the veterinarian's role in honey bee health

HONEY BEES: A GUIDE FOR VETERINARIANS

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INTRODUCTION

Honey bees weren't on veterinarians' radars until the U.S. Food and Drug Administration issued a final Veterinary Feed Directive (VFD) rule, effective January 1, 2017, that classifies honey bees as livestock and places them under the provisions of the VFD. As a result of that rule and changes in the FDA's policy on medically important antimicrobials, honey bees now fall into the veterinarians' purview, and veterinarians need to know about their care.

This guide, available to AVMA members, provides basic knowledge to allow veterinarians to better communicate with beekeepers and serve the needs of these unique patients. The guide includes sections on basic bee and beekeeping terminology and equipment; beehive inspection procedures (including indicators of honey bee health and disease); and relevant honey bee diseases and conditions.

This guide is not meant to provide in-depth coverage of honey bee diseases and conditions, nor is it meant to provide instruction regarding beekeeping. This information was prepared with assistance from the Honeybee VFD Subcommittee of the AVMA's Food Safety Advisory Committee and is provided for informational purposes only. This information has not been approved by the AVMA Board of Directors or the House of Delegates, and it is not to be construed as AVMA policy nor as a definitive statement on the subject, but rather to serve as a resource providing practical information for veterinarians.

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HONEY BEES AND VETERINARIANS

In veterinary school you were taught how to diagnose and treat just about every animal species, but we doubt you had much education – if any – on honey bees. Until the recent changes in labeling and availability of medically important antibiotics, veterinarians in the United States had less involvement in apiculture (beekeeping) and honey bee medicine. As a result of the Veterinary Feed Directive (VFD) final rule and changes in the FDA's policy on medically important antimicrobials, however, honey bees now fall into the veterinarians' purview, and you may need to know about their care.

Honey bees are classified as livestock/food-producing animals by the federal government because a number of products from apiculture enter the human food chain including honey, propolis, pollen, and royal jelly. The requirements for completing a VFD order or prescription for honey bees are the same as for any other food-producing animal.

The federal rule restricts a beekeeper from using any medically important antibiotics (outlined in FDA guidance 152 appendix A) in their honey bees unless they have an either a VFD order or a prescription from a veterinarian. For this VFD order or prescription to be lawful, the veterinarian and the beekeeper must have a valid Veterinarian-Client Patient Relationship (VCPR). A valid veterinarian-client-patient relationship according to the federal definition is one in which the following conditions are met:

1. A veterinarian has assumed the responsibility for making medical judgments regarding the health of (an) animal(s) and the need for medical treatment, and the client (the owner of the animal or animals or other caretaker) has agreed to follow the instructions of the veterinarian;
2. There is sufficient knowledge of the animal(s) by the veterinarian to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s); and
3. The practicing veterinarian is readily available for follow-up in case of adverse reactions or failure of the regimen of therapy. Such a relationship can exist only when the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s) by virtue of examination of the animal(s), and/or by medically appropriate and timely visits to the premises where the animal(s) are kept.

Some states have additional VCPR requirements. To determine what constitutes as a valid VCPR for VFDs in your state, visit the FDA listing of VCPR requirement by state, and visit AVMA's guidelines for VCPRs related to prescriptions and other treatments.

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HONEY BEE BASICS AND TERMINOLOGY

All honey bees in the United States are the same species, the European/Western honey bee (*Apis mellifera*). Honey bees operate as a super organism (colony), made up of individual organisms (bees). All of the functions of the colony occur within a cavity like hive, on multiple sheets of comb, which is made up of a series of **cells**. Within the colony, there are three types of honey bees based on their function:

Queen

Each colony generally contains one queen. The queen is the fertile female of the hive and is the sole source of fertilized eggs that become worker bees, laying up to 2,000 eggs per day during peak production. The queen is the largest bee in the hive, approximately twice the length of a worker bee, and longer than the drones, with a more tapered abdomen. A colony will only produce new queens when it prepares to reproductively split by swarming, when the old queen has died, or to replace a failing queen. Many queen cells will be created, and the first one to emerge will kill the remainder, and will fight with other emerged virgin queens so that only one remains. One to two weeks after hatching the virgin queen will go on several mating flights where she will mate with 10-20 drones, storing the sperm for use over her lifetime. A beekeeper may '**requeen**' or replace a queen to introduce a younger or better performing queen. A colony can only function normally when a queen is present and laying well. A colony is considered "**queenright**" when a queen is present, and "**queenless**" in the absence of a queen.

Drones

Drones are the only male bees in the hive and are haploid (having only one chromosome set) because they arise from unfertilized eggs (queens and workers are diploid because they arise from fertilized eggs). Drones have large, thick bodies – larger than those of the worker bees, but shorter and thicker than that of the queen. Drones perform no functions inside the hive; their sole duty is to search for and mate with virgin queen bees on their mating flights. These flights occur far from their hives. If a drone is fortunate enough to mate, his endophallus is removed in the process and the drone dies. Drones are made whenever the colony has sufficient resources; in the summer, a strong colony can have hundreds of drones, but they are all kicked out of the colony before the winter so they don't consume precious resources.

Workers

Workers are female bees that perform the vital work of the colony. Worker bees have a variety of functions such as providing for the queen's needs; cleaning cells in the comb, nursing larvae, producing wax and forming it into honey comb, guarding and defending the hive, removing dead bees from the hive, cooling the hive or heating the brood, carrying water, gathering and transporting pollen, gathering propolis and using it to coat the hive, collecting nectar, sealing honey, and scouting for resources. Worker bees are incapable of laying fertilized

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eggs that can become queens or other worker bees; they are only capable of laying unfertilized eggs that become drones, but this ability to lay even unfertilized eggs is suppressed in the presence of a laying queen. The lifespan of a worker bee varies with the time of year: they may live only 5-6 weeks during the spring and summer, and may live five months or longer during the inactive winter period.

Image 1: Side-by-side comparison of a worker bee, queen, and drone.

The term **brood** refers to the young, developing bees - the **eggs**, **larvae** and **pupae.** Bees at this stage remain stationary within the cells of the comb. All bees begin their lives as eggs laid in cells in the colony. After 3 days the egg hatches, and a larva emerges. The larvae are fed and grow over the next 6 days, after which the cell containing the larva is capped (the open top is sealed over by worker bees with porous wax). The larva then matures to a pupa inside the capped cell (***capped brood)*** and eventually emerges from the cell as a bee. The total time spent as brood is 16 days for queens, 21 for workers, and 24 days for drones. A colony will contain brood during most of the year. Egg laying ceases in late fall or early winter and in times of stress.

The diet of honey bees is entirely from flowers. Floral nectar provides a source of carbohydrates. Bees prefer to eat fresh nectar when available, but they will store it in cells for when there are no available flowers. To prevent fermentation, the bees dry the nectar to below 18% water content, which then is referred to as honey. Pollen provides a source of protein, vitamins, fats, and minerals. To store pollen, bees will pack it into cells, add nectar, and ferment the pollen into a storable substance called bee bread.

A typical honey bee colony consists of one queen, hundreds of drones (in summer), and tens of thousands of workers. A healthy colony is one where there the queen is laying enough eggs and the workers can raise enough brood to replace the workers that are dying, and there are enough members of each age of worker to perform all the necessary tasks of the colony.

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BEEKEEPING EQUIPMENT AND TERMINOLOGY

Honey bee products

Although honey is the most widely known product of a honey bee hive, there are a number of other products that can be harvested and sold from the hive: beeswax, propolis, royal jelly, pollen, and honey comb. Many beekeepers also use their colonies to produce bees for sale: queens are sold to re-queen a queenless hive or replace a failing queen; while nucs (nucleus colonies: three to six frames from a hive with workers, brood, honey, pollen, and a queen), packages (2 or 3 pounds of bees with a laying queen), and splits (a portion of the original hive) are used to start new colonies.

The hive

Honey bees are cavity dwellers, commonly living in hollowed out trees in the wild. Honey bee hives all have the same basic characteristics: a dark cavity over 40 L volume, a small entrance, and movable frames where the bees can construct honey comb. Although there are several types of hives used, the Langstroth style hive is the most common in the United States. A typical Langstroth hive consists of the following components: a bottom board (which may be solid or screened); a series of boxes or 'supers'; and a lid. There are many variations on the standard hive. The boxes come in different widths (8- or 10-frame), and heights (deep, medium, or shallow). Generally the colony lives in the bottom boxes (the brood nest), and excess honey is stored in the upper boxes (honey supers).

Images 2 and 3: Langstroth hives. Photo © Randy Oliver, ScientificBeekeeping.com

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Image 4: Langstroth hive with top super separated from bottom super.
Photo © Randy Oliver, ScientificBeekeeping.com

All hives must have removable frames to allow for inspection. In image 5 below, one **frame** has been removed from an 8-frame super (hive box); the **foundation** is the central portion framed by the wooden frame. The foundation is a flat piece of beeswax or plastic with the outline of cells embossed into it. The bees will create comb using the foundation as a base, a process beekeepers call '**drawing out'.**

Image 5: Unused frame with foundation. Photo © Jason Morgan, allmorgan.com

Image 6: Frame inspection. Photo © Randy Oliver, ScientificBeekeeping.com

Beekeeping tools

The main tool used by beekeepers is the **hive tool** (image 7), a pry bar used to pry open the lid, separate boxes, and separate and frames for removal. A soft **bee brush** (image 8) may be used to gently brush bees from the removed frame to allow examination of the underlying brood. A **smoker** is used to move bees away from areas to be examined and to calm the bees.

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Image 7:The hive tool (yellow) has been used to safely separate frames so an individual frame can be removed without harming the bees. Photo © Randy Oliver, ScientificBeekeeping.com

Image 8: Bee brush in use to gently remove bees from frame to facilitate visual inspection. Photo © Randy Oliver, ScientificBeekeeping.com

Protection

Protection worn by beekeepers is variable, and may include coveralls (secured at wrists and leg hem); gloves; and a screened head cover with veil. Some experienced beekeepers don't wear any protective equipment when handling their colonies.

