bee movements, the interchange of diseased equipment or by feeding contaminated honey or pollen¹. There are additional ways that the disease can spread: unattended diseased equipment and dead colonies, which can become a source of infection that foraging bees take back to their hives, bees drifting among colonies, infected packages and infected swarms¹. All of the mentioned practices and activities can permit the spread of infectious bacterial spores. Then the infected brood is killed by the vegetative stage when the spore germinates in the larva's gut^{1,2}.

Life Cycle

An AFB infection spreads when bacteria spores in the food of young larvae are ingested¹. It only takes 10 spores fed to a one-day-old larva for it to become infected^{1,2}. Older

larvae require a higher number of spores for infection to occur^{1,2}. Larvae that are older than 48 hours are resistant to infection¹.

When spores reach the midgut of the larvae, they will germinate and begin vegetative multiplication¹. The immature bee will die once the bacteria penetrates the midgut epithelium¹. The decaying larvae often forms a dry, dark brown or black scale, which is usually an indicator a colony is infected with AFB¹. A scale can contain over 2 billion spores¹.

Worker bees will attempt to remove the scale from the hive, further spreading infection throughout the colony¹. Even though adult bees are resistant to infection, they can host spores in their digestive tracts and spread the infection to other bees and young larvae¹.

Diagnostics

The symptoms of AFB often vary between colonies and can easily be confused with other diseases. The symptoms of AFB are typically not present until brood is capped¹. All suspected AFB infections are required, by law, to be reported to the provincial apiculturist. The following are possible symptoms of AFB to look for:

- In most cases, AFB can be reliably distinguished from other honey bee brood diseases using the "ropiness" test¹. Larvae or pupae infected with AFB exhibit a characteristic ropiness when a small stick is used to stir the diseased tissues and then slowly withdrawing the stick from the cell¹. When completing the ropiness test, an infected larva or pupa will extend out to two centimetres from the cell¹. Beekeepers should always carry toothpicks with them to the bee yard to inspect any irregular looking cells. Although a strong indication of AFB infection, beekeepers must remember that this test is not 100% conclusive.



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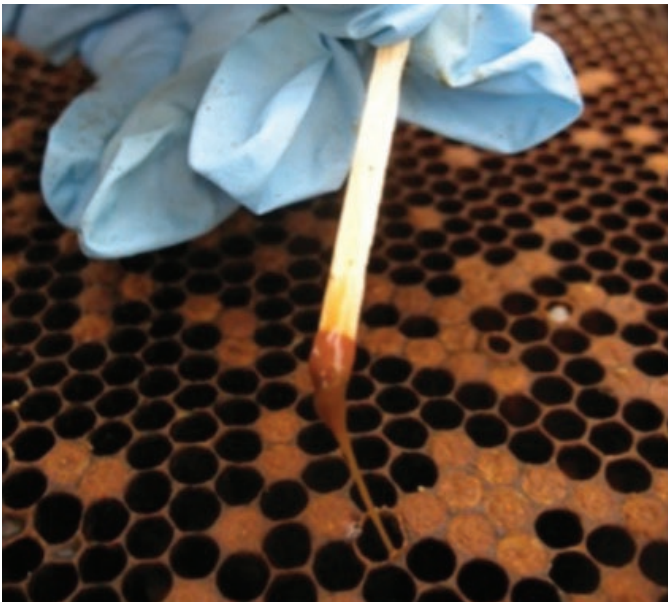


Figure 1. Performing ropiness test on American foulbrood (*Paenibacillus larvae*) infected larva or pupa (©Government of Ontario⁴).

- Presence of scales is an indicator of an AFB infection¹. The scale is usually brown or black in colour and adheres tightly to the base of the cell¹.



Figure 2. American foulbrood (*Paenibacillus larvae*) scales (©Government of Ontario⁴).

- Spotty/irregular brood pattern rather than compact².
- Perforated holes within the brood cappings¹.
- Brood cappings have a sunken and greasy appearance¹.

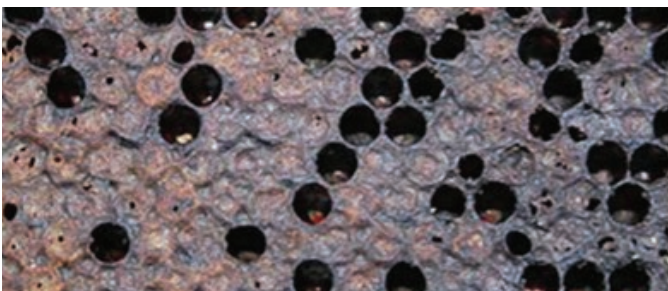


Figure 3. Sunken and perforated cappings have greasy appearance for American foulbrood (*Paenibacillus larvae*) infected brood (©Government of New South Wales⁵).