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HONEY BEE HIVE INSPECTION

Hygiene

When working with a beekeeper, use only their equipment to prevent the transmission of disease between apiaries. The beekeeper should provide a smoker and hive tool, though you should have your own protective gear. Wear disposable nitrile gloves as these will prevent most stings and can be disposed of after use. When you are finished inspecting an apiary, make sure all wax, propolis and honey is removed from your equipment, protective clothing and self. The best way to remove propolis is with the use of denatured alcohol.

Inspecting the records

Just as a good history can set the stage for a successful veterinary visit for pets or large animals, it can also provide clues regarding the colony's overall health and potential problems. Look over the hive records for patterns or sudden changes. If the beekeeper does not have any records, encourage them to keep notes in a journal, on a calendar, or in one of a number of computer applications available. Common records include the following information:

- Age and source of colony and queen
- Is the queen marked, and has she been recently replaced?
- Recent actions by the beekeeper (treatments, adding equipment, removing honey, feeding)
- Changes in size or activity of hive
- Signs of pests or pathogens, including monitoring for the varroa mite

Examining the hives

Always let the beekeeper direct you through the bee yard and allow them to handle the hives themselves. Handling bees is a skill that takes many years to develop, and poor handling of a hive increases the chance that you will damage the colony or agitate the bees, making them less safe to work with in the future.

As with any good physical examination of an animal, the hive exam starts with an external general evaluation. While there will be a lot of variation naturally between hives, some external factors can provide clues to colony health. The activity at the hive entrance is dependent on the size of the colony, the temperature, time of year, time of day, and the food resources that are available. A healthy hive should have bees flying in and out of the entrance in good weather, and most hives in an apiary should have generally the same level of activity. Signs of disease or health issues from outside the colony include large amounts of dead bees in front of a hive, or bees that are crawling or trembling on the landing board or on the ground in front of the hive. A weak or sick colony can also get robbed, where neighboring bees steal the resources. Signs of robbing include a much higher rate of activity at the entrance or other holes, fighting bees, and the presence of yellow jackets or other species getting into the hive. Compare hive activity to

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others in the same yard, keeping in mind that lower activity does not necessarily indicate a problem. The beekeeper may have information about the colony status that would explain lower activity including a recent requeening or split.

When you are near the hive, avoid swatting or agitating the bees. Bees will not be interested in stinging you unless you agitate them or injure or kill a bee. Try not to block the entrance to the hive, as you increase the chances of problems with the bees by blocking their path of travel. Always stand off to the side, or behind the hive, and move slowly.

Allow the beekeeper to perform their usual practices to open the hive. Once the hive is open, note the location of and general size of the colony, known as the **cluster size**. Cluster size is generally estimated in 'frames of bees,' where a frame is counted if both sides of the frame is covered with bees. The cluster should generally fill the boxes, and should be similar for hives that are managed the same way. A small cluster may indicate a health issue, while lack of a well-defined cluster may indicate a queenless colony, or that too much smoke was used.

You will need to see into the brood nest to identify if the colony is healthy. The beekeeper will remove any honey supers that are present to allow you to go down into the lower part of the hive. Ask the beekeeper to remove as many frames as necessary to get a good assessment of brood quality. The presence of eggs, larvae, and capped brood indicate a healthy queen and colony. Look at the ***color, consistency, and pattern*** of the stages in the frame.

Note that the cells of the comb are not flat from front to back; they slope downward toward the foundation, as shown in Image 9. To fully inspect the cells, hold the frame at the top bar and angle it slightly to allow you to view the base of the cells. It is often necessary to view the cells with the sun over your shoulder (face directly towards the shadow of your head) or to use a flashlight to see the bottom of the cells and to view eggs. In order to inspect brood, hold the frame perpendicular to your line of sight.

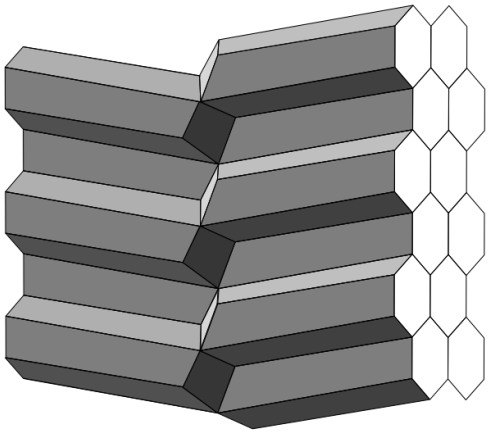

Image 9: Three-dimensional depiction of the cells arranged in comb. Note the downward slope towards the bottom of the cell.

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SIGNS OF HONEY BEE HEALTH

It is important to know what a healthy, normal colony looks like to be able to recognize abnormal colony states.

Adult bee health

As you work through the hive, look at the adult bees. They should be large and uniform. Signs of disease in adult bees include stunted growth; shiny, hairless bodies; deformed wings, or wings held out at odd angles; or weak, trembling behavior. The workers should be highly active, and moving throughout the hive.

Image 10: Healthy adult bees walking along the tops of frames.
Photo © Sarah Scott, USGS

Brood health

Healthy brood should be very consistent in both appearance and position on the frame. In a healthy colony, brood around the same age are near each other with few gaps. Disease or issues are indicated by gaps or inconsistency in the appearance of the brood. Generally, the queen will lay in concentric circles – brood of the same age will be in rings radiating from the center of the frame.

Normal eggs are white in color and upright in the cell. They are attached to the center bottom of the cell. Abnormal positioning of the eggs, including multiple eggs per cell can indicate that the queen is absent and workers have started to lay drones, a terminal condition for the colony. In Image 11, the white eggs are clearly visible against the black of the foundation. Eggs may be difficult to see, so lighting is critical. Some beekeepers will use magnification to see eggs and very young larvae.

Image 11: Healthy eggs, laid by a queen. Note the uniform positioning of a single egg near the center of the cell.
Photo © Randy Oliver, ScientificBeekeeping.com

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Healthy larvae are pearly white in color, plump and glistening, and form a “C” shape in the cell. In image 12, the larvae look healthy because they are white, uniform and lying in large pools of royal jelly. When the larvae first emerge from the egg, they are about the size of the egg and lie in a pool of royal jelly. The larva grows quickly, nearly filling the bottom of the cell before it is capped.

Image 12: Healthy eggs (right) and larvae (left). Photo by Waugsberg, wikicommons

Normal, healthy capped brood (pupae) has a soft, papery looking capping and can that can change from yellow to brow as they age and as the frame is reused. Worker brood is flat to slightly raised, while drone brood tends to be raised (bullet shaped). The capped brood should be very consistent, with many of the same age all together, with few spaces in between cells. A small amount of patchiness (inconsistency) is acceptable, but spotty, “shotgun” patterns and heavy patchiness indicate a brood problem.

Image 13: A frame of healthy capped worker brood. Note the consistent, even appearance.
Photo © Sarah Scott, USGS

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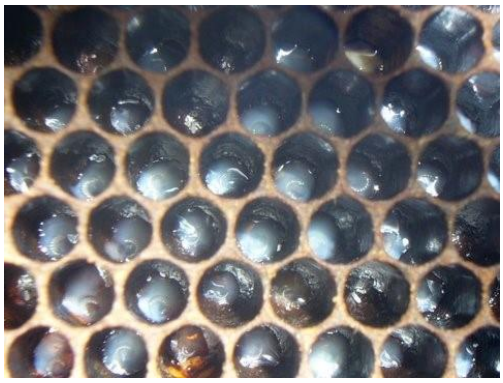
Nutritional Assessment

Colony health is strongly associated with its nutritional status, which is predominately a function of the amount and quality of incoming pollen. Check to see whether there is a band of stored pollen in an arc above and to the sides of the brood (image 14). A 1" arc of bee bread of multiple colors is optimal; a narrower arc, or bee bread of only one color, may indicate a food dearth. A colony should have excess stored nectar/honey and pollen at all times.

Image 14: A healthy frame of brood with ample stored food. Capped honey is visible in the upper left of the photo. A band of stored pollen from various sources separates the honey from the larvae (lower right). Various ages of larvae are seen, with healthy capped pupae in lower right. Photo © Sarah Scott, USGS

The easiest and most direct assessment of nutritional status is to see how much jelly the workers are placing around young larvae.



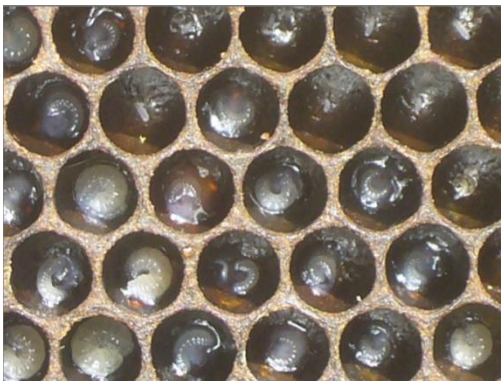


Image 15: Well-fed larvae, as indicated by the ample royal jelly covering the entire bottom of the cell. This colony will likely be in good health. Photo © Randy Oliver, ScientificBeekeeping.com

Image 16: Poorly-fed larvae, as shown by the dry bottoms of the cells (the nurse bees are forced to restrict the amount of jelly being fed). This colony is suffering from nutritional stress, and is more susceptible to pathogens. Photo © Randy Oliver, ScientificBeekeeping.com

It is generally not necessary to find the queen to identify disease within the colony. The presence of a healthy queen can be determined by identifying well laid eggs. If there are no eggs present, cells contain multiple eggs, or if queen cells are present, there may be an issue with the queen.

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Queen cells are immediately recognizable because of their size and perpendicular orientation to the ground (they point towards the earth, while worker and drone brood lie horizontally/ parallel to the ground). Queen cells may indicate that there is an issue with the current queen; that the queen was killed and is being replaced; that the colony is large/crowded and will soon swarm; or they may indicate nothing – sometimes the bees make them, and later tear them down. Queen cups, which look like wax bowls facing down, are not the same as queen cells. Cups are present in the hive throughout the year and are the base for queen cells.

Image 17: Queen cells. Note the downward-pointing, large cells that are often said to resemble peanut shells.
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HONEY BEE DISEASES AND CONDITIONS

Honey bees can be host to a variety of bacterial, viral, fungal, and microsporidial diseases. Only two honey bee diseases, however, are commonly treated with antibiotics, therefore requiring veterinary oversight: European foulbrood (EFB) and American foulbrood (AFB). It is helpful for veterinarians to have a basic understanding of common honey bee diseases, and to recognize them, even if antibiotics are not indicated. It is also important to keep in mind that a colony can have multiple diseases at a time, so identification of key symptoms is vital. With all diseases, it is important to identify how heavily infected a colony is, and if the condition is worsening or getting better. To record disease severity, record the percentage of the brood demonstrating signs of disease. As with any animal, it is common for a colony to have a slight infection that it clears on its own, and slight infections of some diseases are not necessarily cause for concern.

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  - Overly hygienic hive
  - Drone-laying queen
  - Laying Worker
  - Colony Collapse Disorder

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BACTERIAL DISEASES: AMERICAN AND EUROPEAN FOULBROOD

American and European foulbrood are two significant bacterial honey bee diseases that may require veterinary intervention. Both diseases have worldwide distribution, and are commonly treated with antibiotics. The “foulbrood” name originated due to the foul smell arising from the decay of the infected brood, but American and European foulbrood are not closely related. Because these are the two known bacterial infections, these are likely the only two diseases that currently require consultation from a veterinarian.

American foulbrood (AFB)
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European foulbrood (EFB)
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AMERICAN FOULBROOD

American foulbrood (AFB) is caused by *Paenibacillus larvae*, a spore-forming bacteria that generally only affects the pre-pupal and pupal stages of development. The vegetative, infective state of the bacterium is susceptible to oxytetracycline, lincomycin, and tylosin. The spores formed by the bacterium are not affected by antibiotics, are resistant to temperature changes and chemicals, and can live in honey and persist in the environment for up to 70 years.

American foulbrood is a reportable disease in some states. Make sure you are aware of the regulations for AFB in your state (check with your state department of agriculture) before you begin working with beekeepers.

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AMERICAN FOULBROOD DIAGNOSIS: VISUAL INSPECTION FINDINGS

Not all signs need to be present to identify American foulbrood, and some signs can overlap with other diseases.

Foul odor - The odor of AFB is distinctive—something between decomposing adult bees and old gym socks. European foulbrood (EFB) is either odorless or produces a sour milk-like smell. Some other unidentified diseases can also have strong smells, but none the same as AFB. A beekeeper with a good nose can easily smell AFB a few feet away from the colony entrance. A colony with a small infection may not have a noticeable odor.

Shotgun brood pattern - The first sign consistently observed with any disease affecting the brood is a ***spotty (or "shotgun") pattern*** of the brood cells. This is indicative of brood disease, but is not pathognomonic for foulbrood. This indicates that brood are dying before they are capped.

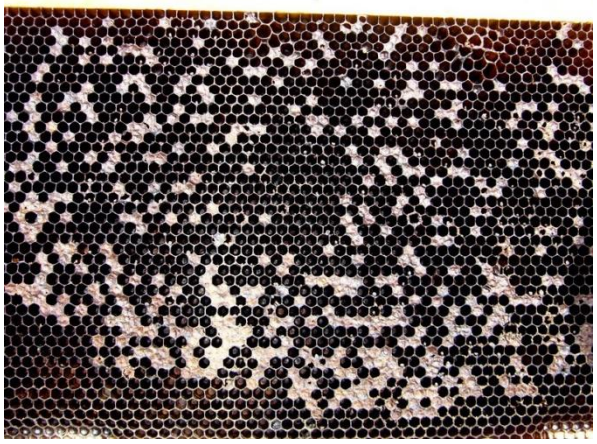

Image 18: A "shotgun" brood pattern typical of American foulbrood. This frame came from a colony with an advanced case of the disease. Photo © Randy Oliver, ScientificBeekeeping.com

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Perforated caps - AFB-affected cells develop sunken, discolored caps. The caps develop a sunken, dark and "greasy" appearance as they liquefy (see center of image 19). Perforated caps can be distinguished from incomplete caps (where the bees are in the process of capping over larvae for normal development) by appearance and location of the hole. Healthy, incomplete caps have a clean-edged, central defect that will be filled if inspected again later, while perforations have irregular edges and may or may not be centrally located.

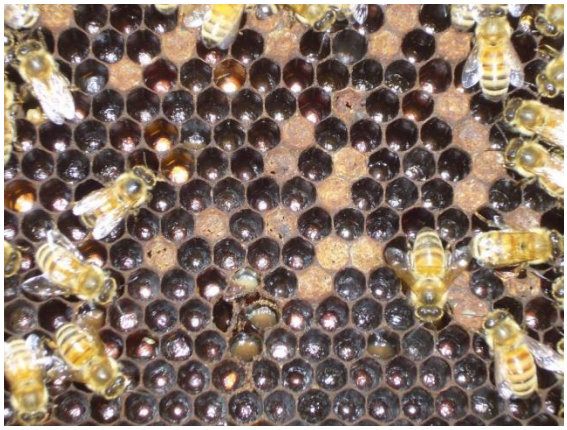

Image 19: Brood exhibiting typical signs of AFB, including the off-center perforations and distinctively-colored melted propupae. The caramel coloring of the melted brood (upper center of photo) is specific to AFB. Photo © Randy Oliver, ScientificBeekeeping.com

Larval scale - As AFB-infected larvae die, they tend to melt flat against the bottom wall of the cell (towards the ground). As they dry, they can form a scale that is visible at the bottom of the cells. Other diseases such as EFB form scales, but AFB scales are much harder to dislodge; removal of AFB scales often results in damage to or destruction of the honey comb. To inspect for signs of AFB scale, hold the frame horizontally.

Image 20: Another presentation of AFB, with propupal segments apparent. Note the melted appearance along the wall of the cell. Photo © Randy Oliver, ScientificBeekeeping.com

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Image 21: Inspecting a frame for AFB scale, looking at the bottom wall of the cell by holding the frame with the top bar towards you. Photo © Randy Oliver, ScientificBeekeeping.com

Image 22: Typical AFB infection. Note the black, raised scales lining the bottom wall of several cells, as well as the sunken, perforate caps. Photo © Randy Oliver, ScientificBeekeeping.com

Pupal tongues – AFB kills young bees at a specific developmental stage, and they sometimes die in a characteristic manner, with the developing proboscis exposed, referred to as the 'pupal tongue', Image 23. The presence of *pupal tongues* is characteristic of AFB, but the absence of pupal tongues does not rule out the disease.

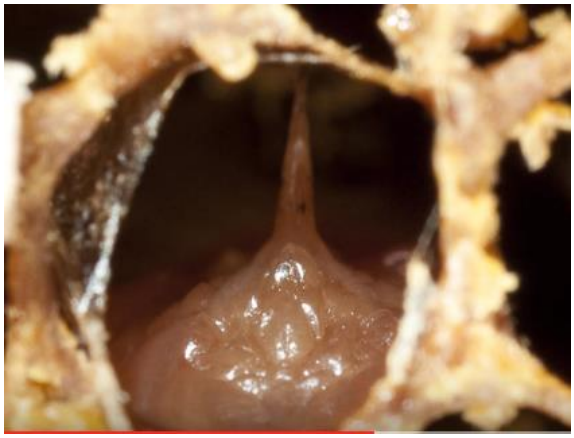

Image 23: Deceased pupae demonstrating characteristic pupal tongue
Image source: The Management Agency, National American Foulbrood Pest Management Plan, New Zealand)

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Next: AFB Diagnosis, Field Tests

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AMERICAN FOULBROOD DIAGNOSIS: FIELD TESTS

Matchstick/ rope test - A *positive rope test* (also called matchstick test) is characteristic of AFB. To perform the test, insert a matchstick, toothpick or similar object into a cell with a discolored, oozing cell cap and then slowly pull it out. The decaying products in the cell will form a viscous string from the tool, and will rope out 2 cm or more, as shown in Images 24 and 25. A negative rope test does not rule out AFB, because the larvae must be in an appropriate stage of decay. A positive rope test is characteristic of only AFB. Other diseased larvae may look similar, but will rope only slightly or be removed as a blob, and will not string to 2cm.

Image 24: Typical AFB "rope" being drawn out with a dry pine needle. Note the viscosity of the ropey pupae, as well as the distinctive caramel color. Photo © Randy Oliver, ScientificBeekeeping.com

Image 25: Positive match stick test, indicating AFB infection. Note the length of the rope from the frame to the matchstick. Diseases other than AFB may pull out, but will not reach past 2 cm as shown here. Photo © Randy Oliver, ScientificBeekeeping.com

The Holst milk test - The Holst milk test can also suggest the presence of AFB. Use two test tubes of highly diluted milk. Add a scale, or infected larvae (or the contents from the rope test) removed from an affected cell to one of the tubes (the other tube serves as a control). Incubate both tubes in your pocket or a warm cup of water for 10-20 minutes, occasionally stirring/shaking both tubes. If the opaque milky fluid in the diseased tube changes to a transparent, brownish fluid, as shown in Image 26, this is suggestive of AFB. As with the other tests described above, a negative test does not rule out AFB, because the larvae must be in the appropriate stage to get a positive result.

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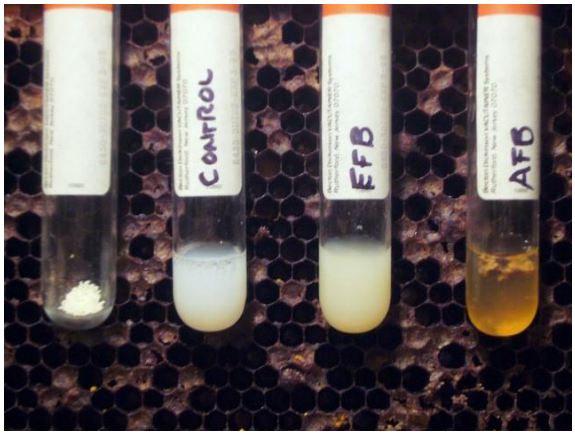

Image 26: Results of the Holst Milk Test over an AFB-infected comb. Although powdered milk will work, it is easier to dilute 2 drops of liquid milk in water. Quicker results will occur if diluted to less than the shown concentration (more transparent). Photo © Randy Oliver, ScientificBeekeeping.com

Field ELISA test - Vita Europe makes an AFB diagnostic test kit, available in the U.S. from most bee supply companies. A vet can carry these kits to the field.