- Larva and pupa can turn from pearly white to brown².



Figure 4. American foulbrood (*Paenibacillus larvae*) infected brown coloured larva (©Government of Ontario⁴)

- Sometimes AFB can be associated with an unpleasant “fish-like” odor². This is a less reliable indicator of an AFB infection as it is subjective from beekeeper to beekeeper.
- There are laboratory techniques that can be used to confirm a suspected AFB infection¹. *Paenibacillus larvae* from an infected honey bee sample can be smeared onto a microscope slide and stained for examination¹. The spores can also be cultured on microbiological media¹. There are also molecular methods to identify the pathogen (ex. polymerase chain reaction)¹.
- Finally, beekeepers can use American Foulbrood Diagnostic Test Kits⁶. These kits can be purchased from beekeeping supply stores. The test reacts specifically to antibodies associated with the pathogen *Paenibacillus larvae*⁶.

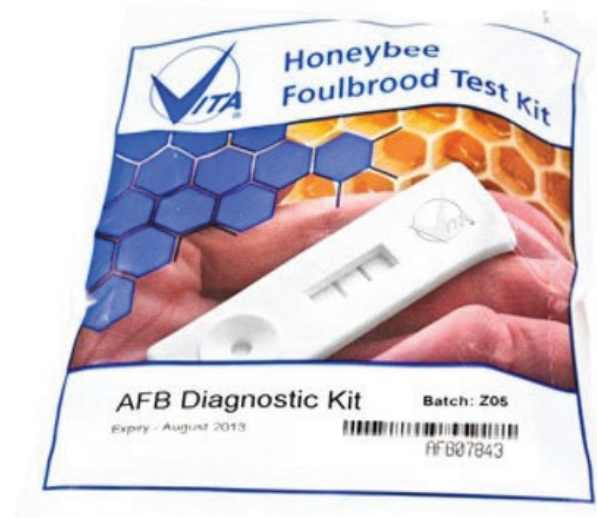


Figure 5. Vita Honey Bee Foulbrood Test Kit (©Vita Bee Health⁶).

Comparing American foulbrood to European foulbrood

There are several important differences to understand between American foulbrood and European foulbrood (*Melissococcus plutonius*) (Table 1).

Table 1. Distinguishing characteristics between American foulbrood (*Paenibacillus larvae*) and European foulbrood (*Melissococcus plutonius*).

Disease	Pathogen	Lifespan of Spores	When Symptoms Appear	Scales	Diagnostics
American foulbrood (AFB)	<i>Paenibacillus larvae</i> *Gram positive spore-forming bacteria	80 years (potentially longer) ¹	After brood capping ¹	An AFB scale is flat and adheres tightly to cell wall (difficult to remove) ¹	Ropiness test can reliably be used to help identify infection ² .
European foulbrood (EFB)	<i>Melissococcus plutonius</i> *Non-spore forming gram positive bacteria	Non-spore forming	Before brood capping ¹	An EFB scale is always easy to remove ¹	Larvae are twisted/coiled in cell, no foul smell and ropiness test does not apply ² .

Overall, AFB is a more serious and contagious disease than EFB, and the management of an AFB infection requires immediate action and reporting.

Management of American foulbrood Infection

Cultural

Beekeepers can adapt good biosecurity practices within their operation that can stop the spread of a potential AFB infection¹. Examples of good biosecurity practices include keeping all beekeeping supplies, tools and equipment within a single operation, flaming hive tools between visiting different apiaries, not catching swarms of unknown origin, feeding during dearth periods, minimizing robbing behaviour by maintaining strong and healthy colonies and protecting weaker colonies with entrance reducers.

Beekeepers should perform regular health inspections on their colonies looking for signs of AFB¹. Early detection of AFB is essential in preventing the spread of the disease throughout an entire operation and to multiple neighboring operations. As mentioned, beekeepers should carry toothpicks with them to the bee yard to inspect any irregular looking cells. If AFB is suspected within a colony the beekeeper should immediately contact their provincial apiculturist/apiarist and/or provincial bee inspector for further examination and to create a mitigation strategy.