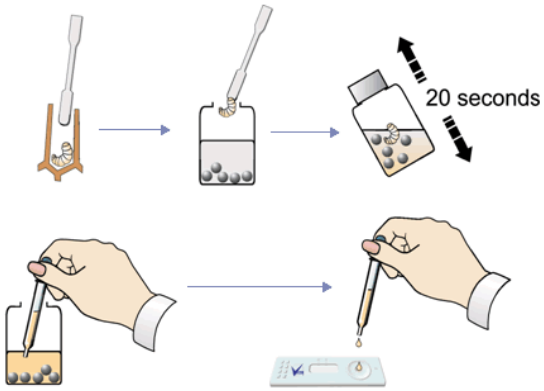

Image 27: Visual instructions for Vita Europe AFB field diagnostic kit, ©Vita Europe

Suspect American foulbrood if the following signs are present:

- Shotgun/patchy brood pattern
- Dying pre-pupae or pupae melting against cell wall
- Capped cells are sunken, discolored, and perforated
- Brown liquid at bottom of cells or oozing out of cap.
- Hard-to-remove scales found on bottom of cells
- Can have foul-smelling odor (also seen with EFB)
- Presence of pupal tongues
- Dying larvae or rope caramel colored
- Positive rope test
- Positive Holst milk test
- Positive field ELISA test

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Next: AFB, Laboratory Testing and Reporting

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AMERICAN FOULBROOD DIAGNOSIS: LABORATORY TESTING AND REPORTING

Samples should be sent to the USDA Agricultural Research Service (USDA-ARS) laboratory in Beltsville, Maryland.

How to send brood samples:

- Comb sample should be at least 2 x 2 inches and contain as much of the dead or discolored brood as possible. NO HONEY SHOULD BE PRESENT IN THE SAMPLE.
- The comb can be sent in a paper bag or loosely wrapped in a paper towel, newspaper, etc. and sent in a heavy cardboard box. AVOID wrappings such as plastic, aluminum foil, waxed paper, tin, glass, etc. because they promote decomposition and the growth of mold.
- If a comb cannot be sent, the probe used to examine a diseased larva in the cell may contain enough material for tests. The probe can be wrapped in paper and sent to the laboratory in an envelope.

Even if you are certain of your diagnosis for AFB, and you are practicing in a state without a mandatory reporting requirement, it is important to provide accurate national incidence data. See the USDA-ARS site for more details on specimen submission.

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Next: AFB, Treatment

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AMERICAN FOULBROOD: TREATMENT

In many states, AFB may be considered a reportable disease, and state apiary law may require destruction of infected colonies. Make sure you communicate with your state apiarist and are aware of state laws regarding American Foulbrood.

Three types of antibiotics are FDA approved to control American foulbrood: oxytetracycline, tylosin, and lincomycin. Oxytetracycline-resistant strains exist, and resistance can be identified by the national laboratories. These antibiotics are not effective against the spores, and their use is not sufficient action against active infection. While antibiotic therapy can be used to prevent the infection from worsening and spreading, further action must be taken to prevent reinfection by the spores remaining on the hive equipment.

Treatment for AFB should occur if even a single infected cell is identified in a colony. Carefully inspect all other colonies in that apiary, being extra cautious about cleaning hive tools and switching gloves as you pass from one hive to another.

Many states require that a colony diagnosed with AFB be destroyed immediately; veterinarians should familiarize themselves and comply with their state's regulations regarding hive destruction for AFB control. The most common method of destruction is burning the hive, but gamma irradiation will also kill AFB spores and may be an available option.

Even if burning the hive is not required in your state, it is recommended because it can fully halt infection and prevent spread of the spores. In areas that do not allow burning or where it would be imprudent to burn a hive, it may be necessary to have the bees killed and the hive boxes put in bags, and then transported as a unit to where they can be burned. Polystyrene and plastic frames may pose environmental risks when burned, and deep burial or irradiation may be indicated.

In states where burning of the affected hive is not required, the frames with affected brood should be burned, but the bees and boxes can be saved with treatment. Shake the bees onto clean/new equipment, burn the old frames, and sterilize the boxes. Treat the colony and all other colonies in that yard with antibiotics.

Once AFB is diagnosed, hive entrances should be screened and any cracks, holes or other egress points should be sealed with tape; this may be more easily accomplished during periods of low bee activity, such as nighttime, rain, colder temperatures, and very early in the morning. Prior to burning the hive, the bees should be depopulated using methods that comply with all regulatory requirements.

Practice fire safety if a colony is to be burned: remember to call utilities before digging a pit in which to place and burn the hive(s); consider calling your local fire department to notify them of

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the planned burn; do not burn near buildings, vehicles, or flammable/combustible materials; do not burn on a windy day; and take other measures as needed for your individual situation. You may be directed to cover the ashes completely with soil. Check with the authorities in your area to ensure proper destruction and site management.

Image 28: A colony being burned and buried after a positive AFB diagnosis. Because of the severity of this disease, this is the required response in some states. In states where burning is not required, it is recommended that the infected equipment is burned, but the bees can be transferred to new, uninfected equipment and treated with antibiotics.
Photo © Randy Oliver, ScientificBeekeeping.com

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Next: Honey bee drug flowchart

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Drug	Amount	Indications	Limitations	Status
Tylosin tartrate (Tylan®soluble, Tylovet®) soluble powder	Mix 200mg in 20g confectioners'/powdered sugar. **Use immediately**. Apply (dust) this mixture over the top bars of the brood chamber once weekly for 3 weeks.	AFB	This drug should only be fed in the early spring or fall and consumed by the bees before the main honey flow begins, to avoid contamination of production honey. Complete treatments at least 4 weeks before main honey flow.	Prescription
Lincomycin powder (Lincomix®)	Mix 100mg with 20g confectioners'/powdered sugar and dust over the top bars of the brood chamber once weekly for 3 weeks.	AFB	This drug should only be fed in the early spring or fall and consumed by the bees before the main honey flow begins, to avoid contamination of production honey. Complete treatments at least 4 weeks before main honey flow.	As of 1/1/2017, transitions from OTC to prescription
Oxytetracycline powder (Terramycin®)	200mg per colony, administered via either a 1:1 sugar syrup (equal parts of sugar and water weight to weight) or dusting with a powdered sugar mixture.	AFB and oxytetracycline-susceptible EFB	The drug is administered in 3 applications of sugar syrup or 3 dustings at 4- to 5-day intervals. The drug should be fed early in the spring or fall and consumed by the bees before main honey flow begins to avoid contamination of production honey. Remove at least 6 weeks prior to main honey flow	As of 1/1/2017, transitions from OTC to prescription
Oxytetracycline (Terramycin® and Pennox 50®)	200 mg/colony, administered via 1:1 sugar syrup, dusting, or extender patty.	AFB and oxytetracycline-susceptible EFB	Remove at least 6 weeks prior to main honey flow.	As of 1/1/2017, transitions from OTC to Veterinary Feed Directive (VFD)

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Next: European Foulbrood (EFB)

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EUROPEAN FOULBROOD

European foulbrood (EFB) is caused by *Melissococcus pluton*, a non-spore-forming bacteria, but infection is associated with a variety of bacterial strains. EFB only affects the honey bee larval stage and is more contagious than American foulbrood (AFB). It more commonly affects stressed colonies, and may resolve spontaneously if stress is reduced and honey bee health is improved. It is less severe than AFB, but can still cause devastating brood loss. In recent years, EFB has shifted its pathogenicity in the U.S.; it no longer spontaneously clears with the advent of a good nectar flow, and EFB infection is likely to persist in the hive.

Visual inspection findings
Field and laboratory testing
Treatment

European foulbrood diagnosis: visual inspection findings

Spotty brood pattern – Similar to AFB and other brood diseases, brood infected with EFB will exhibit a spotty brood pattern as larvae perish, and the healthy pattern of brood is disrupted.

Larval discoloration – EFB tends to only affect older larvae, and does not affect younger larvae or capped pupae. EFB-affected larvae exhibit discoloration (turn yellow or gray), and lose their normal glistening appearance. The trachea of the developing larvae often become apparent, looking like an internal skeleton of the larvae (upper left corner of image 29). EFB is often found with a variety of other bacteria so it is common to see twisted, yellow, and gray larvae together in the same frame. Drone brood may be the first to show signs of EFB, so make sure to inspect drone larvae if present.

Larval deformation – Infected larvae often assume a twisted or corkscrew position as they die. Sometimes the larvae have a melted appearance as they die and decay, or may look deflated or malnourished when infected.

Image 29: A brood comb badly infected with EFB. Note the discoloration of the larvae, as well as their twisted shape in contrast to healthy larvae which forms a flat C-shape at the bottom of the cell. Photo © Randy Oliver, ScientificBeekeeping.com

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Yellow brood food – The royal jelly/ brood food mix surrounding healthy larvae should be pearly white. In EFB colonies, one of the first signs of infection is often a yellowing of the food.

Image 30: A brood frame from a colony heavily infected with EFB. Note the bright yellow liquid surrounding the larvae. Note also the visible trachea in the larvae in the lower right, which looks like a skeleton in translucent larvae. Photo © Randy Oliver, ScientificBeekeeping.com

Image 31: A brood frame from a colony heavily infected with EFB, note the twisted larvae, discoloration, and yellow infected brood food. Note also that only the older larvae display the signs of infection and the younger, smaller larvae look relatively healthy. Photo © Randy Oliver, ScientificBeekeeping.com

Suspect European foulbrood if the following signs are present:

- Shotgun/patchy brood pattern
- Poor colony buildup in spring—diseased larvae may be difficult to detect without thorough inspection
- Discolored larvae (yellow or brown)
- Twisted or corkscrew – shaped larvae
- Visible trachea in larvae
- No scale is formed—the dead larval bodies are easily removed.
- Often a “sour milk” odor
- Yellow royal jelly around larvae

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Next: EFB, Field and Laboratory Testing

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EUROPEAN FOULBROOD: FIELD TESTING

A commercial field test is available for the diagnosis of EFB. Because EFB shares many visual similarities with American foulbrood (AFB), EFB should be among the differential diagnoses when the signs of AFB/EFB are observed but the characteristic tests for AFB are negative.

European foulbrood: Laboratory diagnosis and reporting

Samples should be sent to the USDA Agricultural Research Service (USDA-ARS) laboratory in Beltsville, Maryland.

How to send brood samples:

- Comb sample should be at least 2 x 2 inches and contain as much of the dead or discolored brood as possible. NO HONEY SHOULD BE PRESENT IN THE SAMPLE.
- The comb can be sent in a paper bag or loosely wrapped in a paper towel, newspaper, etc. and sent in a heavy cardboard box. AVOID wrappings such as plastic, aluminum foil, waxed paper, tin, glass, etc. because they promote decomposition and the growth of mold.
- If a comb cannot be sent, the probe used to examine a diseased larva in the cell may contain enough material for tests. The probe can be wrapped in paper and sent to the laboratory in an envelope.

Even if you are certain of your diagnosis for AFB, and you are practicing in a state without a mandatory reporting requirement, it is important to provide accurate national incidence data. See the USDA-ARS site for more details on specimen submission.

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Next: EFB, Treatment

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EUROPEAN FOULBROOD: TREATMENT

Oxytetracycline and tylosin have both been used to treat European foulbrood (EFB), however, ***oxytetracycline is the only drug FDA approved for EFB***. Furthermore, many beekeepers feel that tylosin is not effective, and most chose to use oxytetracycline. Beekeepers also report that EFB disease may resolve without treatment, although this seems to occur less often than previously reported. Stress reduction, including feeding, and re-queening the colony, may aid this process. If the infection is not severe (less than 10% of the brood is infected), the beekeeper may choose to employ watchful waiting, where the infected frames are marked and the colony is re-inspected in a week or so to identify if the infection is spreading or improving. In severe infections, it is often recommended to remove frames with diseased brood, and replace them with new comb in addition to treatment with antibiotics.

Since the bacteria that causes EFB does not have a spore form, frame and equipment destruction is not required; following several months of storage or sterilization with a bleach solution, the frames can be reused. Many beekeepers would choose to destroy equipment, or set them aside for a season, sterilize them or clean with a bleach solution, before reusing equipment from infected hives.

The water soluble forms of oxytetracycline, tylosin and lincomycin are available for use in honey bees by prescription. Although tylosin and lincomycin are only approved for treatment of AFB, and not approved for EFB, extra label drug use is allowed with a prescription under ELDU regulations. Visit AVMA's guidelines for VCPRs when writing a prescription.

Veterinary Feed Directive (VFD) orders do not allow for extralabel drug use. They also must expire within 6 months of issue, although the veterinarian may specify a shorter expiration date. There is only one drug available by VFD for honeybees – oxytetracycline. For use of a VFD, it is necessary to have a valid VCPR and at least a preliminary diagnosis of EFB or AFB prior to initiating treatment. Veterinarians should consult the FDA listing of VCPR requirement by state to determine what constitutes as a valid VCPR in your state.

With a valid VCPR and proper training of the staff, a veterinarian could prescribe an antibiotic to be used when the beekeeper's staff recognizes EFB or American foulbrood (AFB).
With any antibiotic use, make sure records are kept of the dates of treatments, drug, dosage, prescribed withholding time, and identity of the hives.

For treatment of EFB or AFB, oxytetracycline can be mixed with powdered sugar according the labeled instructions and applied to the top of the frames of the brood nest following the dosage and timing on the label. Dusting of uncapped brood cells has been reported to cause death of larval honey bees. Do not dust uncapped brood cells. Oxytetracycline can degrade rapidly when used in a sugar syrup. Therefore, a fresh syrup solution should be prepared daily.

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Antibiotics should be fed early in the spring or fall and consumed by the bees before main honey flow begins to avoid contamination of production honey. Remove at least 6 weeks prior to main honey flow. Due to the potential for residues in honey, it is recommended that tylosin only be used in the fall and not in the spring. Furthermore, tylosin should only be used according to directions as a powdered sugar mixture immediately after mixing. Honey stored during medication periods in combs for surplus honey should be removed following final medication of the bee colony and must not be used for human food. Honey from bee colonies likely to be infected with foulbrood should not be feeding other colonies since it may be contaminated with spores of foulbrood and may result in spreading the disease. Do not use in a manner contrary to state apiary laws and regulations. Each state has specific regulations relative to disease control and medications. Contact the appropriate official or state departments of agriculture for specific inter- and intrastate laws and regulations.

Extender patties (a mix of vegetable oil, sugar, and antibiotics) can also be used as a medicated feed product to administer oxytetracycline. This practice allows the antibiotics to be present in the colony for a longer period of time, which increases the risk of honey contamination and the potential for development of resistance. ***Off label or extralabel use of medicated feed products including VFD products are not permitted.*** However, in general, enforcement action will not be recommended or initiated when the use is consistent with FDA Compliance Policy Guide 615.115 – Extralabel Use of Medicated Feeds for Minor Species.

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Next: Diseases Similar to AFB and EFB

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DISEASES THAT LOOK SIMILAR TO EUROPEAN AND AMERICAN FOULBROOD

Two brood diseases may present similarly to European foulbrood (EFB) and American foulbrood (AFB), but may not require antibiotics; these two diseases are Idiopathic Brood Disease (IBD) and Parasitic Mite Syndrome (PMS). The specific etiologic agents of these two diseases remain unknown, although it is thought that they are caused by multiple viruses, and could be found in the presence of secondary bacterial infections.

Idiopathic Brood Disease (IBD)
- Visual inspection findings
- Diagnosis
- Treatment

Parasitic Mite Syndrome (PMS)
- Inspection and diagnosis
- Treatment

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Next: Idiopathic Brood Disease

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IDIOPATHIC BROOD DISEASE (IBD)

We currently do not know the etiologic agents for every disease that affects honey bees. Some issues affecting bees or adult bees cannot be clearly identified. One example of this is a prevalent brood syndrome called Idiopathic Brood Disease or "snot brood." Likely caused by viruses, IBD appears similar to European foulbrood (EFB), American foulbrood (AFB), and Parasitic Mite Syndrome (PMS). Idiopathic brood disease is often referred to as 'snot brood,' or beekeepers may say that they have "atypical EFB." Colonies with IBD display a host of signs that overlap with both AFB and EFB.

Visual inspection findings
Diagnosis
Treatment

Idiopathic Brood Disease: Visual inspection findings

While AFB and EFB typically infect only certain stages of development, and the bees die in a peculiar fashion, brood frames from a colony with IBD will have a wide range of signs. Brood are often melted, twisted, and can form a scale. Pupal cappings are often perforated or sunken, and larvae can darken to a brown color similar to AFB.

> **Larval Deformation -** IBD / snot brood is characterized by a diverse array of larval outcomes. Like EFB, the older larvae appear affected, and can die in a melted-type appearance. The larvae often appear white and gummy, but can turn dark gray and even form a loose scale, larger and looser than AFB.
> **Perforated/Sunken Cappings -** Similar to AFB, prepupae may also be affected, and it is common to see perforated cappings, and cappings oozing with dark liquid, indicating dead pupae or prepupae underneath.

Idiopathic Brood Disease: Diagnosis

Pupal cappings are often perforated or sunken, and larvae can darken to a brown color similar to AFB. However, the pupal tongue is not present, and IBD will not rope with a match stick test; when a match stick test is performed, the dead pupae generally will come out in a clump. It may rope slightly, but not nearly the 2cm as seen with AFB.

Some samples with IBD that are sent to the USDA laboratory do receive a positive EFB diagnosis, but many do not, indicating that co-infection may be possible, but that it is not necessarily related to EFB. A mite check should be performed to rule out parasitic mite syndrome (next section); IBD is generally associated with colonies with low levels of mites. At the moment, there is no definitive field or lab diagnosis for IBD.

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Suspect Idiopathic Brood Disease if the following signs are present:

- Shotgun/patchy brood pattern
- Larvae may turn yellow but retain 'C' position
- Larval death in propupal stage – sticking straight up parallel to the cell
- Larvae appear melted and gummy
- Larvae eventually melt into dark gray
- Perforated cappings
- May form a loose scale
- Often a foul odor (but different from EFB and AFB)

Idiopathic Brood Disease: Treatment

Many beekeepers report an improvement in colony health after a course of antibiotics (usually oxytetracycline or tylosin). Others report no improvement with antibiotic treatment, and others have reported that signs spontaneously clear. Many beekeepers have reported an association of IBD and exposure to some insecticides, namely fungicides.

While some reports indicate improvement of symptoms with antibiotic treatment, spontaneous clearing also occurs with IBD. Removal of diseased frames, breaking the brood cycle (e.g. by using various methods to prevent the queen from laying eggs for several weeks), and re-queening have been shown to be useful in clearing up infection.

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VARROA MITES AND PARASITIC MITE SYNDROME

Varroa mites (*Varroa destructor*) are ectoparasites with worldwide distribution (reported in the U.S. since 1987) and remain the number one killer of honey bees. According to the USDA, beekeepers have identified varroa mites as their single most serious problem causing colony losses today. The mites feed on the hemolymph and fat cells of the bees. They target larvae that are about to be capped, and move to the bottom of the cell and feed off of the larva once the cell is capped. The mites mate inside the cell and mature; once the bee emerges from the cell, it will already have female mite offspring on it. The mites can cause larval or pupal death and can transmit deformed wing virus, acute bee paralysis virus, Israeli acute paralysis virus, slow bee paralysis virus and other pathogens. Affected larvae may spiral up the cell, eventually curling around the opening of the cell in a half-moon shape.