Physical

If an AFB infection is suspected within a colony, immediately isolate that colony by reducing all entrances until the provincial apiculturist/apiarist and/or provincial bee inspector arrives. Ultimately, the department of agriculture representative will determine how to manage colonies within the operation if an AFB infection is present and the beekeeper must follow their mitigation plan.

AFB infected colonies are routinely destroyed by burning as there is no other practical way to destroy the spores of the bacterium and to stop the spread of the disease¹. Burning infected hives may be the only legislated method of control in some areas of Canada¹. Burning one infected colony does not ensure the eradication of the disease. The department

of agriculture representative will provide directions on how many colonies need to be destroyed and will follow up with additional hive inspections across the operation.

Beekeepers can also reduce levels of spores embedded in wax by regularly replacing frames (recommended every 3-4 years)¹.

Chemical

The antibiotic oxytetracycline hydrochloride does not kill AFB spores but prevents the multiplication of the vegetative form¹. There are reports of some strains of *Paenibacillus larvae* becoming resistant to Oxytetracycline^{1,2}. The use of antibiotics may be used within the AFB infection mitigation plan, with consideration that antibiotics only treat the vegetative state of the disease and antibiotics alone cannot eradicate the disease^{1,2}. It is important to remember that the use of oxytetracycline must be managed through a Veterinarian-Client-Patient Relationship (VCPR) and only a licensed veterinarian can prescribe the use of this antibiotic. Beekeepers must administer the antibiotic following the directions of their veterinarian.

There are other antibiotics approved for use in honey bees in Canada (Tylosin and Lincomycin) which also require a VCPR⁷. It is important to restrict their use as much as possible in order to preserve their efficacy⁷.

Infected Equipment

Most AFB infected equipment will be required to be destroyed by burning. Beyond the regulatory requirements, it is best practice to destroy any equipment suspected of containing AFB spores. Depending on rules and regulations within the region and risk of infection, beekeepers may be permitted to sterilize equipment using gamma radiation. Irradiation has been demonstrated to be effective at killing AFB spores at a dose of 10 kGy¹. Commercial facilities exist in specific locations in Canada that can provide irradiation treatment to equipment¹. There are less recommended ways

to sterilize equipment such as scorching equipment, the use of Sodium hypochlorite and the use of hot parafilm, but these methods are not recommended due to the uncertainty in their effectiveness of killing spores and potential hazards to beekeepers^{1,2}.

Other Products

In Fall 2023, the Canadian Food Inspection Agency (CFIA) conditionally licensed a product developed by Dalan Animal Health which contains inactive *Paenibacillus* larvae and has been demonstrated to provide increased immunity to AFB infections⁸. The product must be provided under licensed veterinarian supervision. To deliver the product, the inactive bacterium is fed to worker bees and their secretions are fed to the queen⁸. Then the next generation of developing bees produced from the queen have increased immunity to AFB⁸. The research trials, funded by Dalan Animal Health, showed between 30% to 50% decrease in AFB infection among colonies that received the product compared to colonies that received the placebo product⁸. As a new product, efficacy is still largely unproven by repeated research trials or through beekeeping practices.



Figure 6. *Paenibacillus larvae* Bacterin (©Dalan Animal Health⁸).

American foulbrood prevalence and detection in Canada

AFB is a listed disease in all provinces of Canada and is an annually reportable disease at the federal level to the CFIA⁷. All provinces have control programs in place to eradicate and/or treat colonies with AFB infections⁷. Most provinces require the destruction of symptomatic AFB colonies and may recommend treating the remaining colonies with antibiotics⁷.

AFB has been detected in up to nine of 10 provinces in Canada in the past years (it is absent in Newfoundland and Labrador)^{7,9}. In certain provinces, if clinical signs are observed, bacteriological testing, sometimes followed with resistance testing, may be conducted⁷. Results of provincial inspections are reported annually in the Canadian Association of Professional Apiculturists reports. To view these reports visit: <https://capabees.com/capa-statement-on-honey-bees/>⁹. Additionally, in 2016 and 2017 Canadian National Honey Bee Health Surveys were conducted which also reported on AFB detected across Canada^{10,11}.

For additional information or potential questions on American foulbrood management in Atlantic Canada, please reach out to the Atlantic Tech Transfer Team for Apiculture at attna@perennia.ca

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