Parasitic mite syndrome (PMS) is caused by viruses transmitted by the varroa mite. In a severely mite-infested colony, bees may express parasitic mite syndrome**.** This syndrome is most commonly seen in the late season in colonies where mites have not been actively managed. Deformed wing virus (DWV) is likely one of the major pathogens, if not the main pathogen, causing disease in PMS.

Visual inspection findings and diagnosis
Treatment

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PARASITIC MITE SYNDROME DIAGNOSIS: VISUAL INSPECTION FINDINGS

Because the mites carry multiple viruses, and because a stressed colony can experience secondary infections, PMS may present with a variety of signs:

Chewed pupae and melted larvae - A first sign is generally a spotty brood pattern, with the presence of chewed pupae, which may have brown or black spots as they begin to decay. Melted larvae may be present.

Image 32. Typical PMS (Parasitic Mite Syndrome) due to a high level of varroa infestation. Note the partially chewed-out pupae and the larvae that appear similar to EFB or AFB, simply slumping down. There are a few cells where a pupa has been recently uncapped. By this point of the virus epidemic, it will be very difficult for the colony to recover, even if varroa is controlled. Photo © Randy Oliver, ScientificBeekeeping.com

Bees dying on emergence from cells – Bees infected with PMS die at many stages, and it is one disease where they die when they are almost fully formed and trying to eclose (exit) from their cells. Typically, you will see some bees that look fully formed in the cells, with their tongues sticking out.

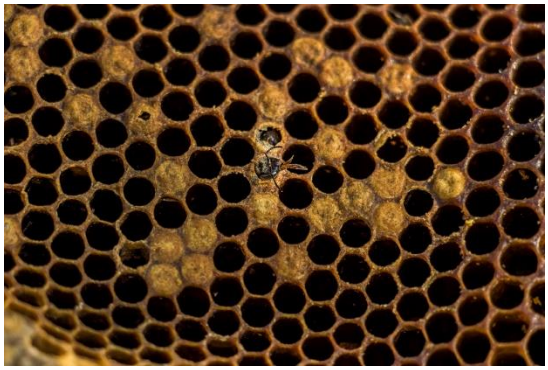

Image 33. Brood frame of colony suffering from an advanced parasitic mite syndrome. Teneral (freshly molted) adults unable to emerge die with characteristic protruding tongue. Photo © Sarah Scott, USGS

Guanine deposits -You may also view guanine deposits caused by mite defecation, which appear like grains of coarse salt stuck to the sides of the cells. To view these deposits, hold the frame horizontal, similar as you would to view AFB scales.

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Image 34. Varroa fecal deposits (guanine) are visible as small white specks on the sides of the cells. Frame is held with the bottom bar towards the viewer. Photo © Randy Oliver, ScientificBeekeeping.com

Deformed wings – Often, young bees will be present exhibiting wrinkled, deformed wings. They may be seen moving on the frames, or crawling outside of the hive. You may not see any bees with deformed wings as they often abandon the hive or are removed.

Image 35. Bees exhibiting signs of deformed wing virus. Upper left bee (marked with red arrow) has characteristic deformed wings and stunted body. Five other bees with deformed wings are visible around the edge of the photo.
Photo © Sarah Scott, USGS

Visible varroa mites - Varroa mites are visible with the naked eye, but may not be observed on inspection because most of the mites are underneath the cappings, or on the underside of bees. Generally, varroa mites on adult bees are only observed when an infestation is very advanced.

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Image 36. Mites on underside of bee abdomen. Two mites are visible on this newly emerged bee (the point of the red arrow is located between the two mites), one with a slightly lighter colored shell. Photo © Randy Oliver, ScientificBeekeeping.com

Collapsing cluster size/ absconding – It is typical for a colony that is heavily infested with varroa to abscond, or for the colony to get much smaller. The beekeeper will report that the colony was "full of bees a week ago," and the upper hive body will be full of honey, but the bees will have disappeared. The telltale signs of the cause are the dead un-emerged adults with their tongues out (they will have deformed wings), and the white mite fecal deposits on the cell ceilings. Generally, the beekeeper will not have records of mite counts from monitoring.

Suspect Parasitic Mite Syndrome if the following signs are present:

- Shotgun/patchy brood pattern
- Melted larvae
- Bees dying on emergence from cells
- Guanine crystals on walls of cells
- Adult bees exhibiting deformed wings
- Chewed pupae
- Uncapped pupae (eyes visible)

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Next: PMS, Treatment

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PARASITIC MITE SYNDROME- TREATMENT

Parasitic mite syndrome can only be prevented and controlled by managing the populations of varroa mites. Depending on the time of year and the location, it may not be possible to save a colony that is showing signs of PMS. Beekeepers in warmer climates can sometimes have a colony rebound with treatment and feeding, while colonies exhibiting PMS before a winter rarely survive. It is recommended to apply a treatment of a miticide to kill the mites and prevent the spread to other colonies.

Management of varroa Mites

Varroa mites are found in every hive in the country and are the most devastating parasites of bees because of their ability to vector other disease agents. Beekeepers must have a strategy in place to manage the varroa mite, and those that do not will experience high mortality of their hives and can transmit disease to nearby colonies. Mite-associated disease remains one of the leading causes of honey bee death, especially among hobby and small scale beekeepers. The honey bee veterinarian should know about the life cycle of this parasite, its monitoring methods, control points, and treatment methods. See the following resources for more information:

- Honey bee health coalition tools to manage varroa
- Michigan State varroa resources

Small- scale beekeepers should be practicing integrated pest management (IPM) to manage for varroa mites. This means that they are actively monitoring mite populations (generally with an alcohol wash or sugar roll), and should employ many physical, mechanical, and if necessary chemical controls. Most commercial beekeepers are able to manage mite populations through repeated applications of chemical miticides.

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Next: Honey Bee Viruses

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HONEY BEE VIRUSES

Honey bees are susceptible to a number of viruses.

Paralytic viruses such as Israeli acute paralysis virus, slow bee paralysis virus, chronic bee paralysis virus and acute bee paralysis virus can cause shivering wings, darkened hairless abdomens and thoraxes, and can progress to paralysis and death. Chronic bee paralysis virus has been becoming more prevalent in recent years. It exhibits as two forms: In "hairless black syndrome" infected adults tremble, are groomed clean of setae (body hairs), and their wing edges are ragged from biting. In the other form, hundreds of trembling, dying bees drop to the bottom board, looking very much like a pesticide kill.

Image 37. Bee exhibiting signs of Chronic bee paralysis virus. Notice that hairless or 'greasy' appearance of the bee. Older bees can lose most of their hair as they age, but it is not so complete on the body while remaining on the head as shown here.
Photo © Randy Oliver, ScientificBeekeeping.com

The Sacbrood virus attacks larvae, resulting in a "shrunken head" appearance and failure to pupate. The larvae are observed on the floor of the cell with discolored or blackened heads angled upward, as shown in the image below.

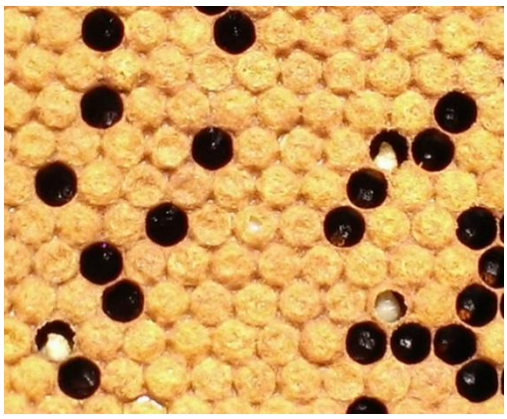

Image 38 - Typical Sacbrood, propupae dying with upraised heads, and the skin forming a sac that can be easily pulled from the comb. Photo © Randy Oliver, ScientificBeekeeping.com

Image 39: fluid sac observed when Sacbrood-infected larvae are pulled from cell. Photo source: Plant and Food Research, NZ. Used with permission.

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When the larvae are pulled out of the cell, there is an obvious fluid sac (Image 39, red circle).

Treatment of Sacbrood –This viral infection generally spontaneously resolves, and the colony does not experience significant setback. Requeening is possible in severe infestations.

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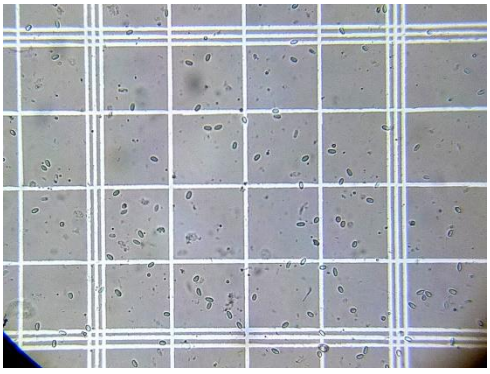
MICROSPORIDIAL DISEASES: NOSEMA INFECTIONS

Two types of Nosema infections are possible, caused by the single-celled microsporidia *Nosema apis* and *Nosema ceranae*. The spores spread from bee to bee by feeding and fecal-oral transmission. The two Nosema diseases have different natural histories. *Nosema apis* tends to be worse in northern climates, and outbreaks occur more often in the fall and winter when bees cluster together to maintain hive warmth, while *Nosema ceranae* can be present year round.

Nosema signs - Signs of Nosema disease can include bees wandering on ground outside colony; "K-wing," (see Image 45), which is an uncoupling of the forward and rear pairs of wings that results in wings that resemble the letter K; yellow, orange or brown fecal staining on front of colony; and poor growth of bees and death.

Nosema diagnosis -. Nosema infection must be confirmed by simple microscopy, either of a sample of adults, or by one-by-one gut squashes.



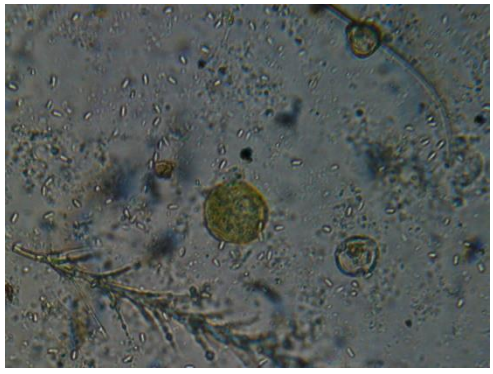


Image 40 - Nosema spores at 400x in a hemacytometer. Photo © Randy Oliver, ScientificBeekeeping.com

Image 41 - A more typical view, with Nosema spores among pollen grains and bee parts. Photo © Randy Oliver, ScientificBeekeeping.com

Nosema treatment - Many beekeepers treat Nosema or treat prophylactically with dicyclohexylammonium Fumagilin Soluble Powder HS (Fumagilin-B®, Medivet), though it is not approved for use in the U.S.

Use of unapproved drugs in food animals is strictly prohibited. Furthermore, use of Fumagilin-B for control of *Nosema ceranae* is not recommended by some experts due to spore hyperproliferation upon withdrawal of the antibiotic. There is currently no approved treatment for Nosema disease, though many colonies clear the infection. In chronic cases, requeening and food supplementation may be useful.

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CHALKBROOD

Chalkbrood is caused by the *Ascosphaera apis* fungus that attacks larvae and pupae. This condition is commonly seen in the spring and is more prevalent during rainy or wet weather, or after the colony has been chilled.

Chalkbrood signs - White fungal growth is observed, followed by white or black "mummies" of dead larvae or pupae. These mummies are seen in the brood cells, and also at the entrance of the hive.

Chalkbrood treatment – There is no chemical therapy for chalkbrood. Stressed hives are at higher risk, and the disease is best controlled by reducing stress through good management practices and by using disease-resistant bees. The hygienic behavior of a strong colony will often clear the infection. In some cases chalkbrood can become persistent, in which case, requeening and removal of diseased brood frames is recommended.

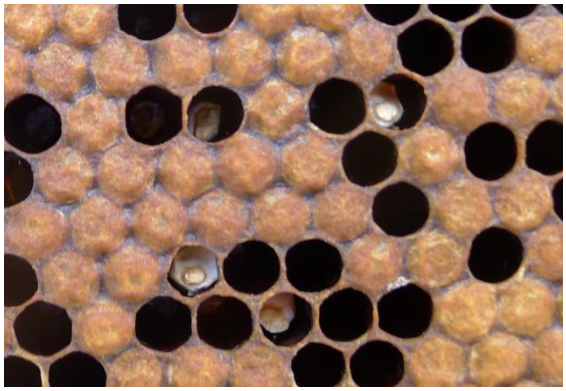

Image 42 - Chalkbrood mummies at the hive entrance. Photo © Randy Oliver, ScientificBeekeeping.com

Image 43 - Chilled brood following an overnight frost—not an indication of disease. Photo © Randy Oliver, ScientificBeekeeping.com

Image 44 - Typical chalkbrood mummies still in the comb. Note the distinctive two-tone appearance, with a tan oval surrounded by white tissue. Photo © Randy Oliver, ScientificBeekeeping.com

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PARASITES

Tracheal mite infestation
Small hive beetles
Tropilaelaps spp

Tracheal mite infestation

Tracheal mite infestation (Acarapisosis) can be difficult to recognize until the damage is severe. Mite females infest 1-2 day old bees and penetrate their thorax and trachea and lay eggs. The offspring mature and mate inside the trachea, causing damage obstruction. Crowded colonies are at higher risk, and infestations tend to peak in the fall and winter when bees are clustered. Affected bees may develop "K-wing," (Image 45) which is an uncoupling of the forward and rear pairs of wings that results in wings that resemble the letter K; however, this is not unique to tracheal mite infestation. Confirmation of infestation is made with microscopic evaluation of affected bees.

> **Tracheal mite control -** This disease has not been widely found in the USA in recent years, which may be related to the high use of miticides for Varroa control. Resistant queens are the preferred method of control, but grease patties or menthol may also be used.

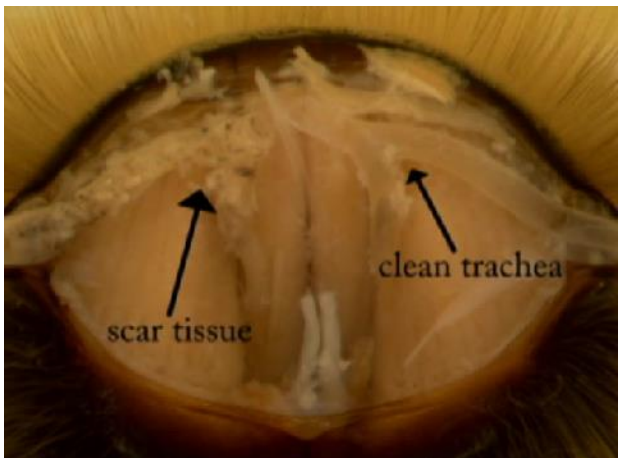

Image 45: K-wing in a honey bee. Photo © University of Florida

Image 46: scar tissue in trachea of bee, viewed under dissecting microscope, with tracheal mite infestation. Photo © University of Florida

Small hive beetles

Small hive beetles (*Nitidulidae* species) originated in Africa and were first observed in the Southeast U.S. in the mid-1990s. The females lay eggs in brood cells and their larvae feed on brood, honey, and pollen before leaving the hive to pupate in the surrounding ground and start the life cycle again. The beetle larvae excrement may ferment honey. These beetles are

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opportunistic scavengers, so the best way to control them is to have strong, healthy colonies that resist their infestation. In northern states with hard winters, beetles are generally indicative of another underlying the issue that weakens the colony, though beetles can severely infest strong colonies in warmer climates. Most beekeepers control beetles with trapping using a frayed fabric or a commercially available trap. Biological control with entomopathogenic nematodes can also be effective means of controlling the beetles. Pesticides such as coumaphos and permethrin are available for use, but can have detrimental effects on the honey bees and should be avoided if possible. If used, it is essential that label directions are followed.

Image 47: Wax moth larvae (wax worms)—a common scavenger in weak or dead hives—not to be confused with SHB larvae. Photo © Randy Oliver, ScientificBeekeeping.com

Image 48: Small hive beetles exposed from under the hive cover. Photo © Randy Oliver, ScientificBeekeeping.com

Image 49: Small hive beetle larvae—bristly and without the distinctive head of wax worms. Photo © Randy Oliver, ScientificBeekeeping.com

Image 50: A "slime out" from SHB larvae and their associated yeast. Photo © Randy Oliver, ScientificBeekeeping.com

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Tropilaelaps spp

Tropilaelaps is the genus name of a mite with four species identified. None have been found in the U.S. When *Apis melifera* has been kept in regions where this mite is found, the mite can create severe infection. Tropilaelaps causes damage similar to varroa, resulting in irregular brood patterns and stunted adults with deformed wings and shrunken abdomens. Tropilaelaps mites are reddish brown, and are smaller than the varroa mite - about 1mm long and 0.6mm wide - and can be seen running on frames in between cells. Tropilaelaps is of high regulatory concern and any suspicious infections should be sampled and the state bee inspector contacted immediately.

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OTHER HEALTH CONDITIONS

A number of additional health conditions can occur in honey bees, and some of these may increase the risk of other diseases or compound existing problems.

Malnutrition
Pesticide toxicity
Diploid drone syndrome
Overly hygienic hive
Drone-laying queen
Laying Worker
Colony Collapse Disorder

Malnutrition

Malnutrition weakens the honey bee's immune system, making them more susceptible to stress and disease. Honey bee nutrition is impacted by loss of diverse plant habitat due to urban and suburban expansion as well as drought and other severe weather conditions. Artificial diets are available, but may not be sufficient in some cases.

Pesticide toxicity

Pesticide toxicity is an ongoing concern. The U.S. Environmental Protection Agency (EPA) has strict regulations in place to protect managed honey bee colonies from pesticide misuse and offers guidance for investigating possible pesticide-related mortalities. In fall 2016, the EPA announced a pollinator risk assessment guidance resource as "part of a long-term strategy to advance the science of assessing the risks posed by pesticides to bees."

Many sub-lethal effects have been identified from pesticides and other environmental chemicals, but they are difficult to identify based on visual inspection. You may suspect pesticide damage if you notice a large number of dead or dying bees at the front of the hive entrance. Make sure that you follow state reporting guidelines, and take care to record the incident (including photos) so that it can be properly reported.

Diploid drone syndrome (inbreeding)

This syndrome results from the queen mating with a close relative drone. The offspring are diploid, but they are homozygous at the sex locus. Despite being fertilized and diploid, the eggs don't develop into worker bees because nurse bees detect the problem and destroy the egg. This usually necessitates re-queening the hive. This situation is very rare in US beekeeping, and will most likely not be seen in most operations.

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Overly hygienic hive

Bees deal with diseased pupae or larvae by removing them from the cells (hygienic behavior). Good hygiene is imperative for a properly functioning hive, but can be damaging if normal brood are unnecessarily destroyed by overly hygienic workers. This resembles diploid drone syndrome in that the nurse bees cull brood, but you can often view uncapped and chewed pupae.

Drone-laying queen

This occurs when the queen lays drone eggs in the worker cells. Although the brood is usually healthy, the pattern is chaotic and can produce excessive drones, which do not contribute work to the hive. This usually necessitates re-queening the hive.

Laying Worker

Normally, there is a queen and brood in a hive, and the pheromones from both inhibit ovary development in the worker bees, preventing them from laying. In the absence of these pheromones (loss of queen and no brood), inhibition is lost and one or more workers develop to the point that they can lay eggs. Because the laying workers never mate, the eggs are haploid and thus become drones.

Signs of a laying worker colony - The brood pattern is spotty, and will be all drone brood laid in worker cells (bullet-shaped). There are typically many eggs in a cell, and they are likely to be poorly laid, and attached to the sides of the cell.

Image 51: laying worker colony, with many eggs in each cell and eggs attached to the sides of the cells. Photo © Randy Oliver, ScientificBeekeeping.com

Image 52: laying worker colony, with spotty brood pattern and bullet-shaped caps on cells. Photo © Randy Oliver, ScientificBeekeeping.com

Treatment of a laying worker colony – Laying worker colonies are under severe stress and are likely to exhibit signs of disease, malnutrition, and pest invasion, so it is best to deal with them to prevent disease transmission in the apiary. This is a terminal condition for the colony, and cannot be righted with the addition of a queen. Small

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laying worker colonies can be shaken out and the equipment removed (the bees will enter other hives). They can also be directly added to other colonies in the yard. Large laying worker colonies should be combined with a nuc or other colony using a newspaper combine.

Colony Collapse Disorder (CCD)

In 2006, beekeepers began observing dramatic declines in honey bee colonies. Further investigation revealed that the situation was different from previously known causes of loss. Colony Collapse Disorder is defined as a dead colony with no adult bees and no dead bee bodies but with a live queen, and usually honey and immature bees (brood), still present. It does not include honey bee colonies that are lost due to any reason, such as losses due to poor management or nutrition; varroa mites; or other diseases described in this section, even though the colony size may seem to 'collapse' as in the case with varroa/virus infections.

Although intensive research continues and numerous rumors and theories have been suggested, the cause for CCD has not yet been proven. According to the USDA, most research has pointed to a complex of factors being involved in the cause of CCD, though these are not uniform in all CCD incidents.

As research continues and more is learned about CCD, the USDA recommends that beekeepers focus on improving general honey bee health and habitat and use best management practices to reduce stress and mitigate the known causes of reduced production and honey bee death. An emergency response kit is available to evaluate a collapsing hive and protocols are available for sampling live bee and salt water solution and pollen.

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SUBMISSION OF SPECIMENS FOR DIAGNOSIS

The following instructions have been excerpted from the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Bee Disease Diagnosis Service site.

General Instructions

- Beekeepers, bee businesses, and regulatory officials may submit samples.
- Samples are accepted from the United States and its territories; samples are NOT accepted from other countries.
- Include a short description of the problem along with your name, address, phone number or e-mail address.
- There is no charge for this service.
- For additional information, contact Sam Abban by phone at (301) 504-8821 or e-mail at samuel.abban@ars.usda.gov.

How to Send Adult Honey Bees

- Send at least 100 bees and if possible, select bees that are dying or that died recently. Decayed bees are not satisfactory for examination.
- Bees should be placed in and soaked with 70% ethyl, methyl, or isopropyl alcohol as soon as possible after collection and packed in leak-proof containers.
- USPS, UPS, and FedEx do not accept shipments containing alcohol. Just prior to mailing samples, pour off all excess alcohol to meet shipping requirements.
- Do NOT send bees dry (without alcohol).

How to send brood samples

- A comb sample should be at least 2 x 2 inches and contain as much of the dead or discolored brood as possible. NO HONEY SHOULD BE PRESENT IN THE SAMPLE.
- The comb can be sent in a paper bag or loosely wrapped in a paper towel, newspaper, etc. and sent in a heavy cardboard box. AVOID wrappings such as plastic, aluminum foil, waxed paper, tin, glass, etc. because they promote decomposition and the growth of mold.
- If a comb cannot be sent, the probe used to examine a diseased larva in the cell may contain enough material for tests. The probe can be wrapped in paper and sent to the laboratory in an envelope.

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Send samples to:

Bee Disease Diagnosis
Bee Research Laboratory
10300 Baltimore Ave. BARC-East
Bldg. 306 Room 316
Beltsville Agricultural Research Center - East
Beltsville, MD 20705

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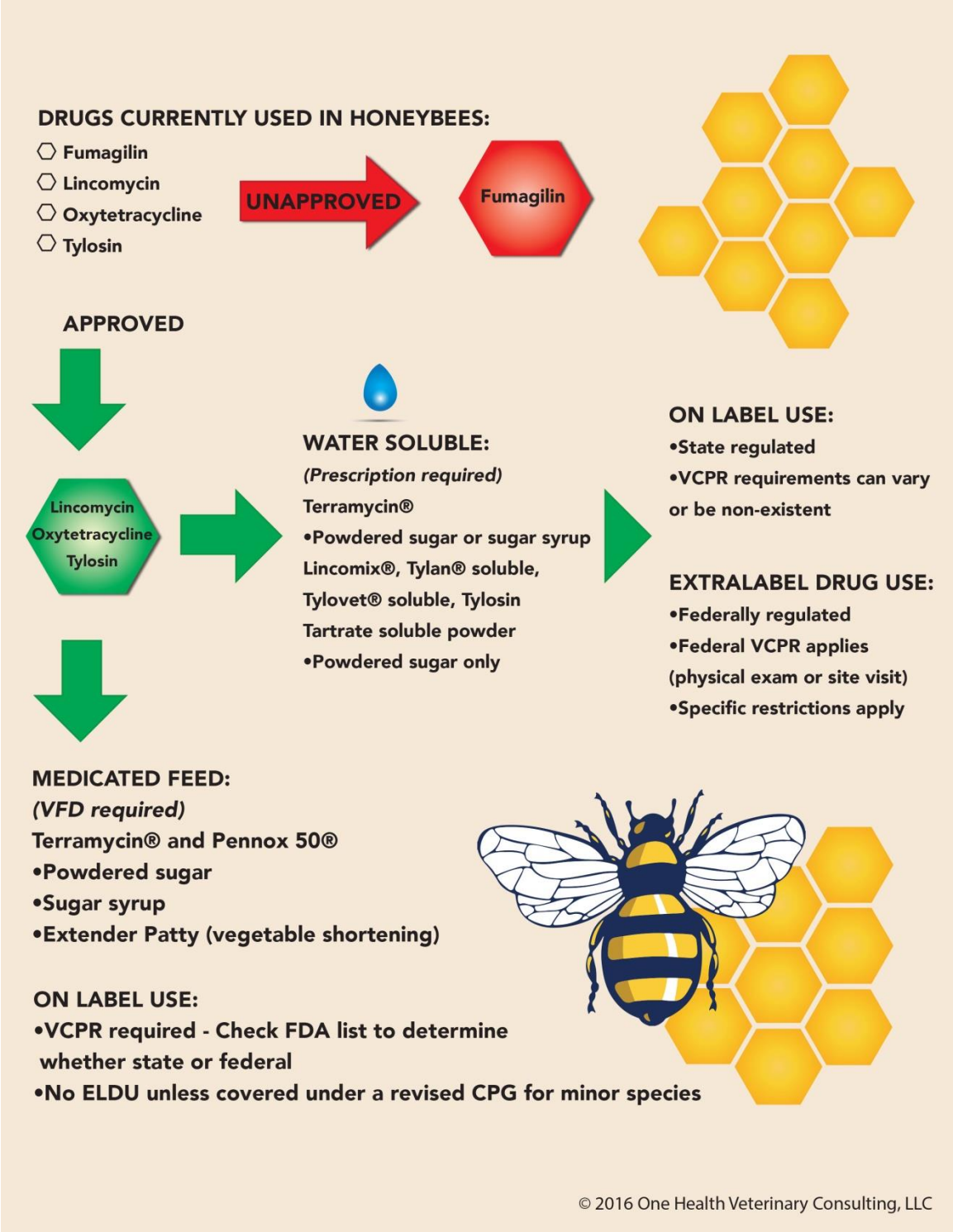

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ADDITIONAL RESOURCES

General honey bee information, biology and terminology

USDA: Honey Bees
Honey bee lifecycle (South Carolina Mid-State Beekeepers Association)
Anatomy and physiology of honey bees (South Carolina Mid-State Beekeepers Association)
Principles of honey bee genetics (Glenn Apiaries)
Bees need vets (Michigan State University)
Beeinformed.org
American Beekeeping Federation
Apiary Inspectors of America
Eastern Apicultural Society
List of beekeeping organizations
Western Apicultural Society

Beekeeping equipment

Parts of a beehive (Bees-and-Beekeeping.com)
Basic components of the Langstroth bee hive

Hive inspection and health

(video) Examining bee hives for disease (DPI Agriculture, New Zealand)
(video) Hive inspection, part 1: general principles (Honey Bee Honey)
(video) Hive inspection, part 2: how to find the queen (systematically) (Honey Bee Honey)
(video) Hive inspection, part 3: evaluating the queen (Honey Bee Honey)
Honey comb identification (Backyardhive.com)

Honey bee diseases

Beeinformed.org
A guide to the field diagnosis of honey bee brood diseases (Agriculture Victoria)
BeeAware.org (Australia)
Field guide to honey bees and their maladies
University of Florida Honey Bee Research & Extension Lab
Beeinformed.org
A guide to the field diagnosis of honey bee brood diseases (Agriculture Victoria)
Honey bee helpers: It takes a village to save a colony (USGS)

Bacterial diseases

American Foulbrood (CABI Invasive species compendium)
European Foulbrood (CABI Invasive species compendium)
(video) Hive inspection, part 4: American Foulbrood (Honey Bee Honey)

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(video) European and American foulbrood in honey bee colonies: Part 1 (University of Florida Honey Bee Research & Extension Lab)
(video) European and American foulbrood in honey bee colonies: Part 2 (University of Florida Honey Bee Research & Extension Lab)
(video) Identifying American Foulbrood (DPI Agriculture, Australia)
(video) American Foulbrood (Crispin Boxhall)
(video) AFB elimination 8 – how to recognise AFB (New Zealand)
(video) European foulbrood (Crispin Boxhall)

Viral diseases

Sacbrood (BeeAware.org)

Fungal diseases

Chalkbrood disease (BeeAware.org)

Nosema
(video) Nosema (University of Florida Honey Bee Research & Extension Lab)

Parasitic diseases

Honey bee viruses, the deadly Varroa mite associates
Honey Bee Health Coalition Tools for Varroa Management
MSU - Managing the varroa mite
(video) Honey bee apiary official inspection by the PA Department of Ag – Varroa destructor test
Tracheal mites (University of Florida Honey Bee Research & Extension Lab)
Small hive beetles (University of Florida Honey Bee Research & Extension Lab)
Bee Biosecurity Video Series, Plant & Food Research (NZ)

Colony Collapse Disorder

Honey bee health and Colony Collapse Disorder (USDA)

Other conditions

Diploid drones
Drone-laying queen

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